Rôle du récepteur 5-HT4 et de la protéine beta-arrestine 1 dans la modulation des processus émotionnels et cognitifs dans un modèle d'anxiété-dépression

Flavie Darcet

NNT : 2016SACLS126

Thèse de Doctorat
de l'Université Paris-Saclay
préparée à
l'Université PARIS SUD

ÉCOLE DOCTORALE N°569
INNOVATION THERAPEUTIQUE : DU FONDAMENTAL A L'APPLIQUE

Spécialité de doctorat : Sciences pharmacologiques

Par

Mme Flavie DARCET

Rôle du récepteur 5-HT_4 et de la protéine β-arrestine 1 dans la modulation des processus émotionnels et cognitifs dans un modèle d'anxiété/dépression

Thèse présentée et soutenue à Châtenay-Malabry, le 18 mai 2016

Composition du Jury :

M. Jean-Martin Beaulieu, Professeur, Université de Laval, Québec, Canada, Président
M. Tangui Maurice, Directeur de Recherche, INSERM U.1198, Université de Montpellier, Rapporteur
M. Michel Boulouard, Professeur, Université de Caen Basse Normandie, UCBN, Rapporteur
M. Bruno Giros, Professeur, UMR 8246, Neurosciences, Université Pierre et Marie Curie, Examinateur
M. Denis David, Professeur, Univ. Paris Saclay, Univ. Paris Sud, INSERM UMRS 1178, Directeur de thèse
M. Jean-Philippe Guilloux, Maître de Conférences, Univ. Paris Saclay, Univ. Paris Sud, INSERM UMRS 1178, Directeur de thèse

Remerciements

- ***A Messieurs les membres du jury,***

Je remercie Monsieur le **Professeur Michel Boulouard**, Doyen de l'U.F.R des Sciences Pharmaceutiques à l'Université de Caen Basse Normandie pour m'avoir fait l'honneur d'accepter d'évaluer et de juger mon travail de thèse en qualité de rapporteur.

Je remercie Monsieur le **Docteur Tangui Maurice**, Directeur de recherche (CNRS U1198) à l'Université de Montpellier pour m'avoir suivie depuis le comité de mi-thèse et pour avoir accepté de juger mon travail final en tant que rapporteur.

Je remercie Monsieur le **Professeur Jean-Martin Beaulieu**, Directeur de recherche à l'Université de Laval (I.U.S.M.Q) pour m'avoir donnée l'opportunité de travailler sur les souris génétiquement modifiées pour la β-arrestine 1 et pour notre fructueuse collaboration autour de cette thématique. Merci également d'avoir accepté de faire partie de mon jury de thèse.

Je remercie Monsieur le **Professeur Bruno Giros**, Directeur de recherche à l'Université Pierre et Marie Curie (UMRS113- INSERM - UMR8246-CNRS) d'avoir accepté de siéger à ce jury de thèse.

- ***A Monsieur le Professeur Alain Gardier,***

Je tiens à vous exprimer toute ma reconnaissance pour m'avoir accueillie au sein de votre laboratoire et pour m'avoir soutenue dès le master 2 et tout au long de ces 3 années de thèse. Je vous adresse mes remerciements les plus sincères pour m'avoir donné l'envie d'explorer le domaine de la pharmacologie et des neurosciences. Merci enfin pour tous vos conseils avisés et votre implication dans mon travail de thèse.

- ***A Monsieur le Professeur Denis David,***

Je peux affirmer sans nul doute que c'est votre passion et votre enthousiasme pour la recherche qui m'ont poussés à m'engager dans ce travail de thèse. Je tiens donc à vous remercier sincèrement pour m'avoir permis de vivre cette expérience humaine et professionnelle très formatrice et pour m'avoir confié des projets de recherche très intéressants et ambitieux. Merci également pour votre soutien et votre grande disponibilité tout au long de mon parcours. Souvenez-vous, vous m'aviez dit en début de thèse que vous accepteriez que je vous tutoie lorsque je deviendrai Docteur en Sciences. A l'heure où vous lisez ces quelques lignes, c'est chose faite (ou presque !), alors aujourd'hui Denis, officiellement, je TE remercie pour ces 3 années de collaboration !

- ***A Monsieur le Docteur Jean-Philippe Guilloux,***

Un merci tout aussi grand te revient JP. Je tiens à te remercier chaleureusement pour ta présence, ta disponibilité et ton énorme investissement pour l'ensemble de mon travail de thèse. Merci beaucoup également pour ton aide précieuse autant dans les nombreuses manipulations de qPCR, d'hybridation ou de prélèvements de structures que dans les tâches plus … informatiques ! Et même si nos univers musicaux se confrontent (« c'était mieux avant ! »), je trouve qu'on a formé une plutôt bonne équipe ;-) !

- ***A Monsieur le Docteur Laurent Tritschler,***

J'ai beaucoup apprécié travailler avec toi à l'occasion de notre brève mais agréable collaboration sur le Frontiers. Merci pour ta bonne humeur communicative et pour tous tes coups de main qui m'ont été bien utiles, Mac Giver du labo ! Tu resteras pour moi l'homme au chapeau, qui raconte comme personne d'autre l'histoire du parapluie, et quelle histoire !

- ***Aux chercheurs et docteurs avec lesquels j'ai pu collaborer durant la thèse,***

Je tiens à remercier les Docteurs **Alexandra Barbelivien** du Laboratoire de Neurosciences Cognitives et Adaptatives de Strasbourg (UMR7364) ainsi que **Marianne Leger** de l'Université de Caen Normandie pour les conseils constructifs qu'elles m'ont apportés sur la mise au point des tests de cognition.

Je remercie l'équipe du Docteur **Sandrine Humbert** (Institut des Neurosciences, Grenoble) pour avoir participé à mon travail de thèse et pour m'avoir permis de découvrir une nouvelle technique *in vitro*. Je tiens à remercier tout particulièrement **Fabienne Agasse** et **Caroline Benstaali** pour leur aide précieuse et leur enthousiasme dans ce projet de recherche.

Je remercie l'équipe de **Fabrice Chrétien** de l'Unité d'Histopathologie humaine et modèles animaux de l'Institut Pasteur à Paris pour avoir pris le temps de scanner les lames d'hybridation *in situ* dans le cadre du projet sur le protéine β-arrestine 1 et pour m'avoir conseillée sur la méthode de comptage.

Je remercie Monsieur le Professeur **René Hen** pour m'avoir permis de travailler sur des souris Nestine-CreER^{T2} afin de créer les animaux tissus-spécifiques. Je remercie également les Docteurs Mazen Kheirbek, Ben Samuels et Christine Denny pour tous leurs conseils constructifs en réponse à mes problématiques de thèse.

- ***Aux doctorants, post-doctorants ou stagiaires, d'hier et d'aujourd'hui, qui ont croisé mon chemin,***

Merci à Maryam, Daniela, Ha, Charlène, Christelle, Yannick, Indira, Gaël, Sophie, Guillaume, Lin, Hai, Asmaa, Clarisse, Délia, Flavien pour votre dynamisme et votre bonne humeur. Pour ceux qui commencent ou sont en cours, COURAGE ! Pour ceux qui ont fini … BRAVO ! Merci à Louise, Nicole et Françoise pour leur aide.

- ***A tous les membres de l'ADIT,***

Romain, Benjamin, Hélène, Stéfania, Marie L., Marie D., Géraldine, Marie-Eliane, Rami, Chloé. Continuez à faire vivre cette association ;-)

- ***Au personnel de la plateforme Animalerie,***

Je tiens à remercier particulièrement **Valérie Dupont-Domergue**, **Ayma Galland** et **Pauline Robert** pour leur aide, leur gentillesse et leur professionnalisme. Merci également à Oliver, Julie, Aurélie qui ont également permis que mes études de comportement se passent dans les meilleures conditions.

- ***Au personnel de la plateforme Trans-Prot,***

Je tiens à remercier **Claudine Delomenie** et **Céline Boursier** pour leur formation et leurs conseils.

- ***A l'équipe du Palais de la découverte,***

Je tiens à remercier **Tanguy Schindler** et **Aurélie Massaux** pour m'avoir donné l'opportunité de vivre ma première expérience de vulgarisation scientifique à l'occasion de la Semaine du Cerveau 2014.

- ***A mes amis,***

Merci pour vos encouragements pendant ces 3 années et votre préoccupation sans faille pour le sort de mes petits compagnons de travail : « comment vont tes souris ? » ! A l'heure qu'il est, je vous laisse deviner la réponse …

- ***A ma petite famille,***

Merci à mes parents, mon frère Thomas et ma sœur Cécile pour m'avoir écoutée parler de mon sujet de thèse en boucle, pour m'avoir encouragée sans cesse et reboostée devant les difficultés pendant ces 3 longues années. Votre intérêt pour la « Arestide Beta Plus » comme vous le disiez m'a beaucoup touché ! Enfin, un merci tout particulier aux deux hommes qui partagent ma vie. Pierre, merci d'avoir partagé avec moi cette folle période et d'avoir été compréhensif devant TOUS les aspects de cette thèse, de l'excitation de la publication à la morosité générée par la perspective d'un week-end de manips, sans oublier l'agréable odeur de ta femme à la fin d'une journée avec les Souris ! Tu as été mon repère, qui m'a permis de garder les pieds sur terre et de prendre du recul sur cette incroyable expérience, merci pour tout. Valentin, défi à atteindre pour tes 18 ans, lire la thèse de Maman et m'en faire un résumé en Anglais ;-). Votre soutien à tous a été le moteur de ma réussite.

Publications scientifiques

Revue

Darcet F, Gardier AM, Gaillard R, David JD, Guilloux JP. Cognitive dysfunction in major depressive disorder: A translational review in animal models of the disease *(Pharmaceuticals, February 2016)*

Articles de recherche

Darcet F, Mendez-David I, Tritschler L, Gardier AM, Guilloux JP, David DJ. Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Frontiers in Behavioral Neurosciences* (2014) (**Article 1**).

Mendez-David I, David DJ, **Darcet F**, Wu MV, Kerdine-Römer S, Gardier AM, Hen R. Rapid anxiolytic effects of a 5-HT4 receptor agonist are mediated by a neurogenesis-independent mechanism. *Neuropsychopharmacology*, Nov 28. doi: 10.1038/npp.2013.332 (2014) (**Article 2**).

Darcet F, Gardier AM, Guilloux JP, David DJ Chronic 5-HT_4 receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression *(Neurosciences Letters, February 2016)* (**Article 3**).

Darcet F, Beaulieu JM, Hen R, Agasse F, Benstaali C, Mendez-David I Gardier AM, Guilloux JP, David DJ. Conditional β-arrestin 1 deletion in stem cells of the dentate gyrus alters emotional state and dampens antidepressant effects in adult mice *(in preparation)* (**Article 4**).

Darcet F, Beaulieu JM, Hen R, Gardier AM, Guilloux JP, David DJ. Selective β-arrestin 1 deletion in stem cells of the dentate gyrus alters cognitive phenotype and prevents pro-cognitive-like effects of chronic fluoxetine and 5-HT_4 receptor agonist treatments in adult mice *(in preparation)* **(Résultats complémentaires**).

Communications affichées

Darcet F, Gardier AM, Guilloux JP, David DJ. *Chronic 5-HT4 receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression.* XV^{ème} Journées de l'école doctorale, Châtenay-Malabry, France (Juin 2015)

Darcet F, Beaulieu JM Hen R, Gardier AM, Guilloux JP, David DJ. "*Selective β-arrestin 1 deletion in stem cells of the dentate gyrus alters emotional state and dampens antidepressant effects in adult mice"*, Society for Neuroscience, Washington DC, USA (Novembre 2014)

Darcet F, Mendez-David I, Tritschler L, Gardier AM, Guilloux JP, David DJ**.** *"Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression"* Young Researchers in Life Sciences, Institut Pasteur, Paris, France (Mai 2014)

Darcet F, Mendez-David I, Tritschler L, Gardier AM, Guilloux JP, David DJ**.** *"Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression"* XIV^{ème} Journées de l'école doctorale, Châtenay-Malabry, France (Juin 2014)

Mendez-David I, David JD, El Ali Z, **Darcet F**, Wu M, Kerdine-Romer S, Hen R, Gardier A. "*Fast onset and neurogenesis independency of a 5HT4 receptor agonist in an animal model of anxiety/depression*", Society for Neuroscience, San Diego, USA (Novembre 2013)

Darcet F, Gardier AM, Guilloux JP, David DJ. "*Cognitive performances in a neuroendocrine mouse model of anxiety/depression*" ULLA Summer School, Londres, Angleterre (Juillet 2013)

Sommaire

Abréviations

5-CSTT: 5-choice serial reaction time task
5-HT: sérotonine
5-HT$_{1A, 1B, 2C, 3, 4, 5, 6, 7}$: récepteurs de la sérotonine 1A, 1B, 2C, 3, 4, 5, 6, 7
5-HTT/SERT: transporteur de la sérotonine
AC: adénylate cyclase
Ach: acétylcholine
ACTH: hormone corticotrope
AMPc: adénosine monophosphate cyclique
ARN/ARNm: acide ribonucléique/acide ribonucléique messager
β-CD: β-cyclodextrine
BM: Barnes maze
BDNF: brain derived neurotrophic factor
CA: Corne d'Ammon
CFC: contextual fear conditioning
CMS: stress chronique modéré
CORT: corticostérone
CRH: corticotropin released hormone, ou corticolibérine
CREB: c-AMP response element-binding protein
CpG: cytosine-phosphate-guanine
DA: dopamine
DCX: doublecortine
DLP: dépression à long terme
DSM: diagnostic and statistical manual of mental disorders
EDM: épisode dépressif majeur
EPM: labyrinthe en croix surélevé
FDA: food and drug administration
FST: test de la nage forcée
GD: gyrus dentelé
GPCR: récepteur couplé aux protéines G
GR: récepteur aux glucocorticoïdes
GRK: kinase des récepteurs couplés aux protéines G
HPA: hypothalamo-hypophyso-surrénalien
HRSD: échelle d'évaluation d'Hamilton
IMAO: inhibiteur de la monoamine oxydase
IP: intrapéritonéale
ISRN: inhibiteur sélectif de recapture de la noradrénaline
ISRS: inhibiteur sélectif de recapture de la sérotonine
PLT: potentialisation à long terme
KO: knock out
MDD: major depressive disorders
MWM: Morris water maze
NA: noradrénaline

NORL: novel object recognition location
NORT: novel object recognition test
NRI: inhibiteur de recapture de la noradrénaline
NSF: novelty suppressed feeding
OF: test du champ ouvert
OMS: organisation mondiale de la santé
PCR: réaction en chaîne par polymérase
PFC: cortex préfrontal
SNC: système nerveux central
SRE: promoteur sélectif de la recapture de la sérotonine
TCA: antidépresseur tricyclique / tricyclic antidepressant

Liste des figures

Liste des tableaux

Résumé

Souvent précurseurs d'épisodes dépressifs caractérisés, les troubles cognitifs (difficultés à se concentrer, raisonnement déformé face aux stimuli normalement agréables, indécision, diminution du temps de réaction et/ou pertes de mémoire) constituent des symptômes quasi constants, retrouvés parmi les patients souffrant de troubles de l'humeur. La persistance de certains symptômes cognitifs, même après rémission complète des symptômes dépressifs et pendant les épisodes récurrents de dépression, souligne l'importance d'une évolution dans la prise en charge thérapeutique de ces patients. Une réelle évaluation aussi bien préclinique que clinique est nécessaire afin de déterminer quel traitement pourrait le mieux bénéficier aux symptômes de la dépression et autre signes de co-morbidité associés. L'efficacité modeste des antidépresseurs conventionnels, tels que les Inhibiteurs Sélectifs de Recapture de la Sérotonine (ISRS), quant à la correction du déficit cognitif appelle à de nouvelles approches thérapeutiques. De récentes études indiquent que les troubles mentaux tels que l'anxiété et/ou la dépression pourraient bénéficier de la modulation de la signalisation du récepteur sérotoninergique 5-HT_4. Si les études précliniques montrent un certain attrait pour l'utilisation des agonistes du récepteur 5-HT_4 dans le traitement des épisodes dépressifs, la quasi majorité de ces travaux ont été réalisées chez des animaux naïfs. Il est donc nécessaire de procéder à une caractérisation complète des conséquences d'un traitement chronique par un agoniste du récepteur 5-HT_4 aussi bien sur le plan émotionnel que cognitif pas seulement chez des animaux naïfs comme la plupart des études le font, mais bel et bien dans un modèle d'anxiété/dépression. Au laboratoire, nous avons développé un modèle animal d'anxiété/dépression basé sur l'élévation des concentrations en corticostérone et mimant les effets d'un stress chronique (modèle CORT)

Les premières questions posées dans ce travail sont donc les suivantes : L'application d'un stress chronique à la corticostérone (CORT) peut-il induire-il des troubles cognitifs chez les animaux ? Si oui, quelles stratégies thérapeutiques sont efficaces pour améliorer ces altérations cognitives ? Dans ce but, après induction du phénotype anxio/dépressif chez la souris, nous avons caractérisé de façon complète les performances d'apprentissage et de mémoire (mémoire de type épisodique, mémoire spatiale, flexibilité mentale, mémoire associative) chez les animaux et tenté de les corriger à l'aide de composés de différentes classes thérapeutiques. Nous avons démontré que l'administration chronique de corticostérone induit un déficit global de toutes les fonctions cognitives testées en plus des symptômes d'anxiété/dépression classiquement générés dans ce modèle (**Article 1**).

J'ai ensuite participé à l'évaluation des propriétés antidépressives et anxiolytiques suite à une administration infra-chronique ou chronique d'un agoniste du récepteur 5-HT_4 dans notre modèle d'anxiété / dépression, le modèle CORT. Notre étude a montré que dans ce modèle, un agoniste du récepteur 5-HT_4, le RS67333 (1,5 mg/kg/jour pendant 4 semaines), possède des propriétés anxiolytiques d'action rapide (**Article 2**).

Ensuite, toujours en utilisant ce modèle, nous avons évalué si la correction du phénotype anxio/dépressif par un traitement chronique de RS67333 (1,5 mg/kg/jour pendant 4 semaines) comparé à un traitement à la fluoxetine (18 mg/kg/jour), s'accompagne aussi d'une amélioration des fonctions cognitives. Alors que le traitement chronique avec le RS67333 restaure l'intégralité des déficits cognitifs induits par la corticostérone, le traitement avec la fluoxetine ne permet qu'une amélioration partielle, dépendante du type de mémoire étudié (**Article 3**).

Des données de la littérature indiquent que la cascade de signalisation de β-arrestine 1 (impliquée également dans la désensibilisation et l'internalisation du récepteur 5-HT_4) serait un biomarqueur potentiel préclinique/clinique des états dépressifs et de la réponse au traitement antidépresseur. Dans la mesure où la neurogenèse hippocampique adulte est un processus en partie nécessaire à la réponse aux antidépresseurs, nous avons cherché à caractériser le phénotype anxio/dépressif des souris tissus-spécifiques conditionnelles, dont l'expression de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé a été supprimée (ARRB1). Puis, de façon à affiner plus précisément le rôle de la β-arrestine 1 dans les effets comportementaux et neurogéniques des antidépresseurs ISRSs, nous avons administré un traitement chronique de fluoxetine (18 mg/kg/jour) aux animaux dépourvus de β-arrestine 1 et procédé à une batterie complète de tests comportementaux anxiolytiques/antidépresseurs.
Si le phénotype anxio/dépressif semble dans l'ensemble peu altéré par la délétion spécifique et conditionnelle de la β-arrestine 1, la réponse à la fluoxetine a été bloquée dans certains tests comportementaux tels que le test des 4 plaques et le Novelty Suppressed Feeding. La protéine β-arrestine 1 apparait donc comme un acteur clé dans certains effets antidépresseurs de la fluoxetine. D'autre part, l'étude du phénomène de neurogenèse hippocampique révèle que l'expression de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé de l'hippocampe est nécessaire à la survie des jeunes neurones et essentielle pour obtenir des effets bénéfiques de la fluoxétine sur la prolifération et la survie de ces jeunes neurones. En revanche, la protéine β-arrestine 1 ne semble pas impliquée dans la réponse de la fluoxetine sur l'étape de maturation des jeunes neurones.

Enfin, afin de déterminer l'importance de la protéine β-arrestine 1 dans le phénotype cognitif et dans la réponse aux traitements classiques et innovants, nous avons évalué les performances

cognitives chez les animaux ARRB1$^{-/-}$ dont la β-arrestine 1 a été supprimée sélectivement dans les cellules souches du gyrus dentelé. Nous avons ensuite examiné à nouveau les conséquences d'un traitement chronique avec un agoniste du récepteur 5-HT$_4$ (RS67333) comparé à un traitement à la fluoxetine. Contrairement au phénotype anxio/dépressif, le phénotype cognitif est altéré chez les animaux déficitaires en β-arrestine 1. De plus, les traitements chroniques au RS67333 et à la fluoxetine ne sont pas capables de corriger ces déficits cognitifs chez les souris ARRB1$^{-/-}$. Ces résultats suggèrent non seulement que la protéine β-arrestine 1 est nécessaire aux différents mécanismes de mémoire, mais surtout que son expression dans les cellules souches du gyrus dentelé est nécessaire pour que l'agoniste du récepteur 5-HT$_4$ puisse induire ses effets pro-cognitifs.

Ce travail de thèse a mis en avant le rôle prépondérant du récepteur 5-HT$_4$ dans la réponse aux antidépresseurs non seulement dans sa capacité à corriger les troubles d'anxiété/dépression, mais aussi les troubles cognitifs associés à la dépression dans un modèle d'anxiété/dépression. D'un point de vue mécanistique, ces données confirment l'implication de la protéine β-arrestine 1 dans la réponse à la fluoxetine au niveau comportemental et directement au sein du processus de neurogenèse hippocampique chez la souris adulte. Enfin, la protéine β-arrestine 1 se révèle être un acteur déterminant dans les mécanismes cognitifs et indispensable dans la réponse aux traitements étudiés.

Introduction

1 Les épisodes dépressifs majeurs et leurs traitements

1.1 La pathologie de la dépression majeure

L'Organisation Mondiale de la Santé (OMS) prévoit que d'ici 2030 la dépression majeure sera la deuxième cause d'invalidité dans le monde. Les troubles de l'humeur touchent 7% (prévalence annuelle) de la population mondiale, tandis que les formes sévères de dépression ont une incidence sur 2-5% de la population américaine (Kessler et al., 2005). En outre, environ 32 à 35.000.000 d'adultes dans la population américaine (prévalence vie entière : 16%) connaitront un épisode dépressif majeur au cours de leur vie. En Europe, une méta-analyse fondée sur 27 études cliniques, comprenant plus de 150.000 sujets de 16 pays européens, a estimé la prévalence de la dépression entre 3 à 10 % au cours des 12 derniers mois (Kessler et al., 2005). Quant à la France, selon un rapport de l'Institut de Veille Sanitaire, les épisodes dépressifs majeurs affecteraient 7,8% de la population (Sapinho et al., 2008).

La dépression majeure se caractérise par plusieurs symptômes biologiques et psychologiques qui affectent de nombreux aspects du quotidien. Selon le manuel diagnostique et statistique des troubles mentaux (American Psychiatric Association. and American Psychiatric Association. DSM-5 Task Force., 2013), le trouble dépressif caractérisé se définit par une modification persistante de l'humeur pendant une longue période accompagnée d'une souffrance morale et d'un ralentissement psychomoteur

Plus précisément, un épisode dépressif majeur (EDM) est caractérisé par une humeur ou une perte d'intérêt ou de plaisir généralisé pendant au moins deux semaines consécutives, et ce pratiquement toute la journée et presque chaque jour. L'EDM est avéré si, durant cette période, apparaissent au moins 4 des symptômes suivants : fatigue, ralentissement psychomoteur, perte de poids ou d'appétit, sommeil altéré, difficultés à se concentrer ou à prendre des décisions, idées de dévalorisation ou de culpabilité et idées de mort récurrentes ou tentatives de suicide, et qu'ils entrainent une perturbation des activités habituelles (Tableau 1). Sa classification est en perpétuelle évolution de façon à catégoriser au mieux chaque cas clinique et améliorer ensuite la prise en charge.

Tableau 1: Critères diagnostics d'un épisode dépressif majeur d'après le DSM-5

Diagnostic criteria for Major Depressive Disorder :
A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure. **1**. Depressed mood most of the day, nearly every day **2**. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day **3**. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) **4**. Significant weight loss or weight gain (> 5% of body weight in a month), or decrease or increase in appetite **5**. Insomnia or hypersomnia nearly every day. **6**. Psychomotor agitation or retardation **7**. Fatigue or loss of energy nearly every day. **8.** Diminished ability to think or concentrate, or indecisiveness **9**. Recurrent thoughts of death, recurrent suicidal ideation **B.** The symptoms do not meet criteria for a mixed episode. **C.** The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning. **D.** The symptoms are not due to the direct physiological effects of a substance (e.g. a drug of abuse, a medication) or a general medical condition (e.g.,hypothyroidism). **E.** The symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation

La dépression majeure se caractérise également par la récurrence de ses épisodes. Les liens sociaux, professionnels et personnels du patient sont altérés par ces symptômes. La dépression est clairement un changement dans la biologie normale d'une personne. Afin de pouvoir quantifier la sévérité du symptôme dépressif ainsi que la réponse du patient au traitement, plusieurs échelles d'évaluation clinique existent, dont l'échelle d'Hamilton qui reste l'un des outils les plus utilisés. Ce questionnaire, dans sa version complète, regroupe 24 items évaluant quantitativement la sévérité des symptômes et des atteintes somatiques associées (Hamilton, 1967). Une personne diagnostiquée comme dépressive dès que son score atteint « 15 ». Le score d'un patient évolue proportionnellement avec l'intensité de l'épisode dépressif et du nombre de symptômes présentés

par le patient. Cet outil permet également l'évaluation de la réponse au traitement. Classiquement, la réponse au traitement est définie par une diminution d'au moins 50% du score obtenu lors de la première évaluation du patient. L'échelle de Montgomery et Asberg (MADRS) (Montgomery and Asberg, 1979) est un outil également fréquemment utilisés en pratique par les psychiatres ainsi que dans les études cliniques incluant des patients déprimés.

En complément des critères diagnostics fondamentaux décrits dans le Tableau 1, le DSM-5 définit au sein des épisodes dépressifs majeurs différents sous-types de dépression : la dépression mélancolique, la dépression atypique. Celles-ci différent selon plusieurs paramètres cliniques et biologiques identifiées grâce aux études cliniques et permet une meilleure classification dès la première prise en charge des patients. Ainsi, la dépression mélancolique sera caractérisée par une perte permanente d'intérêt et de réactivité à des activités qui procuraient en temps normal du plaisir et est accompagnée d'épisodes dépressifs plus intenses le matin, de réveils matinaux, de retards moteurs et une prévalence accrue des idées suicidaires (DSM-5 ;(Caldieraro et al., 2013)). De plus, les données neurochimiques abondent dans le sens d'une hyperactivité de l'axe hypothalamo-hypophysaire adrénalien (HPA) dû à un défaut de rétrocontrôle inhibiteur puisque 40 à 55 % des patients seraient non-suppresseurs dans le test à la dexaméthasone suivi d'une injection de CRH (Coryell, 2007). Cette hyperactivité de l'axe génère alors une augmentation des concentrations d'hormone corticotrope (ACTH), de corticolibérine (CRH) et de cortisol et induit *in fine* une hypercortisolémie (Antonijevic, 2008; Carroll et al., 2007; O'Keane et al., 2012). A l'inverse, la dépression atypique est caractérisée par une hypofonctionnalité de l'axe HPA, avec une sécrétion de CRH et de cortisol pouvant être diminuées (Antonijevic, 2008). De plus, elle s'accompagne des symptômes suivants : une meilleure réactivité émotionnelle aux évènements positifs, une hypersomnie et une augmentation du poids et de l'appétit (DSM-5).

1.2 Les traitements antidépresseurs & leurs limitations

1.2.1 Prise en charge clinique des épisodes dépressifs caractérisés

La prise en charge des épisodes dépressifs a pour objectif principal la rémission des symptômes dépressifs, tout en réduisant les possibles complications et les risques de rechutes (Figure 1). Une médication par des antidépresseurs est classiquement initiée pour les formes modérées à sévères d'EDM. La prise en charge thérapeutique diffère cependant selon l'intensité des symptômes et le stade du patient (premier épisode, rechute ou récidive). De plus, le choix précis de la stratégie thérapeutique sera orienté en fonction des antécédents du patient, l'efficacité et les effets pharmacologiques recherchés, les effets indésirables connus, les possibles propriétés complémentaires des composés (sédatives, stimulantes) et la présence de comorbidités psychiatriques diagnostiquées.

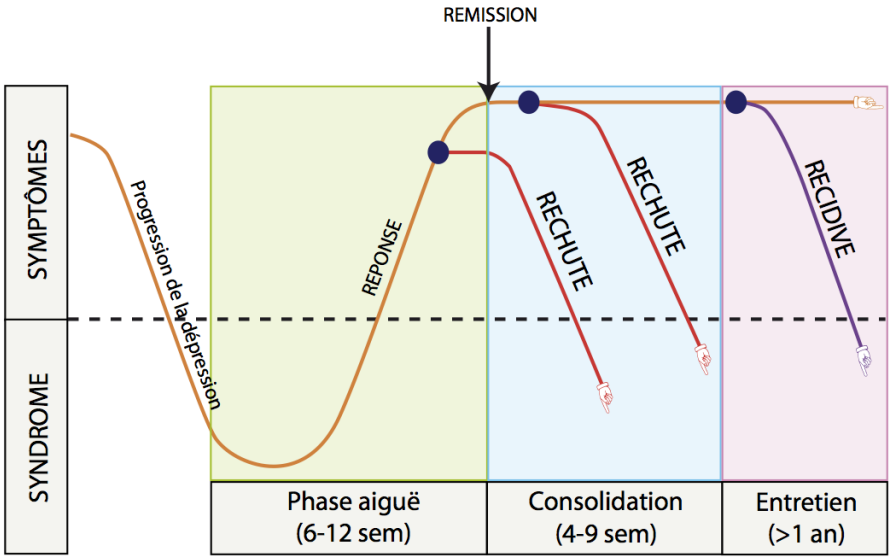


Figure 1: Réponse, rémission, rechute et récidive pendant les phases de traitement antidépresseur (d'après Kupfer et al., 1991). La réponse au traitement s'observe pendant la phase aiguë. Les rechutes peuvent survenir avant la rémission pendant la phase aiguë ou après rémission pendant la consolidation. La récidive se manifeste pendant la phase d'entretien.

De plus, même parmi les patients qui reçoivent un traitement adéquat et étroitement surveillé avec la psychothérapie ou des antidépresseurs, tous ne répondent pas complètement à ce traitement. Dans le traitement de la dépression, une réponse complète ou une rémission, est définie comme la résolution complète des symptômes dépressifs et un retour complet de fonctionnement, généralement définie comme la réalisation d'un score <8 sur l'échelle standardisée « Hamilton Rating

Scale for Depression » (HRSD). Cependant, certains inconvénients sont encore observés : une réponse thérapeutique se développant lentement (2 à 3 semaines) et un pourcentage significatif (30%) de patients non répondeurs, résistants au traitement ou qui récidivent (Wong and Licinio, 2001).

Dans la dépression caractérisée, le traitement de consolidation à privilégier est le médicament qui a permis d'obtenir la rémission symptomatique tout en maintenant les mêmes posologies. Le traitement de maintien a pour but d'éviter les récidives (ou rechutes). Lors d'un épisode de rechute, il est recommandé de choisir un antidépresseur qui s'est avéré efficace et bien toléré dans le passé par le patient (Recommandations AFSSAPS – 2006). Une uniformisation des stratégies de diagnostic, de prise en charge thérapeutique et de suivi de la dépression a été proposée récemment sous la forme d'un algorithme universel de façon à optimiser la prise en charge des patients (Figure 2) (Nutt et al., 2010).

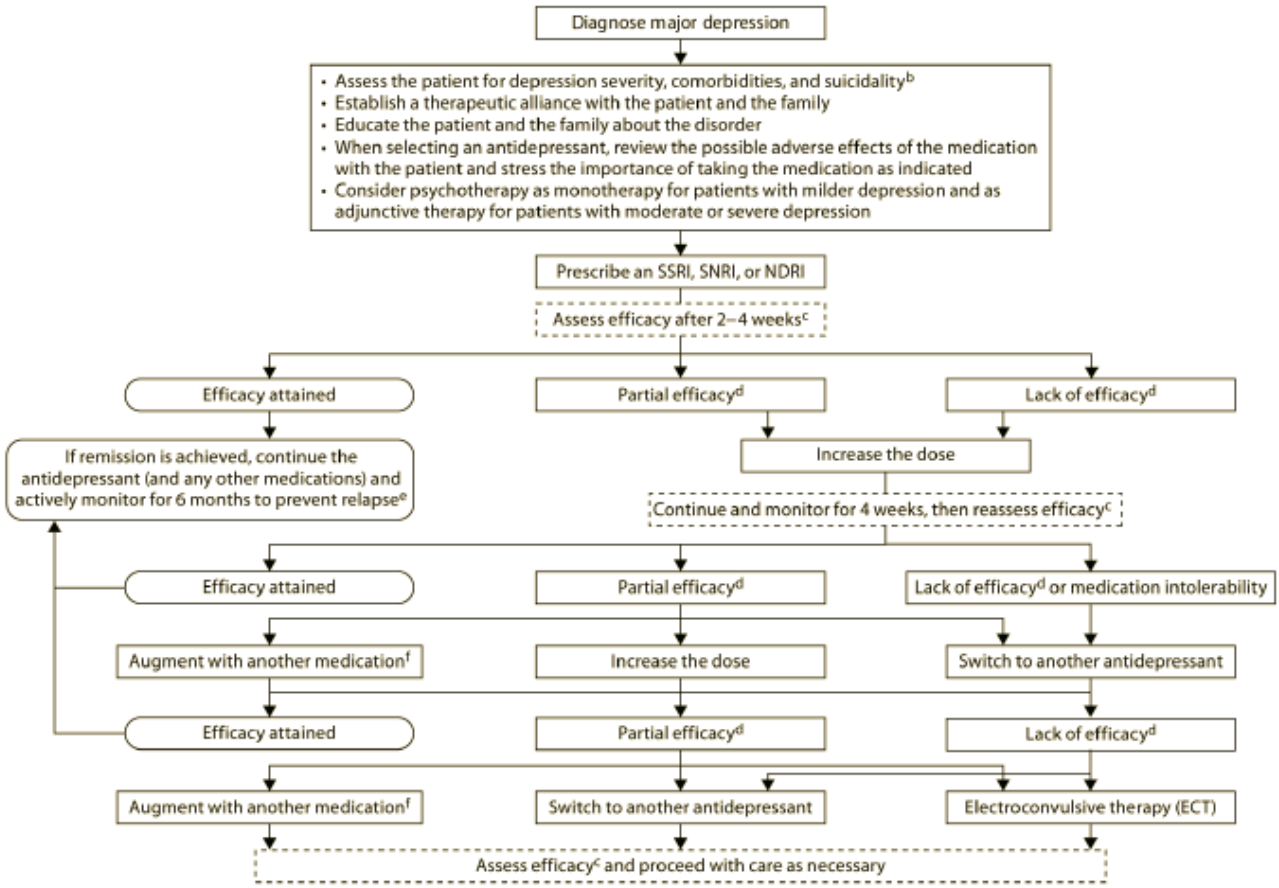


Figure 2: Proposition d'un algorithme universel des décisions thérapeutiques utilisées par les médecins dans la prise en charge des patients dépressifs (Nutt et al., 2010).

1.2.2 Traitements historiques

Différentes classes d'antidépresseurs ont été développées selon leur sélectivité vis-à-vis des transporteurs de recapture monoaminergiques. Les antidépresseurs tricycliques (par exemple, l'imipramine), qui inhibent la recapture de la sérotonine et de la noradrénaline, et les Inhibiteurs de la MonoAmine Oxydase (exemple l'iproniazide, $IMAO_{A/B}$ non sélectif irréversible), apparaissent dans les années 1950 à 1960 et sont les premiers médicaments utilisés en clinique pour traiter la dépression (Nestler et al., 2002) (Tableau 2). Ces médicaments ont été depuis remplacés par de nouveaux antidépresseurs ayant une efficacité comparable, mais une tolérance supérieure et un meilleur profil de sécurité.

La découverte de ces effets antidépresseurs et de leurs cibles moléculaires a conduit à la conception de médicaments de deuxième et troisième génération, tels que les Inhibiteurs Sélectifs du Recapture de la Sérotonine (ISRS) et les Inhibiteurs Sélectif du Recapture de la noradrénaline (ISRN). Depuis cette découverte, l'hypothèse monoaminergique de la dépression a été émise. La dépression serait une atteinte de la neurotransmission sérotoninergique, noradrénergique et, dans une moindre mesure, dopaminergique centrale. Aujourd'hui, la plupart des médicaments utilisés dans le traitement de la dépression, et notamment les ISRS (comme par exemple, la fluoxetine), sont efficaces pour traiter à la fois l'anxiété et la dépression (Samuels et al., 2011; Schatzberg and Nemeroff, 2009). Néanmoins, les derniers éléments de recherche montrent que cette hypothèse monoaminergique ne peut expliquer à elle seule la physiopathologie de la dépression (Chaudhury et al., 2015). C'est pourquoi l'hypothèse neuroendrocrinienne avec une perturbation de l'axe hypothalamo-hypophysaire (HPA) et la théorie de la plasticité ont été énoncées.

Tableau 2: Classification des antidépresseurs actuels

Classe pharmacologique des antidépresseurs		Molécules	Mécanisme d'action	Ref
Inhibiteurs d'enzymes	Inhibiteurs des Monoamine Oxidase (IMAOs)	Phenelzine, tranylcypromine, moclobemide, selegiline	Les IMAOs empêchent la dégradation des neurotransmetteurs monoaminergiques, tels que la 5-HT et la NA	(Tollefson, 1983)
Inhibiteurs de recapture	Antidépresseurs tricycliques (TCAs)	imipramine, desipramine, trimipramine, clomipramine, amytriptyline, nortriptyline, protriptyline, doxépine, amoxapine , maprotiline	Les TCAs inhibent le transporteur de la NA et de la 5-HT, respectivement	(Gillman, 2007)
	Inhibiteurs Sélectifs de la Recapture de la Sérotonine (ISRSs)	fluoxetine, paroxétine, citalopram, escitalopram, sertraline, fluvoxamine	Les ISRSs inhibent sélectivement le transporteur de la sérotonine	(Vaswani et al., 2003)
	Inhibiteur Sélectif de la recapture de la Noradrénaline et de la Dopamine (ISND)	bupropion	Les ISNDs bloquent l'action du transporteur de la NA et de la DA	(Kinney, 1985; Preskorn and Othmer, 1984)
	Inhibiteur Mixte de la recapture de la Noradrénaline et de la Sérotonine (ISRNSs)	venlafaxine, desvenlafaxine, levominalcipran, duloxétine	Les ISRNSs inhibent le transporteur de la NA et de la 5-HT	(Gorman and Kent, 1999)
	Inhibiteur de recapture de la Noradrénaline (IRNs)	lofepramine; reboxétine	Les IRNs inhibent sélectivement le transporteur de la NA	(Brunello et al., 2002)
	Antagoniste des récepteurs adrénergiques α2 et des récepteurs sérotoninergiques	mirtazapine	La mirtazapine bloque les autorécepteurs et hétérorécepteurs α2 adrénergiques et bloque les récepteurs postsynaptiques 5-HT_2 et 5-HT_3	(Stimmel et al., 1997)
Antidépresseurs innovants	Antidépresseurs à action multimodale	vortioxetine, vilazodone, trazodone	La vortioxetine est un antagoniste des récepteurs 5-HT_7, 5-HT_3 et 5-HT_{1D}, un agoniste partiel du 5-HT_{1B}, un agoniste du 5-HT_{1A} et un inhibiteur de SERT. La trazodone est un antagoniste des récepteurs 5-HT_2 et un agoniste partiel des récepteurs 5-HT_{1A}. La vilazodone est un ISRS ainsi qu'un agoniste partiel du récepteur 5-HT_{1A}.	(Nemeroff, 1994; Stahl et al., 2013)
	Antidépresseurs à action multicible	Agomelatine	L'agomelatine est un agoniste des récepteurs MT_1 and MT_2 de la mélatonine et un antagoniste des récepteurs 5-HT_{2C}	(Le Strat and Gorwood, 2008)
	Promoteur Sélectif de la Recapture de la Sérotonine	Tianeptine	La tianeptine augmente sélectivement la recapture de la 5-HT	(McEwen et al., 2010)

En plus de l'approche pharmacologique, d'autres stratégies thérapeutiques qui n'impliquent pas les antidépresseurs sont disponibles et ont prouvés leur efficacité dans le traitement de la dépression. Par exemple, la thérapie par électrochocs (électro-convulsivo thérapie ou ECT), la stimulation électrique du nerf vague, la stimulation cérébrale profonde et certains types de psychothérapie ciblée comme la thérapie cognitivo-comportementale et interpersonnelle (Guidi et al., 2016) peuvent être mises en place pour soulager les symptômes de la dépression mais sont encore en cours d'évaluation clinique. D'autres interventions telles que la stimulation chronique de la région cingulaire subgénuale (aire 25 de Brodmann) ont également montré des effets prometteurs lors d'essais cliniques (Mayberg et al., 2005). Ces stratégies non pharmacologiques sont généralement envisagées en cas de dépressions sévères résistantes à toutes autres stratégies thérapeutiques.

1.2.3 Limites actuels des traitements

Malgré les progrès évidents apportés par les antidépresseurs actuellement disponibles (sélectivité d'effet, rapport bénéfices/risques), 30 à 60 % des patients ne répondent toujours pas de manière adéquate et souffrent toujours de symptômes résiduels incapacitants (Trivedi et al., 2006). Au cours des dernières décennies, des milliards de dollars ont été dépensés pour cibler de nouveaux médicaments plus sélectifs que les récepteurs de la sérotonine ou de la noradrénaline, agonistes ou antagonistes ayant des effets semblables aux médicaments antidépresseurs déjà sur le marché, mais avec une réponse plus rapide et présentant moins d'effets indésirables. Malheureusement, en dépit de la recherche et des connaissances acquises sur le mécanisme d'action des antidépresseurs classiques et de leurs propriétés thérapeutiques, le traitement pharmacologique de la dépression reste peu satisfaisant. En effet, bien que les antidépresseurs actuellement disponibles aient une réelle efficacité curative (démontrée par des études cliniques « *contre placebo* » ou en comparaison à la thérapie de référence, l'ECT) dans le traitement de cette pathologie, il faut i- attendre plusieurs semaines pour qu'ils démontrent une pleine efficacité, ii- de nombreux patients répondent peu au traitement, iii- les symptômes concomitants sont souvent peu contrôlés et iv- certains antidépresseurs peuvent engendrer des problèmes de tolérance ou de dépendance (pour revue,(Martinowich et al., 2013)). Environ 60% des patients souffrant de dépression ne répondent pas de façon adéquate aux antidépresseurs ou sont résistantes à ces médicaments. Moins de 50 % des patients souffrant de dépression montrent une complète amélioration (Trivedi et al., 2006). Les effets indésirables des ISRS sont fréquemment décrits lors d'un traitement chronique, notamment l'insomnie, somnolence, sensation vertigineuse, akathisie et dysfonction sexuelle à long terme (par

exemple, diminution de la *libido*, l'éjaculation retardée, etc…). Comme les antidépresseurs de première génération, les ISRS nécessitent au moins 2 à 4 semaines d'administration avant l'obtention de bénéfices thérapeutiques (Wong and Licinio, 2001). Le fait que la réponse au traitement antidépresseur soit si imprévisible chez un individu et qu'il soit souvent nécessaire d'essayer plusieurs antidépresseurs pour obtenir un effet optimal, peut causer de la frustration chez le patient, favoriser une mauvaise observance de ce traitement, ce qui en limitera son efficacité et involontairement servira à renforcer les symptômes de la dépression (Fava, 2000; Trivedi et al., 2006). L'écart entre les effets aigus des ISRS (*in vitro,* le blocage des transporteurs SERT et/ou NET est rapide) et l'apparition tardive de leurs effets *in vivo* après administration chronique chez l'animal et chez l'Homme suggèrent un mécanisme d'action plus complexe que prévu. Cette distinction apparente entre les effets aigus et chroniques des ISRS a fait émerger l'hypothèse de la nécessaire activation de plusieurs mécanismes pharmacologiques dans le cerveau lors d'un traitement chronique avec des ISRS. L'efficacité modeste des antidépresseurs conventionnels, et notamment des ISRSs, appelle à de nouvelles approches pour traiter les différentes formes légères, modérée ou sévères des épisodes dépressifs associés ou non à des troubles anxieux. Des études cliniques mettant en œuvre des thérapies pharmacologiques combinées, telles que le blocage de certains récepteurs monoaminergiques centraux (antagoniste de l'autorécepteurs 5-HT_{1A}, par exemple) en plus de l'inhibition d'un des transporteurs des monoamines, ont déjà été proposées afin de raccourcir le délai d'apparition de l'effet antidépresseur et/ou d'augmenter l'efficacité de ces médicaments.

1.2.4 Nouveaux traitements innovants

Les efforts actuels de développement de médicaments visent à découvrir de nouvelles cibles et de nouvelles classes d'antidépresseurs dans l'espoir d'identifier de nouveaux composés ayant une efficacité plus large et / ou d'apparition des effets plus rapide avec un meilleur profil d'effets indésirables (Tableau 2). Pour exemples, les inhibiteurs de recapture doubles ou triples des monoamines sont des molécules nouvelles capables d'agir sur plusieurs systèmes de neurotransmetteurs à la fois (Roose et al., 1994). Autre exemple, la vortioxetine (Lu AA21004; 1-[2-(2,4-diméthyl-phénylsulfanyl)-phényl]- pipérazine) est un nouvel antidépresseur ayant une activité antidépressive « multimodale » développé par des Laboratoires Lundbeck. Fin Septembre 2013, la « Food and Drug Administration » (FDA) américaine et fin octobre 2013, l'Agence Européenne du Médicament (EMA) ont approuvé la mise sur le marché de la vortioxetine (BRINTELLIX®) avec pour indication le traitement des adultes souffrant de troubles dépressifs majeurs. En 2015, l'EMA et la

FDA ont reconnu les propriétés bénéfiques de la vortioxetine sur la correction des déficits cognitifs lors d'EDM.

Autre exemple, le développement de l'agomelatine (S20098, N-[2 - (7- méthoxynaphthalén-1-yl) éthyl] acétamide), médicament antidépresseur ayant à la fois des propriétés agoniste mélatoninergique et antagoniste du récepteur 5-HT_{2C}, est prometteur car les troubles affectifs sont caractérisés par des rythmes circadiens anormaux (pour revue, voir (de Bodinat et al., 2010)). L'agomelatine (Valdoxan®/ Thymanax® développé par les Laboratoires IRIS) a obtenu l'autorisation de commercialisation en 2009 pour le traitement des épisodes dépressifs majeurs en Europe, devenant ainsi un des premiers antidépresseurs approuvés avec un mécanisme d'action non exclusivement monoaminergique.

Enfin, les antagonistes du récepteur ionotropique glutamatergique NMDA, en particulier la (±)-kétamine sont comme des candidats à la prochaine génération d'antidépresseurs d'action rapide (pour revue, voir (Maeng et al., 2008; Martinowich et al., 2013). De façon étonnante, il semble que les effets d'un anesthésique, la (±)-kétamine sur le comportement reposent sur l'activation du récepteur AMPA, un autre type de récepteur canal ionique du L-glutamate (l'isomère S- est en cours d'évaluation car il aurait moins d'effets indésirables « psychomimétiques ») et, par conséquent, des médicaments capables d'activer directement le récepteur AMPA ou un de ses sites modulateurs allostériques (Ex : essais cliniques en cours du GLYX-13 ou rapastinel, un agoniste partiel du site glycine du récepteur NMDA) pourraient produire des actions antidépressives rapides et de longue durée.

Enfin, malgré les énormes progrès réalisés en recherche avec l'utilisation de traitements physiques ou somatiques comme la psychothérapie, la thérapie par électrochocs (ECT) ou la pharmacologie des antidépresseurs visant à élucider la physiopathologie des troubles anxio/dépressifs, de nombreuses questions restent en suspens concernant le mécanisme d'action des antidépresseurs et leur lien avec la dépression (Nestler et al., 2002).

2 Les troubles cognitifs dans la dépression majeure

Bien que seule la « difficulté de concentration » apparaisse clairement dans la liste des critères de diagnostic de la dépression majeure définie par le DSM-5, de nombreux autres troubles cognitifs associés aux symptômes dépressifs sont reportés par les patients souffrant de troubles de l'humeur. Parmi eux, des altérations dans l'attention, la mémoire de travail, les performances d'apprentissage et de mémoire et les fonctions exécutives alourdissent significativement le poids de la maladie chez ces patients.

La **Revue "Cognitive dysfunction in major depressive disorder : A translational review in Animal models of the disease »** qui suit, présente dans un premier temps les déficits cognitifs observés chez les patients déprimés, en fonction de leur âge et la nature de dépression. Les différents outils cliniques permettant de déceler ces troubles cognitifs sont également évoqués. Dans un second temps, les troubles d'apprentissage et de mémoire associés à différents modèles animaux d'anxiété/dépression sont détaillés. Pour chaque domaine cognitif étudié, nous avons déterminé si les troubles cognitifs observés sont communs aux différents modèles animaux ou si d'autres facteurs non-spécifiques (tels que l'espèce animale, le sexe ou l'âge) pouvaient dessiner des altérations communes à travers les différents modèles. Enfin, le rôle de la neurogenèse hippocampique chez les Rongeurs adulte dans les déficits cognitifs a également été développé dans cet article.

pharmaceuticals

MDPI

Review

Cognitive Dysfunction in Major Depressive Disorder. A Translational Review in Animal Models of the Disease

Flavie Darcet [1], Alain M. Gardier [1], Raphael Gaillard [2,3,4], Denis J. David [1,*] and Jean-Philippe Guilloux [1]

1 Université Paris-Saclay, University Paris-Sud, Faculté de Pharmacie, CESP, INSERM UMRS1178, Chatenay-Malabry 92296, France; flavie.darcet@u-psud.fr (F.D.); alain.gardier@u-psud.fr (A.M.G.); jean-philippe.guilloux@u-psud.fr (J.-P.G.)

2 Laboratoire de "Physiopathologie des maladies Psychiatriques", Centre de Psychiatrie et Neurosciences U894, INSERM, Université Paris Descartes, Sorbonne Paris Cité, Paris 75014, France; R.GAILLARD@ch-sainte-anne.fr

3 Service de Psychiatrie, Centre Hospitalier Sainte-Anne, Faculté de Médecine Paris Descartes, Université Paris Descartes, Sorbonne Paris Cité, Paris 75014, France

4 Human Histopathology and Animal Models, Infection and Epidemiology Department, Institut Pasteur, Paris 75015, France

* Correspondence: denis.david@u-psud.fr; Tel.: +331-468-359-68; Fax: +331-468-353-55

Academic Editor: Guy Griebel

Received: 4 December 2015; Accepted: 1 February 2016; Published: 17 February 2016

Abstract: Major Depressive Disorder (MDD) is the most common psychiatric disease, affecting millions of people worldwide. In addition to the well-defined depressive symptoms, patients suffering from MDD consistently complain about cognitive disturbances, significantly exacerbating the burden of this illness. Among cognitive symptoms, impairments in attention, working memory, learning and memory or executive functions are often reported. However, available data about the heterogeneity of MDD patients and magnitude of cognitive symptoms through the different phases of MDD remain difficult to summarize. Thus, the first part of this review briefly overviewed clinical studies, focusing on the cognitive dysfunctions depending on the MDD type. As animal models are essential translational tools for underpinning the mechanisms of cognitive deficits in MDD, the second part of this review synthetized preclinical studies observing cognitive deficits in different rodent models of anxiety/depression. For each cognitive domain, we determined whether deficits could be shared across models. Particularly, we established whether specific stress-related procedures or unspecific criteria (such as species, sex or age) could segregate common cognitive alteration across models. Finally, the role of adult hippocampal neurogenesis in rodents in cognitive dysfunctions during MDD state was also discussed.

Keywords: major depressive disorder; cognitive dysfunctions; animal models of anxiety/depression; neurogenesis

1. Introduction

Cognitive dysfunction is a common feature of major depressive disorder (MDD), contributing to the serious decline in patients' quality of life. Described in the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V, [1]) as "significantly affecting the individual's capacity to function", these cognitive changes are associated with the set of emotional and behavioral alterations (including persistent depressed mood and loss of pleasure) that characterizes MDD pathology. Many clinical studies have focused their work on the nature and the magnitude of cognitive

alterations during the clinical course of MDD [2–6]. During clinical observation, patients mostly report their difficulty to concentrate, to make decisions, the feeling that their brain is slowed down or the fact that "they forget everything" (tasks, meetings, *etc*.) [7]. These subjective complaints relate to a broad range of cognitive impairments reported during depressive episodes, from executive functions (attention, processing speed, cognitive flexibility) to working and visual learning and memory. However, contradictory findings from neuropsychological tests have encouraged clinicians to examine whether the heterogeneity of the MDD population would prevent from identifying a specific neurocognitive profile in depressed individuals.

The DSM-V clearly defines subtypes in major depression such as melancholic and atypical subtypes of MDD. Comparing different cognitive functions across MDD subtypes may help in the identification of neurocognitive patterns according to the specificity of MDD markers. Additionally, few studies aimed at delineating between trait- and state-like cognitive alterations, *i.e.*, the deficits observed exclusively during depressed episodes and those occurring prior, between and after MDD episodes [7]. One meta-analysis performed across first-episode MDD subjects, and including 13 studies, segregated state-dependent cognitive alterations in MDD subjects (psychomotor speed and memory functioning) from trait-markers (attention and executive functioning). However, the large clinical heterogeneity of MDD subjects may dampen this report.

In order for basic research to provide potential advances in this field, it is essential to use animal models that present behavioral, neurochemical and brain morphological phenotype reminiscent of some symptoms of MDD. Indeed, animal models exhibiting deficits in one or more of the relevant domains of cognition are useful to investigate mechanisms underlying impaired cognitive processes observed in MDD and their dependence on mood pathology. In rodents, many anxiety-depression models, including chronic early-life stress and adulthood models, have been validated for the study of anhedonic behaviors, modeling the negative mood symptomatology of MDD. It has been reported that adverse experiences during pregnancy or early stress life events in childhood could lead to an increased sensitivity to the effects of stress in adulthood life, directly enhancing vulnerability to depression [8,9]. Rodent models such as prenatal stress (chronic stress exposure during gestational period), early post-natal handling and maternal separation (chronic pups-dams separation during weaning period), have been validated to produce depression-like behavior in adulthood [10,11]. However, in most clinical cases, the apparition of MDD pathology occurs during adulthood and is typically caused by a succession of adverse stress episodes in life, leading progressively to the core of the pathology. Several adult animal models have been validated as anxiety-depression models such as social defeat model (SD) [12], learned helplessness (LH) [13], unpredictable chronic mild stress (UCMS) [14] and chronic corticosterone administration (CORT) [15]. Among these models, UCMS procedure, based on a chronic exposure of unpredictable stressors, has been reported as one of the most robust animal models of depression thanks to good predictive, face and construct validities [16,17].

In this review, the first part will be focus on clinical cognitive dysfunctions depending on the type and stage of the MDD illness. The second part of this work will gather preclinical studies observing cognitive deficits in different rodent models of anxiety/depression. For each cognitive domain, we will highlight which deficits can be shared, across models, and, whether specific stress-related procedures or non-specific criteria (species, sex, type of cognitive parameter measured) can segregate common cognitive alteration. Finally, the role of adult hippocampal neurogenesis in rodents in cognitive dysfunctions during MDD state will be discussed.

2. Cognition in Patients Suffering from MDD

2.1. Cognitive Performances in MDD through Different Ages

Cognitive abnormalities in many various cognitive domains have been reported among patients suffering from MDD [4,18–22]. Specifically, cognitive alterations in attentional processes [23–25], executive functioning [6,26–28], working memory [27,29,30], verbal or visual learning and

memory [29,31] and emotional processing [31,32] were noted in MDD patients. While early-onset depression is associated with higher disease severity and with higher levels of recurrence [33], limited data are available regarding children, adolescents or young adults cognitive performances during MDD episodes. Deficit in attention, memory and problem solving could have a serious impact in these populations on daily activities, especially when individuals are involved in education or academics programs, during which their achievement depends on these skills [34]. Among the few studies examining cognitive performances in pediatric, adolescent or young adults depressed subjects, all of them agreed on a general cognitive degradation but none managed to extract specific impairments related to the early-onset of depression [33,35,36]. It remains currently unclear whether or not cognitive impairments should be considered as vulnerability markers of depression, potentially preceding the development of depressive symptoms or, whether cognitive symptoms develop only after the onset of a major depressive episode [33].

Further studies involving larger, homogenous cohorts of patients are needed to provide new understanding regarding this issue. Cognitive dysfunctions in elderly depressed people have been widely investigated, but no specific cognitive impairments have been observed due to the physiological decline of cognitive process with age and to potential neurodegenerative disorders appearing in late-life. However, late-life depression has been particularly associated with a slower speed in information processing, executive functions difficulties and working memory deficits [37–39]. Alternative treatments strategies including cognitive training, psychotherapy, assistive devices, interventional procedures, physical/speed therapy and others emerging therapies are employed to treat cognitive dysfunctions in elderly depressed patients, in addition to a classical pharmacological therapy [40].

2.2. Cognitive Neuropsychological Assessments Instruments Used for MDD Patients

The Hamilton Depression Rating Scale (HAM-D) and the Montgomery-Asberg Depression Rating Scale (MADRS) are clinician-administered assessments of depressive symptoms that are the most frequently used methods in depression clinical trials. However, neither of these scales evaluates cognition in any depth and both rely on a clinician's subjective opinion based upon a patient's report. In the HAM-D, a single item assesses psychomotor functioning, whereas a single item on the 10-item MADRS assesses concentration [41].

The number of studies investigating cognition performances in depressive disorder has grown during the last decade, reflecting the interest in cognition as a therapeutic target [42]. Given the extent and the magnitude of cognitive dysfunctions in MDD, a greater assessment of cognitive performances may help in MDD evaluation. However, little is known about current clinical routine practice and specific available assessment tools to assess cognitive symptoms in a depression context. A recent cross-sectional survey interrogating psychiatrists from different countries investigated the strategy and routine methods facing cognitive evaluation in MDD patients [43]. When psychiatrists were asked to share their assessment method to explore cognitive function in MDD, 61% of them exclusively relied on patient history interview, 32% of them used solely cognitive instruments and only 7% of them used both methods. Most of the psychiatrists who reported using instruments specifically cited the Mini Mental Status Examination (MMSE, preferentially used in dementia disorder such as Alzheimer's disease) or instruments assessing depression severity rather than cognitive assessments tools (HAM-D, MADRS, Beck Depression Inventory or Geriatric Depression Scale). Only six appropriate cognitive assessment tools were mentioned, including the Trail Making Test, the Stroop test or the Digit span task. While this study showed psychiatrists' awareness of cognitive dysfunction in MDD patients, few were actually using appropriate instruments and most of instruments cited were inappropriate for the intended population and disease state, evoking a general misuse and confusion regarding instruments for assessing cognitive dysfunction in MDD.

Through a review highlighting the nature of cognitive assessment instruments used in MDD trial, the California Verbal Learning task (CVLT), the Trail Making Test (TMT-A), the Wechsler Memory-Scale

(WMS) and the Wechsler Adult Intelligence Scale (WAIS) were listed as the most frequently used in clinical studies [44]. Among cognitive existing batteries that assess several cognitive domains rather that one domain represented by a single task, the CANTAB battery (Cambridge Neuropsychological Test Automated Battery) is the most widely used in MDD trials.

Classified by cognitive domains, some of the most familiar used tests in the MDD context are as follows [41,45]:

- Attention processing monitoring: the Digit Span test and the Continuous Performance test,
- Processing speed: the Trail Making Test-Part A, the Digit symbol test and the Finger Tapping test,
- Executive functions and verbal memory: the Stroop Color Word test, the Trail Making Test-Part B and the Wisconsin Card Sorting Test,
- Memory functions: the Rey Auditory Verbal Learning Test, the Wechsler Memory Scale and the California Verbal Learning task

Despite the variety of accessible methods, there is still a lack of harmony regarding the practical use of appropriate instruments or batteries to assess cognitive functions in major depression. The development of a validated and standardized cognitive battery to use in a specific manner in MDD clinical trials is necessary to improve both assessments and treatments of MDD patients.

2.3. Progression of Cognitive Symptoms along the Course of MMD

The course of MDD pathology is characterized by a first-episode of major depression either followed by a complete remission or break periods without any mood-related symptoms and recurrent episodes separated by short time periods between relapse. In order to better visualize the progression of cognitive symptoms associated with depressive symptoms, Figure 1 simultaneously describes the course of depressive symptoms and the potential course of cognitive symptoms through different steps of the illness.

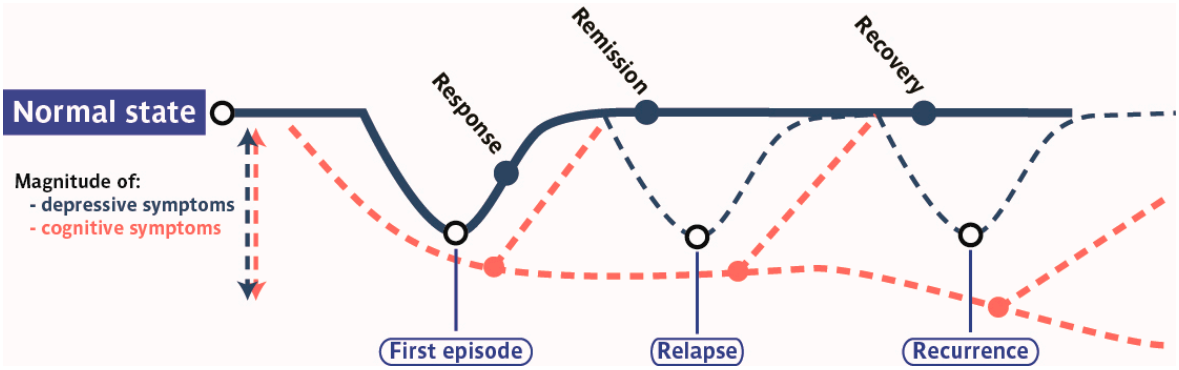


Figure 1. Schematic representation of a common trajectory towards chronic recurrent depression. Blue full and dotted lines represent various forms of progressions of depressive symptoms during the course of MDD. The red dotted line represents the magnitude of cognitive symptoms associated to MDD pathology according to MDD state. An early onset of cognitive symptoms depression has been reported before the clinical diagnosis. Those cognitive signs can persist even after remission or recovery of MDD symptoms.

Although many studies found that a wide range of antidepressant treatments were able to improve both mood and cognitive-related symptoms in MDD [46–48], others failed to find an association between antidepressant treatment and improvement in cognitive functions in depressed patients [49–51]. Interestingly, the speed of remission of MDD seems related to an improvement in the patients' cognitive functioning after a successful treatment. Indeed, unlike slow-remitters, the subjects with a rapid remission pattern presented specific cognitive benefits, with significant improvements in speed of information processing, working memory, and executive functions [52].

Although description of cognitive alterations in the context of mood disorders has been extensively analyzed in the literature [6,18,19,24,28,31,53], understanding why some cognitive dysfunctions are still present in remitted or recurrent state of MDD and how specifically progress these deficits remains unanswered. Indeed, recent reviews, focused on cognitive functioning in the remitted state of depression, confirmed that cognitive deficits persist in depressed patients despite the stabilization of mood-related symptoms [5,54]. These deficits seem to reside more within the cognitive domains of attention and executive functions (mental flexibility, decision-speed) than within other domains, although studies in additional patient cohorts are needed to strengthen this statement. The involvement of many factors associated to the progression of MDD pathology (number, duration and severity of MDD episodes) and other clinical features (age of onset, time elapsed since the last episode of depression, treatment interventions, sex or co-morbid psychiatric disorders) make the identification of a specific neurocognitive profile in remitted patients even more complex [5].

Although many studies found that a wide range of antidepressant treatments were able to improve both mood and cognitive-related symptoms in MDD [46–48], others failed to found an association between antidepressant treatment and improvement in cognitive functions in depressed patients [49–51].

The proportion of MDD patients that encountered recurrence episodes is estimated at 50%–60% [55]. Cognitive symptoms in recurrent depressive disorder (rDD) represent the largest range of cognitive impairments during the MDD pathology with decrements in memory, learning, attention, spatial visualization, visual-motor coordination, verbal fluency and memory, psychomotor retardation and most of the executive functions (planning, problem solving, behavioral inhibition, mental flexibility) [56–58]. A way to examine the degree of cognitive deficits in recurrent depressive disorders is to gather studies that compare first-MDD episode cognitive symptoms to recurrent depressive cognitive symptoms. A recent study evaluated the following cognitive functions: information processing speed (Digit Symbol from WAIS-R), executive functions and working memory (TMT, Stroop test), verbal memory (immediate and delayed memory) and learning ability (CVLT), and verbal fluency (VFT) in first-MDD episode and in recurrent episode patients [58]. Although no difference was found in the severity of the depressive symptoms between first-episode and rDD individuals, the patients from the latter group recorded significantly lower scores in all the cognitive tests. These findings are in line with a previous report, in which recurrent patients exhibited worse performances in all the tests (executive functions and perseverative tendency) compared to controls subjects [59]. Moreover, the number of recurrent episodes seems correlated with the severity of perseverative behavior. The cumulative duration of depressive episodes and their repetition have a detrimental effect on the severity of the associated-cognitive deficits.

Otherwise, evidence suggesting that rDD was associated with negative biases and increased sensitivity to negative events. While first-episode MDD patients can show an impairment in emotional cognitive function [31,32], rDD subjects displayed a deficit in identifying facial expression at a more severe degree, even demonstrating excitation and exaggeration for sad emotions [60]. Thus, neural mechanisms involved in the perception of negative or happy/neutral faces may differ, confirming the abnormal neural processing of emotional stimuli in depressed patients all along the course of the disease.

Taken together, rDD are characterized by a proportional aggravation of cognitive disturbances compared to first-episode MDD (Figure 1). This progressive decline of cognitive functioning through MDD illness may contribute to the failure to reach a full recovery in patients and considerably widens their risks of developing over time dementia and neurodegenerative disorders.

Many questions about cognitive deficits associated with MDD pathology remain unanswered: could cognitive impairments be a premature behavioral marker of susceptibility to depression and be used as a prevention tool? Are residual cognitive dysfunctions in remitted patients a risk factor for relapse? New discoveries in this field could significantly improve therapeutic care in MDD patients. Relieving cognitive dysfunctions may be a necessarily step to treat major depression.

2.4. Cognitive Dysfunctions According to the MDD Subtypes

Most of clinical studies investigating cognitive dysfunctions in MDD patients did not detail the heterogeneous condition of depression symptomatology. Melancholia and atypical disorders are the main subtypes in depression. Specifically, melancholic depression can be distinguish from non-melancholia by a set of physical symptoms occurring during clinical depressive episodes such as anhedonia and/or lack of reactivity to usually pleasurable stimuli, observable psychomotor retardation, weight loss, worsening of symptoms in the morning hours and early morning awaking (DSM-V, [1,61,62]). On the other hand, atypical subtype is rather characterized by mood reactivity and positivity, a younger onset of age, less severe and fewer depressive episodes, longer duration of episodes, hypersomnia, weight gain and more co-morbidity with anxiety and substance abuse [1,63]. In addition to the DSM-V definition, distinct biological correlates differentiate melancholic subtype from others subtypes of depression. Melancholic depression is associated with HPA-axis hyperactivity (hypercortisolemia) and specific sleep patterns (reduced REM latency, increased REM time and reduced deep sleep) [64,65].

After two decades of research, increasing evidence suggests that melancholic patients exhibit greater cognitive dysfunctions than non-melancholic patients but no qualitative review focusing on cognitive impairments in melancholic subtype has been written yet. Table 1 summarizes clinical studies evaluating cognitive functions within melancholic patients through various neuropsychological tests. Classical clinical assessments to identify melancholic patients in these studies were determined by DSM-IV or DSM-V criteria for melancholic features Checklist (DSM-IV) and the CORE index for melancholia by behavioral observation. The CORE index was developed in 2007 when observable psychomotor disturbance was judged as the most relevant clinical markers of melancholic depression and psychomotor retardation was announced as the "core" behavioral pattern defining melancholic MDD [61,66]. This assessment was preceded by a 17-item HAM-D evaluation and, occasionally, the Mini Mental State Examination (MMSE) was employed as a screening tool to exclude potential subjects with early onset dementia.

Table 1. Differential cognitive impairments in melancholic and non-melancholic patients.

MDD Type	Sex	Mean Age (Years)	Cognitive Domains Tested	Assessment Methods	Main Results	Reference
MEL (*n* = 20) Non-MEL (*n* = 18) CTRL (*n* = 38)	♂/♀	39	Psychomotor tasks	Fitt's task Figure-copying task Symbol digit substitution task	MEL patients were slower performing all the tasks compared to non-MEL and CTRL patients.	[67]
MEL (*n* = 7) Non-MEL (*n* = 8) CTRL (*n* = 8)	♂/♀	40	Response selection Attention Executive function	Choice reaction task (T1) Spatial Stroop task SRC task Spatial Stroop + SRC task	- MEL patients were significantly slower than non-MEL patients in T2, T3, and T4.MEL patients were slower than CTRL in all tasksNo difference between non-MEL and CTRL in all tasks	[68]
MEL (*n* = 11-20) Non-MEL (*n* = 11–20) CTRL (*n* = 11–20)	♀	50	Executive function Memory	CANTAB battery: ID/ED set shifting task, SOC Spatial Recognition Memory, PAL	- MEL patients showed deficit in executive function in the ID/ED set shifting compared to non-MEL patients;No difference between groups in SOC, PAL, SRC	[69,70]
MEL (*n* = 26) Non-MEL (*n* = 9) CTRL (*n* = 26)	♀	34	Explicit episodic memory Implicit (procedural) learning	WMS-R SRTT	- No difference between MEL and non-MELSequence-specific implicit learning was lower in MEL compared to non-MEL	[71]
MEL (*n* = 17) Non-MEL (*n* = 17)	♂/♀	41	IQ Executive function Attention/working memory Learning/long-term verbal memory Prospective memory Attention, response inhibition Set shifting, feedback use Semantic memory/ verbal fluency Planning, self-monitoring, multi-tasking	NART Donders Simple Reaction Time Digit Span test CVLT Prospective memory task SCWT Shortened WCST COWAT SET	*Baseline MDD*: MEL patients showed deficits in memory tasks (CVLT trial 1, CVLT total trials) and prospective memory (delayed free recall). MEL group recalled fewer words overall; MEL patients performed more poorly than non-MEL group in executive functions tests: digit span backwards, SCWT interference score, WCST categories completed, WCST perseverative errors and modified Six Elements Test; *Remitted MDD*: Same cognitive impairments in MEL patients were observed.	[72]
MEL (*n* = 20) Non-MEL (*n* = 20) CTRL (*n* = 20)	♂/♀	47	Working memory Emotion classification Arousal and valence rating	Emotion face paradigms	MEL patients showed better memory for sad faces (sad benefit).	[73]
MEL (*n* = 65) Non-MEL (*n* = 59) CTRL (*n* = 124)	♂/♀	39	Memory recall Attention Short-term working memory Executive functioning Semantic knowledge, language Spatial visual memory Cognitive flexibility, selective attention	Verbal recall, recognition task Time estimation task Reverse digit span task Executive maze task Word Generation test Span of visual memory task Verbal Interference test Switching of attention task	MEL patients showed poorer performances in spatial visual memory and attention task compared to non-MEL group.	[74])

Table 1. *Cont.*

MDD Type	Sex	Mean Age (Years)	Cognitive Domains Tested	Assessment Methods	Main Results	Reference
MEL (n = 142) Atypical (n = 76) Undifferentiated (n = 91) CTRL (n = 200)	♂/♀	34	Processing speed Attention Shifting Planning Verbal fluency Visual spatial memory Verbal working memory	TMT-A, Digit symbol coding subtest Digit Span Forward of the WAIS-RC Modified WCST, TMT-BTower of Hanoi (TOH) Animal naming WMR-RC Digit span backward subtest of the WAIS-RC	MDD state: In the domains of processing speed (TMT-A and Digital Symbol Coding of WAIS-RC) and verbal fluency (animal naming), MEL patients performed significantly worse than atypical patients; Remitted state : MEL patients performed worse than controls in processing speed, shifting and verbal fluency.	[63]
MEL (n = 25) Non-MEL (n = 63)	♂/♀	48	Attention Information processing speed/Mental flexibility Psychomotor speed Response inhibition Planning Working memory Semantic verbal fluency	TMT A-B; WAIS-ITMT A-B ; SCWT I/IIFinger Tapping test SCWT III Tower of London WAIS-I/WAIS-IINaming	Baseline: MEL patients performed worse than non-MEL patients in most of neuropsychological tests (TMT-B; WAIS I/II, SCWT, TOL and in the Finger Tapping Test); Remitted state: Overall, MEL patients performed worse than non-MEL patients. Significant differences were found in the TMT-B and the semantic verbal fluency.	[62]
MEL (n = 279) Non-MEL (n = 544) CTRL (n = 247)	♂/♀	37	Motor coordination Response inhibition Sustained attention Decision speed Information processing Verbal memory Working memory Executive function Cognitive flexibility Explicit emotion identification Implicit emotion identification	Finger tapping Go-NoGo Continuous performance task Choice reaction time task Switching attention Memory recall and cognition Digit span task Maze task Verbal interference Identification accuracy/RTPriming RT	MEL patients performed worse at switching attention, decision speed and verbal interference compared to non-MEL patients; MEL patients were significantly slower than non-MEL patients to identify happy faces in explicit and implicit emotion identification.	[75]

Abbreviations: MEL: melancholic; non-MEL: non-melancholic; CTRL: Controls; SCR: Stimulus-Response Compatibility; CANTAB: Cambridge Neuropsychological Test Automated Battery; SOC: stocking of Cambridge task; ID/ED: intradimensional/extradimensional attention set shifting task; SRM: spatial recognition memory; PAL: paired associated learning task; WMS-R: Wechsler Memory Scale-Revised; SRTT: Serial Reaction Time Task; NART: National Adult Reading test; CVLT: California Verbal Learning task; PM: Prospective Memory task; SCWT: Stroop Color Word Test; WCST: Wisconsin Card Sorting Test; COWAT: Controlled Oral Word Association Test; SET: Modified Six Elements Test; TMT (A or B): Trail Making Test; WAIS-RC: Wechsler Adult Intelligence Scale—Revised by China; TOH: Tower of Hanoi; WMR-RC: Wechsler Memory Scale-Revised by China; RT: reaction time.

Conducted in small cohorts of patients, the oldest studies mainly focused on psychomotor and motor coordination performances such as Finger tapping task, fast walk, finger-thumb apposition, hands movements, foot tapping, finger-copying task or reaction time tasks. In accordance to the CORE concept, psychomotor performances were diminished in melancholic patients compared to non-melancholic patients [67,68,76].

The major finding emerging from the variety of cognitive domains tested is that all studies assessing attentional processes and more globally executive functions invariably showed deficits in one or several associated tasks in melancholic patients compared to non-melancholic patients [62,63,68,70–72,74,75]. These specific impairments are already a key component in melancholic profile. It also appears that the differentiation between melancholic and non-melancholic groups relies on increased task difficulty. Indeed, simple reaction task [68], spatial recognition memory [69,70], explicit episodic memory [71], semantic memory [72], memory recall or working memory [74,75] were similar across depression subtypes. In contrast, complex tasks that required set-shifting [62,69,70], attentional processes [62,68,71,72,74], cognitive flexibility [72,75], planning [62], decision-speed [63,75] distinguish melancholic and non-melancholic MDD patients.

As mentioned previously, cognitive abnormalities in MDD patients can persist even after clinical remission [5,54,77,78]. So far, only two studies compared cognitive performances of MDD patients with or without melancholic features longitudinally, from admission to recovery [62,72]. At baseline, both investigations agreed that cognitive performances in melancholic were impaired in most of the cognitive domains tested (attentional processes, information processing, psychomotor speed, mental flexibility, learning verbal memory, planning) compared to non-melancholic subtype. Interestingly, these memory and executive dysfunctions in melancholic patients were maintained despite the remitted state of patients. In contrast, some of cognitive disturbances observed in non-melancholic patients were improved after remission [62,72]. The persistence of some cognitive dysfunctions, mostly reported in melancholic patients, could represent a marker of a distinct depressive subtype instead of being secondary to the severity of depression. Further investigations are needed to determine why melancholic patients require a longer period of recovery in order for their cognitive functions to be restored to control levels. Only one study investigated cognitive deficit in the three different MDD subtypes defined by DSM-V: melancholic, atypical and undifferentiated patients. Major depressive subtypes displayed similar cognitive deficits in most of the tested domains but differed in some cognitive tasks. Melancholic patients performed significantly worse in domains such as processing speed (TMT-A, Digit symbol coding subtest) and verbal fluency (animal naming) compared to atypical and undifferentiated patients [63]. After remission from depression, while all the subtypes could recover their visual spatial memory and verbal working memory, clinically remitted melancholic patients exhibited specific deficits in processing speed, set-shifting measures and verbal fluency, in line with previously discussed results [62,72], suggesting once again a distinct profile in neurocognitive performance in baseline and remitted MDD, depending on the subtype.

There is substantial evidence showing that perception in patients with MDD is characterized by blunted responsiveness to emotionally positive information as well as an increased tendency to perceive emotionally neutral visual information as negative [79]. However, relatively few studies have used neurobehavioral measures to examine emotional disturbances and loss of positive affect in melancholic MDD population. When melancholic subjects were asked to classify emotional faces previously encountered, they showed either a better memory for sad faces [73] or significantly more time at explicitly identifying happy faces [75] compared to non-melancholic patients. These findings support that melancholic patients display a hypersensitivity to sad stimuli, reflected in a greater tendency to recall or identify sad expressions. Perceptual emotion biases appear to be specific in melancholic patients and could open up the debate about a possible primary neurocognitive feature of melancholic depression instead of a consequence of symptomatic mood. Further longitudinal neuroimaging studies combined with cognitive measures in melancholic patients could provide new insights in this field.

Taken together, the majority of studies agreed on both generalized and specific cognitive impairments in the melancholic subtype, characterized by a greater degree of these alterations compared to non-melancholic patients. Specifically, melancholic patients were distinguished by specific impairments in decision speed and positive emotion processing [63,73–75]. Moreover, given that most of cognitive deficits persist in remitted state in melancholic patients, the debate about the rehabilitation process of this type of depressive patients warrants an important focus.

Although subtyping depressive patients can be a useful tool in identifying specific profiles in depressive symptomatology, its clinical utility regarding predictive antidepressant drug efficacy must be hampered by recent exploratory [80] or translational research [81] investigations.

Even though, anhedonia and dysfunctional reward processing are unspecific to MDD, and may be observable in other psychiatric disorders [82], this is one of two required symptoms for a diagnosis of MDD [1]. This decrease in engagement in rewarding activities may be related to the complex disturbances in the emotional, motivational and cognitive domains that are associated with depression. Changes in reward-motivated learning in depression may exacerbate the clinical symptoms, or delay recovery. MDD and especially MDD-anhedonic subjects can display deficits in motivational processing and dysregulation of the brain's reward system [83]. Studies in MDD subjects showed a deficit in establishing a reward bias [84], and in reward-related decision-making [85]. If the assessment of "motivational anhedonia" is improved, it could help patient making behavioral choices that are likely to increase exposure to positively reinforcing experiences. Indeed, alterations in reward-related learning failed to appropriately guide or motivate subsequent behavior. These forms of incentive learning depend on several factors, including reward processing, motivation and the neuroplasticity underlying formation of new or strengthened memories. However, anhedonia is a particularly difficult symptom to treat, especially with current first-line pharmacotherapies (e.g., SSRIs), that do not adequately address motivational anhedonia in depression ([86] for review). If little is known about reward-motivated learning in animal models, a recent study using chronic exposure to corticosterone-induced depression-like behavior in Rat produced lasting deficits in the acquisition of reward-related learning tested on a food-motivated instrumental task [87]. Interestingly, in the same study, authors showed that amitriptyline increased instrumental performance compared in corticosterone-exposed rats. This may suggest that chronic exposure to amitriptyline *per se* facilitates this form of reward-related learning. The interrelationship between specific components of motivational dysfunction and specific cognitive systems in the context of depression remains to be studied in order to allow for a greater understanding of mood disorders [88].

3. Cognitive Behavioral Paradigms Used to Assess Learning and Memory Performances in Rodent Models of MDD

Cognition is a complex brain function that involves processes including attention, processing speed, learning and memory, working memory, verbal fluency and executive functions. However, some of these cognitive domains cannot be modeled in Rodents because of the higher cognitive demands specific to Humans, particularly in executive functions such as planning, problem solving, multi-tasking or decision-making and verbal fluency.

Collective efforts from preclinical researchers and clinicians are currently in progress to improve the translation of fundamental research tools into clinical application in Psychiatry. For example, the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) initiative was introduced to identify tasks with construct validity across species to improve the "translational-ity" of behavioral tests from rodents to non-human primates and humans [89,90]. More recently, the Research Domain Criteria (RDoC) project, established by the National Institute of Mental Health, propose a biologically-valid framework for the understanding mental disorders. This latter project relies on functional dimension of behavior based on genes, molecules, cells, circuits, physiology, behavior and self-reports, like the "Cognitive Systems" domain, and will allow a better crosstalk between clinical and preclinical studies [81,91]. In preclinical research, the development of

visual touchscreen-based memory procedures to assess cognitive functions in animal models will improve standardization of testing approaches, stimuli and conditions and will minimize experimenter involvement and potential bias. These types of behavioral tasks already exist to assess working memory in Rodents [92] but they are not used yet in animal models of anxiety/depression.

Despite this growing awareness for translational research across species, classical learning and memory behavioral paradigms remain widely used in fundamental research and in the context of anxiety-depression. Table 2 summarizes typical behavioral paradigms used in Rodents to assess cognition in anxiety/depression models of animals.

Table 2. Behavioral paradigms in rodents used to assess cognitive functions in anxiety/depression models.

Cognitive Domains and Functions	Behavioral Paradigms in Rodents
Attention	5-choice serial reaction time task (5-CSRTT)
Executive function - cognitive flexibility - inhibitory learning	Attentional set-shifting task (ASST) Reversal Morris water maze Prepulse inhibition (PPI)
Learning and memory	
Working memory	Delayed alternation Y-maze Delayed alternation T-maze Delayed match-to-sample with odors, subjects Modified MWM, BM, RAWM, RAM
Episodic memory	Novel object recognition test Object location recognition Passive avoidance place Social discrimination procedure
Reference spatial memory	Morris water maze (MWM) Barnes maze (BM) Radial arm water maze (RAWM) Object location recognition/ Object-in-place
Associative memory	Contextual/cued fear conditioning Extinction fear conditioning Passive/active avoidance place

Among them, few have a strong translational value from rat to non-human primates to humans (5-choice serial reaction time task to assess attentional process). In addition, fear extinction conditioning is the only task that presents a direct translational assessment from rodents to humans. Except these paradigms, the vast majority of preclinical assessment tools does not exhibit particular translational features. In exchange, they offer the advantage to be very well-validated through literature history, thus allowing relevant comparisons between studies using the same cognitive task.

Finally, some of the behavioral tests appear several times in different cognitive domains (*i.e.*, object location recognition, passive avoidance), confirming that a unique categorization of a cognitive task into a specific domain of memory is not always appropriate.

4. Episodic Memory in Rodent Models of Anxiety/Depression

In humans, episodic or autobiographical memory refers to the recollection of personally experienced past events and facts, which can be ranged from specific and general memories. Specific memories occur on a particular day at a specific place and time, whereas general memories recall events that occurred repeatedly or events that lasted more than a day. Overgeneral memory, characterized by subjects recalling fewer specific episodic details and prioritizing general schematic events has been observed in individuals with depression [93,94]. Thus, impairment in episodic

autobiographical memory has become a consistent feature in MDD patients. In addition, overgeneral autobiographical memory was found to be a predictor of the course of depression in a meta-analysis [95] and to be persistent in remitted or recurrent phases of MDD [96,97]. Finally, a recent work found that children of mothers with a history of MDD during their child's life, recalled less-specific memories, pointed out overgeneral memory as a cognitive vulnerability for depression [98]. However, the majority of neuropsychological tests used to evaluate cognitive functions in depressed patients do not include episodic memory assessment.

Considering the conscious approach of episodic memory assessment in Humans, translational research in Rodents required an adaptation in terminology, finally using "episodic-like" memory to designate animal's performances. Recognition memory tasks are widely used tools to assess episodic-like memory in rodents. These behavioral paradigms rely on animal's ability to determine whether or not a stimulus has been previously encountered. The type of stimulus (nature of objects, location of objects, order of presentation) can vary to evaluate the various components of episodic-like memory (What? Where? When?). The spontaneous nature of this task, exploiting the curiosity of rodents and their propensity for novelty seeking, confers to recognition tests many advantages, especially in an anxiety-depression context. Unlike many others learning or memory paradigms, no positive (food reward) or negative (punishment, water) reinforcements are needed, minimizing other psychological parameters that could influence performances during the task [99]. However, modeling MDD conditions in rodents induces some modifications in parameters such as anxiety, motivation and motor activity [15,100,101] that cannot be excluded for the interpretation of the performance in recognition tasks. The involvement of these parameters is systematically corrected with a measure of motor activity either during the test itself (total ambulatory distance, number of crossings in the arena) or in other behavioral paradigms applied before memory assessment (rotarod, Open Field) [99,102]. When the anxious state overly restricts exploration behavior, adaptations in the protocol test must be set up to re-establish a sufficient amount of exploration. For example, introducing one or several habituation periods (animals exploring the arena empty without objects) preceding the acquisition and the retention period of the test can fix this issue [99].

4.1. WHAT?

Many preclinical studies have investigated the performances of anxiety-depression models on object recognition memory (Table 3). The novel object recognition test (NORT), initially described by Ennaceur and Delacour [103] usually comprises an acquisition session with two identical objects and a retention session during which animals have to discriminate between a familiar object previously faced and a recently introduced new object (different in size, shape, texture, colors). Overall, findings differ strongly across and inside animal models [104]. Antenatal and early post-natal chronic stress procedures (PNS, MS, MD) present the most conflicting findings when compared to chronic adult stress procedure (SD, UCMS and CORT models). Independently of the model, the main source of heterogeneity in discrimination tasks arises from protocol variations across laboratories, such as the nature of objects (size, shape, color, texture), the discriminability of the stimulus (low, moderate or high degree), the time and episodes of familiarization, sessions/inter-trial interval durations and environmental conditions (light, noise, cage size, sawdust). Additional inevitable variables like species, strain or sex add even more variability across findings and differences inside each depression models, especially in prenatal stress and maternal separation/deprivation procedures.

Studies using prenatal stress or early-life stress procedures showed either impairing [105–113], or improving [114,115] or no effects [105,106,108–110,114,116–118] on object recognition test. Despite a thorough analysis of all the parameters characterizing studies presented in Table 3, no common features in pre/perinatal manipulations were observed.

Table 3. Episodic-like memory performances in rodent models of anxiety/depression.

Behavioral Test	Animal Model	Species	Sex	Age When Tested	Interval Intertrial	Effect on Discrimination Index (DI)	Reference
WHAT?	PNS	Mouse	♂/♀	Juvenile (PN23)	4 h	No effect	[105]
			♂	Adult (PN45)		No effect	
			♀			Impairment	
		Rat	♂/♀	Juvenile (PN23)	2 h	No competent for the task	[106]
			♂	Adult (PN56)		Impairment	
			♀			No effect	
			♀	Juvenile (PN28)	1 h	No competent for the task	[107]
				Adult (PND90)	1 h	Impairment	
			♂	Adult (PND80)	1 h	Impairment	[108]
			♀		1 h	No effect	
			♂	Adult (PND60)	40 min	Improvement	[114]
			♀		40 min	No effect	
			♂/♀	Adult (PND63)	15 min	No effect	[109]
			♂		1 h	No effect	
			♀			Impairment	
			♂		3 h	Impairment	
			♀			Impairment	
			♂/♀	Adult (PND63)	24 h	Impairment	[110]
	MS	Mouse	♀	Adult (PND85)	6 h	Impairment	[111]
			♂/♀	Adult (PND60)	24 h	Impairment	[112]
		Rat	♂	Adult (PND60)	1 h, 4 h	Impairment	[113]
			♂	Adult (PND70)	1 h	No effect	[116]
			♂	Adult (PND75)	1 h	No effect	[117]
					24 h		
			♂& ♀	Adult (PND90)	2 h	No effect	[118]
			♀	Adult (PND55)	1 h	Improvement	[115]
					24 h		
	MD		♂	Juvenile (PND35)	1 h	No effect	[119]
			♀			Impairment	
			♂/♀	Adult (PND60)	4 h	Impairment	[120]

Table 3. *Cont.*

Behavioral Test	Animal Model	Species	Sex	Age When Tested	Interval Intertrial	Effect on Discrimination Index (DI)	Reference
WHAT?	Early stress life	Mouse	♂/♀	Adult (PND > 90)	24 h	Impairment	[121]
	Social defeat	Mouse	♂	Adult	1 h	Impairment	[122]
					24 h	Impairment	
	UCMS	Mouse	♂		1 h, 2 h	Impairment	[123–125]
					24 h	Impairment	[126]
		Rat	♂		1 h	Impairment	[125,127,128]
	CORT	Mouse	♂		5 min	Impairment	[102]
					1 h	Impairment	[129,130]
					24 h	Impairment	
		Rat	♂/♀		1 h	No effect	[118]
WHERE?	PNS	Rat	♂	Adult (PND80)	1 h	Impairment	[108]
			♀		1 h	No effect	
	Early stress life	Mouse	♂	Adult (PND > 90)	24 h	Impairment	[121]
			♀		24 h	No effect	
	MS	Rat	♂	Adult (PND75)	1 h	No effect	[117]
					24 h	Improvement	
	UCMS	Mouse	♂	Adult	24 h	Impairment	[126]
		Rat	♂		4 h	Impairment	[127]
WHEN?	PNS	Rat	♂	Juvenile (PND30–40)	1 h	No effect	[131]
	MS			Adult (PND75)	1 h, 3 h	Improvement	[117]
				Adult (PND60)	3 h	Impairment	[132]

On the contrary, object recognition memory is predominantly impaired in chronic stress procedures applied during adulthood, such as social defeat, UCMS or the CORT model. This alteration is indicated by a diminution of the discrimination index (DI) [102,122–125,129,130], regardless of the duration of the inter-trial delay. One of these studies assessing long-lasting effects of UCMS procedure showed that anxio-depressed animals still displayed a discrimination deficit one month after the end of the stress treatment [125], suggesting that episodic-like alterations persist even after the cessation of the stress. These findings are in line with episodic memory deficits remaining in remitted or recurrent MDD patients [96,97]. A single study performed in rats disproves this impairment in the CORT model observing no effect in anxio-depressed animals [118]. Besides a likely species-dependent performance, the 2-week corticosterone protocol used in this study compared to a 4-week one in others studies [102,129,130] could explain in this case the lack of effect on recognition memory.

4.2. Where and When?

Although the novel object recognition test is widely used in laboratories, place recognition (the "Where" component) and temporal ordering (the "When" component) tasks can help to refine the degree of evaluation of episodic-like memory. The place recognition task examines the animal's ability to discriminate between an object that was moved during the delay between the acquisition and the retention phase and an object that remained stationary, adding a spatial component to the task. On the other hand, temporal ordering task consists in presenting a set of two identical objects during a first acquisition session. After a defined interval, another set of two new identical objects is presented to animals for exploration. During the retention session, animals have to discriminate between the "old object" from the first acquisition session and the "recent" object from the 2nd session. An intact temporal order memory is observed when an animal has spent more time with the "old" object [99].

The same pattern emerges from place recognition memory compared to object recognition with conflicting pre/peri-natal findings (impaired: [108,121]; improved: [117]; no effect: [108,117,121]) *versus* adult chronic stress procedures (impaired: [126,127]). In this type of recognition, female animals across models seem to be less vulnerable than male animals [108,121].

Very few studies evaluated the "when?" component of episodic-like memory *via* the temporal ordering task. Only works in maternal separation were performed, once again presenting discrepancies in the results. One study showed no effect of MS procedure on temporal order memory [133], while another one found an impairment [132]. At this time, there is not enough information available in the study of place recognition and temporal ordering tasks to clarify the impact of anxiety-depression models on these episodic-related tests.

Independently of the nature of task, antenatal and early post-natal procedures were the only models in which an improvement in episodic-like performances was observed [114,115,117]. For example, Makena *et al.* [117] showed that object location and temporal ordering task performances were enhanced in maternally separated rats, suggesting that perinatal manipulations can positively modulate brain development and neuro-adaptative responses to a stimulus, influencing the animal behavior reactivity as adults. Furthermore, post-natal manipulations have been reported to increase the intensity of maternal care (during reunion after a given period of dams-pups separation) of maternally-separated pups after a brief separation [134,135], and to provide greater skills to adapt to psychological and physiological stressors in adulthood [115,136].

Taken together, except an overall impairment in all discrimination tasks observed in adult chronic stress procedures, results are too inconsistent in pre/perinatal manipulations to extract relevant advances in the area of episodic-like memory. Further investigations focusing on place and temporal recognition may contribute to progress in the discovery of fundamental mechanisms underlying episodic-like memory in rodents in a pathological context. Finally, efforts should be intended to integrate episodic memory in clinical neuropsychological tests performed in MDD patients to fully assess cognitive functions.

5. Working Memory Deficits in Rodent Models of Anxiety/Depression

In humans, the concept of working memory refers to the ability to get and temporarily store specific information during a variable delay in which the stimulus is absent, and to return the message when requested by the context. Clinically, the majority of neuropsychological tests assessing working memory relies on verbal tasks such as the Digit Span task (typically remembering a phone number or a list of word items and repeating them back after a defined delay) [72,75]. In rodents, working memory can be defined as a "short-term memory for an object, stimulus or location used within a testing session, but not typically between sessions" [137]. Unlike reference memory (long-term association between the stimulus-response pairing), working memory is characterized by its transience because it needs to function on a particular trial and then must be forgotten and ignored in following trials. Although using language as a parameter is not achievable in rodents, many behavior paradigms have been created to model this type of memory. The most widely used preclinical tools to assess working memory are maze type tasks which require spatial working memory to be solved. Some of the common variants of these tasks used in MDD context are T-maze and Y-maze alternation tasks, which rely on the natural exploratory behavior of rodents and exploit animals' inherent tendency to choose an alternative arm over an arm that has been previously explored on consecutive trials [11,137]. Moreover, protocol adaptations in spatial learning tasks such as the radial arm maze (RAM) or Morris water maze (MWM) are often used to assess working memory in chronically stressed animals [137,138]. For example, whereas the spatial version of MWM evaluates reference memory (the location of the hidden platform remains the same across all the trials), the cued version of MWM enables the assessment of working memory (the location of the hidden platform is switched between each session), forcing animals to recruit different neuronal pathways and mechanisms to succeed the task.

As illustrated in Table 4, working memory performances in rodents in various anxiety-depression contexts appear to be model-dependent. Indeed, cognitive performances vary according to the nature of the chronic stress procedures. While prenatal stress and unpredictable chronic mild stress procedures both induce global impairment in working memory, maternal separation and chronic corticosterone methods seem to have no impact on this type of memory. Interestingly, impairment or absence of effect in working memory is consistent in each different preclinical model whether alternation tasks or adapted RAM or MWM were used, suggesting that working memory stress-induced effects are not task-dependent.

Table 4. Working memory performances in different models of anxiety/depression.

Cognitive Domain	Model	Species	Gender	Effect on Working Memory	Reference
Working alternation task: T-maze or Y-maze	PNS	Rat	♂/♀	↓ alternation	[139–141]
	MS	Rat	♂/♀	No effect	[118,119,142–144]
		Mouse		No effect	[112]
			♂	Strain-specific effects	[145]
	Social defeat	Mouse	♂	↓ alternation	[146]
		Rat	♂	Delay-specific effects	[147]
			♂/♀	No effect	[148]
	CMS	Rat	♂	↓ alternation	[149–152]
		Mouse		↓ alternation	[153]
	CORT	Rat	♂/♀	No effect	[118]
			♂	No effect	[118,142,144,154,155]

Table 4. *Cont.*

Cognitive Domain	Model	Species	Gender	Effect on Working Memory	Reference
Working spatial memory: cued MWM/BM/RAW	PNS	Rat	♂/♀	No effect	[109]
				Learning impairment	[140]
	MS	Rat	♂/♀	No effect	[156]
			♀	No effect in learning	[115]
				Enhancement in retention	
	Social defeat	Rat	♂	Delay-specific effects	[157,158]
	CMS	Rat	♂	Learning impairment	[159]
				Retention impairment	[160]
	CORT	Rat	♂	No effect	[161,162]

Bidirectional findings are observed when analyzing early chronic stress life procedures effects on working memory. Indeed, while prenatal stress mostly impaired alternation tasks and spatial working memory [139–141], these cognitive performances are unchanged in most maternal separation studies [112,115,118,119,142,143,145,156]. Specifically, working memory following maternal separation performed in mice was found to be strain-specific. In the delayed alternation T-maze, Balb/cJ mice displayed a spatial working memory deficit whereas C57Bl/6J mice were not affected [145]. In prenatal stress, all studies describing an alteration in working memory were performed in juvenile rats (approximately 1 month of age, from PND 26 to PND 36) [139–141]. The only study performed in PNS-young adult rats failed to demonstrate any behavioral changes in the radial-arm maze [109], suggesting that cognitive working functioning may be more sensitive to PNS procedure in juvenile than in adult rats. In addition, Markham *et al*. provided a full characterization of cognitive consequences in PNS-rats [106] in which working memory tools were based on discrimination processes. In this study, all the learning and retention parameters of visual/spatial discrimination or visual discrimination alone were negatively impacted by chronic gestational stress, suggesting in this case as a perseverative behavior as a consequence of prenatal stress.

Regardless of the nature of the task, a robust altered effect in unpredictable chronic mild stress animals was found on working memory. Indeed, all of the preclinical studies demonstrated either a decrease in alternation behavior [149–153] or a decrease in learning and retention abilities in spatial working memory after a chronic unpredictable environmental stress [159,160]. On the opposite, working memory was not affected in rats submitted to a chronic corticosterone administration [118,142,144,154,155,161,162]. This could be explained by the cessation of the chronic CORT exposure before behavioral testing, as found in a recent study examining prior effects of chronic CORT stress in the cued version of MWM [162]. Moreover, working memory may be sensitive to the duration of chronic CORT, as a longer duration of CORT regimen (56-days CORT treatment instead of a usual 21 to 28 days treatment duration) can induce a spatial working memory impairment in the Y-maze in rats [154].

Interestingly, several studies initially focused their attention on neonatal maternal separation and chronic CORT procedures independently before combining these two methods (MS + CORT) to assess their behavioral effects in delayed alternation tasks [118,142,144]. While all of these works agreed on the absence of working cognitive effects of these procedures separately, it seems that results concerning the combined MS + CORT procedures are task- and delay-dependent. Following the combined MS + CORT procedure, working memory assessment via the Y-maze demonstrated a decrease in the percentage of time spent in the novel arm after a 2 h-delay [118,142], whereas no cognitive effect in alternation rate was found in any of the tested delays (30 and 60 s) in the T-maze [144].

Finally, working memory performances in social defeated animals varied, in delay- and species-dependent manners. In the T-maze alternation task, mice displayed a decrease in alternation rate as soon as the 5-seconds delay [146] whereas the alternation rate was not affected in rats, neither at

10 or 30 or 60 s delays [147,148]. The delay must rise to 90 s to induce a deficit in working memory in adult defeated-rats compared to controls [147]. This observation supports that: (1) the longer the delay between sessions, the better the probability to forget the information; and (2) chronic social stressed mice are more vulnerable than defeated-rats in working memory tasks.

Taken together, chronic environmental stress procedures lead to a decline in working memory whether it was applied during gestational or adulthood periods. On the contrary, early maternal separation or chronic corticosterone regimen do not cause any damaging effects separately, but demonstrated conflicting delay-dependent effects when associated. Socially-defeated animals displayed species and delayed-dependent behaviors in working memory. Other non-spatial behavioral paradigms evaluating working memory with operant tools are currently in development such as Delayed Match to Sample, Delayed non-Match to Sample Delayed [137], but so far, no reports were found in an anxiety-depression context.

6. Attention and Executive Functions in Rodent Models of Anxiety/Depression

Attention deficits are referenced as a diagnostic criterion in DSM-V [1] and are often reported in patients suffering from MDD, increasing the need to better understand mechanisms underlying this cognitive process through rodent models. Attention processes can be divided in three sub-domains including: the ability to sustain attention on a specific task over a long period of time (sustained attention), the ability to respond simultaneously to multiple tasks (divided attention) and the ability to focus on selective environmental information while ignoring distractions (selective attention) [163]. However, a small amount of studies examined attentional abilities in animal models of anxiety/depression (Table 5). In preclinical studies, one of the most widely used tasks measuring attention is the 5-choice serial reaction time task (5-CSRTT). Variations in this protocol can be arranged in order to assess the three subtypes of attention in a single behavior paradigm [163]. Briefly, this task consists in presenting five spatial different apertures to animals in an operant chamber, with a food cup located on the opposite wall of the five location holes. The animal initiates a trial by opening the food cup door. After a short delay, a visual signal consisting of a brief illumination of one of the five apertures is presented. If the animal nose pokes in the corresponding hole, a food pellet is delivered into the food cup and this action is counted as a correct response to the test. Conducted through several trials, attention performance is usually measured by examining when the animal responds to a correct or incorrect hole where the light appears (accuracy *versus* omissions) and the speed with which the animal responds (time reaction). Complexity in the task can be increased by varying the stimulus light or inter-trial interval duration for example, assessing in this case parameters such as premature or perseverative responses (nose poke that occurs prior to light presentation or repeated nose pokes in a previously lit aperture), giving further information on impulsive behavior (see [164–166] for details in methodology). Among few available reports presented in Table 5, all of them agreed about some deleterious effects of anxiety-depression on attention performances in the 5-CSRTT [167–170], increasing accordingly as cognitive challenges in the task are getting more complex. For example, under standard 5-CSRTT conditions (fixed inter-trial interval (ITI) and stimulus durations (SD)), there were few differences in performances between rats submitted to prenatal stress procedure and controls. However, when ITI and SD vary across trials, there was a decrease in accuracy and an increase in number of premature and timeout responses, respectively, indicating an impairment in sustained attention, increased impulsivity and cognitive inflexibility in prenatal stressed rats [168,171].

Accumulating evidence suggests that unrestrained secretion of corticotropin-releasing hormone (CRH) can contribute to the apparition of depressive symptoms as well as cognitive deficits ([172] for review). Interestingly, attentional abilities have been evaluated with the 5-CSRTT in a genetic mouse model based on overexpression of CRH throughout development. Disrupted attention processes were observed once the task was acquired, as CRH transgenic mice showed a decrease in the percentage of correct responses, a longer correct response latency and an increase in omitted responses

compared to wild-type animals [167]. In line with this work, others studies using pharmacological cerebral CRH administration in mice demonstrated attention alterations [173,174].

Table 5. Attention and executive functions performances in different rodent models of anxiety/depression.

Executive Function	Task	Model	Species	Gender	Behavioral Effect	Reference
Attention/Impulsivity	5-CSRTT	CRH-KO	Mouse	♂	Impairment	[167]
		PNS	Rat	♂/♀	Impairment	[168]
		CMS		♂	Impairment	[169]
		CORT		♂	Bidirectional effects	[170]
Attentional set-shifting task	ASST	MS	Mouse	♂/♀	Strain-specific impairment	[145]
			Rat	♂	Impairment	[132]
		UCMS			Impairment	[175,176]
		CRS			Impairment	[177]
		CORT			Impairment	[178]
Reversal learning	MWM	MS	Mouse	♂	Reversal learning impairment	[111]
				♀	No effect	
			Rat	♂/♀	Reversal learning impairment	[179]
					Reversal learning enhancement	[180]
		Social defeat	Mouse	♂	Reversal learning impairment	[181]
				♀	No effect	
		UCMS	Mouse	♂	Reversal learning Impairment	[182]
			Rat	♂	Reversal learning impairment	[183,184]
		CORT	Mouse	♂	Reversal learning/retention impairment	[102]
			Rat	♂	Reversal learning impairment	[161]

A recent study further investigated impulsive behavior in adolescent CORT-treated rats in the 5-CSRTT and others attentional/impulsive tasks derived from multiple-choice serial reaction tasks [170]. Intriguingly, adolescent chronic exposure to corticosterone induced in bidirectional effects on impulsivity behavior. CORT-treated rats were slightly less impulsive on measures of impulsive action, but also markedly displayed an increased impulsive choice, selecting the immediate small reward faster and more frequently instead of waiting for the larger reward after a longer delay. This suggests that chronic CORT treatment in adolescent rats enhances impulsive choice while simultaneously decreases impulsive action [170]. Collectively, these findings showed that an anxio-depressed state in rodents can alter attentional processes across models.

Executive functions refer to higher cognitive processes in mental functioning such as: cognitive flexibility, planning, decision-making, reasoning, multi-tasking, concept formation or response inhibition and represent by far the most complex and challenging domain to model cognitive deficits in rodents. Similarly to attentional deficits, executive dysfunctions are commonly listed as cognitive difficulties in MDD patients and are progressively pointed out as specific neurocognitive abnormalities observed in remitted, recurrent or melancholic subtype of depression. Table 5 gathers available literature about executive functions performances across different anxiety-depression rodent models.

Among all the aspects of executive functioning, cognitive flexibility remains the most widely used task and can be modeled in preclinical studies in different ways. Particularly, the most described task used to assess cognitive flexibility in rodents in anxiety-depression models is reversal learning in spatial learning and memory tests. Classically, animals learn, during an acquisition phase, the specific location of an escape platform, which is then switched to the opposite location during the reversal phase, asking them to adapt and change their behavior to succeed in the task. Overall, the majority of studies showed impairments in cognitive flexibility in reversal learning and memory [102,111,161,179,181–184], evoked by the incapacity for stressed animals to decrease the time to find the platform along reversal trials and to spend more time in the new target quadrant instead of the previous target quadrant during the retention trial. These findings suggest that anxio-depressed animals lost their ability to unlearn a previously acquired rule and to adapt their behavior to the new instruction, in accordance with clinical observations.

Interestingly, some of these studies reported that chronic stress procedures, independently from their nature, induced deficits in spatial learning reversal without affecting acquisition learning [161,182–184]. It is assumed that chronic stress can induce a perseverative behavior in MWM, preventing animals from re-learning a new location. This possibility was supported by the measurement of a decrease in escape latency during acquisition (confirming that a correct learning occurs) followed by an increase in the percent of time spent in the initial training quadrant during reversal learning [184]. Other authors suggest that dissociation between acquisition and reversal cognitive performances could be due to the involvement of specific brain regions. Indeed, whereas the hippocampus is primarily involved in spatial learning tasks, the prefrontal cortex, implicated in reversal learning, reveals a greater vulnerability to the deleterious effects of chronic environmental stress [182,183,185].

Sex-specific differences have been observed in studies performed in mice, showing that female animals were resilient to cognitive alterations effects observed in male animals [111,181]. While some expected elements, based on sexual differences such as gonadal hormones modulation, could explain the male vulnerability to cognitive effects of stress, other causes directly inherent to the nature of the chronic stress procedure or the cognitive task itself might are open to discussion. For example, supporting the brain region-specific hypothesis previously evoked, spatial acquisition was unaffected in socially defeated mice in both sexes, but only males failed to succeed in the reversal learning phase, suggesting that neuronal circuits involved in cognitive flexibility are differently impacted in male and female mice by chronic social stress [181]. On the other hand, in a chronic early-life stress context, differences in dams' interaction towards male or female offspring [186] may explain why female offspring are less vulnerable to the effects of MS than males [111]. Further investigations are required to identify mechanisms underlying sex-specific effects of anxiety-depression context on cognitive reversal performances.

As well as episodic-like memory, maternal separation was the only model in which an enhancement of cognitive flexibility was found. In the only study showing improved reversal learning effects, authors tested cognitive performances in maternally-separated rats at three different ages of development: PND21 (after weaning), PND35 (juvenile) and PND56 (young adult). Their results highlighted that enhanced behavioral effects generated by MS procedure do not emerge until adolescence [180].

The attentional set-shifting task (ASST) is a paradigm used to assess cognitive flexibility in anxiety-depression models. Probably less employed than reversal tests because of its complexity to set up, this task refers to animal's ability to recognize and to adapt their behavior to various changing rules or environmental circumstances. In the most common version of rodent ASST, rodents are trained to dig for a food reward and make a simple discrimination based on both odor and digging medium. During the test, at each stage, rats are required to attend to one of two perceptual dimensions (odor or digging medium), and to shift between stimuli dimensions in order to learn the rule to successfully retrieve the food reward. The ability to extract knowledge from different stimuli dimensions suggests

that animals are capable of using some aspects of higher-order cognitive functions present in human executive functioning [187,188].

Although the nature of parameters varies according to the study (discrimination, compound discrimination, reversal or extra-dimensional phases), an alteration in cognitive flexibility has been observed across anxiety-depression models [132,145,175,178]. A study performed in mice revealed a strain-specific impairment, where an increase in days to reach the criterion in the extra-dimensional stage was observed in maternally-separated Balb/c but not in C57Bl/6 mice [145]. This strain-specific performance could be explained by differences in maternal care (more nursing, licking and grooming in C57Bl/6 mice than Balb/c) and genetic susceptibility to stress.

Studies performed in rats highlighted interesting differences in performance between chronic stress procedures. The first work studying the consequences of MS on cognitive flexibility showed that maternally-separated rats displayed weaker overall performances in the ASST compared to control animals, as indicated by significant deficits in compound discrimination (SD), reversal 1 (REV1) and extra-dimensional set-shifting (ED) [132]. These three stages are characterized by the appearance of a new level of complexity never encountered before by the rat, illustrating their reduced ability to adapt to the introduction of new complex rules and perform strategy changes. Animals exposed to a chronic environmental stress procedure during adulthood (UCMS or chronic restraint stress) displayed a constant deficit in extra-dimensional shift (ED) in ASST [175–177]. To evaluate the persistence of this cognitive deficit, the performance of separate groups of rats was assessed on the 4th, 7th, 14th and 21st day following the cessation of chronic restraint stress [177]. Stressed rats exhibited long-lasting ED set-shifting impairments, since these deficits were observed at all the time points, even three weeks following stress termination. Finally, CORT-treated rats were less efficient in all the reversal learning stages of the ASST (REV1, REV2, and REV3) than vehicle-treated animals, due to a failure to learn the new rule or to unlearn the old rule. Moreover, CORT-treated rats required more trials at the intradimensional shift stage (ID) suggesting that they treated each set of cues independently and did not use their prior experience as an indicator of which dimension was the salient one [178].

Despite the complexity of assessment instruments and interpretation, cognitive deficits in attention and executive functions are shared across all anxiety-depression existing rodent models, strengthening clinical findings in MDD patients. Developing further preclinical behavioral tools to explore other aspects of executive functions than cognitive flexibility is obviously the next challenge in the field.

7. Spatial Learning and Memory Deficits in Rodent Models of Anxiety/Depression

Spatial memory is one of the most documented types of memory in laboratories, thanks to the development of various spatial behavioral paradigms through decades (i.e. Morris water maze, 8-radial arm (water) maze, Barnes maze, Y-maze, novel object recognition location). This particular form of reference memory has already been the target of a bibliographic review focusing on the consequences to chronic stress effects on spatial learning and memory performances, but mostly limited to chronic restraint stress or social exposures in several species [189]. To our knowledge, no synthesis gathering data about spatial memory performances in anxiety-depression rodent models was constituted. One of the main interests of studying spatial memory in rodents lies into the intimate relationship between hippocampus, chronic stress and spatial skills, allowing investigators to dissect mechanisms underlying spatial learning and memory processes *via* rodent models. It is now well known that long-term exposure to a chronic stress can induce deleterious changes in hippocampal structure, including in the neurogenesis process, thus deteriorating some aspects of spatial behavioral experience [121,190].

Historically, the three main mazes, mentioned in Table 6 used in preclinical studies in anxiety-depression models, were created between the late 1970s and early 1980s. The Morris water maze (MWM), certainly the most widely used spatial memory paradigms, relies on distal extra cues surrounding the pool to guide animals to navigate from start locations to locate a hidden escape platform [138,191]. The Barnes maze (BM), a dry-land version of the MWM, requests animals to escape from a brightly lit exposed circular open platform surface to a small dark recessed chamber located

under one of the 20 holes around the perimeter of the platform [192]. Finally, the radial arm maze (RAM) consists in eight equidistantly spaced arms radiating from a circular central platform. Reference spatial memory is assessed when the rodent only traverses goal arms of the maze containing a food reward [193]. Common features in spatial tasks rely on a spatial learning phase (acquisition phase) and a reference memory probe (retention phase). Spatial learning is usually assessed across repeated trials along several days of training and reference memory is determined by preference for the platform quadrant/hole when these ones are removed. These spatial tests distinguish from each other by the nature of the motivation to perform the requested task: either aversively motivated task (water in MWM, RAWM or high open arena in BM), or appetitive motivated tasks with reward (RAM) [189,191].

Table 6. Visuo-spatial learning and retention performances in different rodent models of anxiety/depression.

Model	Test	Species	Gender	Age When Tested	Learning	Retention	Reference
PNS	MWM	Rat	♂	Juvenile	Impairment	Impairment	[141]
			♀		No effect	No effect	
			♂/♀	Adult	Impairment	Impairment	[106,194,195]
			♂		No effect	Impairment	[195]
			♂/♀		No effect	Impairment	[196]
					No effect	Improvement	[109]
					No effect	NA	[197]
		Mouse	♂/♀		No effect	Impairment	[198]
	BM	Mouse	♂		No effect	No effect [a]	[199]
						Improvement [a]	
MS	MWM	Rat	♂/♀	Juvenile	No effect	No effect	[180]
				Adult	Impairment	Impairment	
					No effect	No effect	[200]
			♂		No effect	No effect	[132,179]
					No effect	Impairment	[201,202]
					No effect	Improvement	[203]
			♀		No effect	Improvement	[115]
Chronic early life stress	MWM	Mouse	♂	Adult	Impairment	Impairment	[121]
			♀		No effect	No effect	
Social defeat	MWM	Mouse	♂	Adult	No effect	No effect	[122,146]
	BM	Mouse	♂		No effect	NA	[181]
			♀		No effect	NA	
	RAWM	Rat	♂		NA	Impairment	[157]
Learned helplessness	MWM	Mouse	♂	Adult	Impairment	Impairment	[190]
UCMS	MWM	Mouse	♂	Adult	Impairment	Impairment	[123,182,190,204]
		Rat			Impairment	Impairment	[128,159,205]
CUR	RAWM	Rat	♂	Adult	No effect	Impairment [b]	[206]
					No effect	Improvement [b]	
					Impairment	No effect [b]	[207]
			♀		No effect	No effect [b]	
CORT	BM	Mouse	♂	Adult	Impairment	Impairment	[102]
	MWM	Mouse			Impairment	Impairment	
		Rat			Impairment	Impairment	[208]
	RAM	Rat			Impairment	Impairment	[209]
	BM	Rat			Impairment	Impairment	[154,160,209]
					No effect	Improvement	[162]

[a]: depending on the presence of a recovery period between chronic stress cessation and behavioral testing; [b]: depending on the presence of a recovery period between chronic stress cessation and behavioral testing.

The vast majority of studies uses latency to reach the platform in MWM and BM tasks as a parameter to measure learning across training days. However, latency is sensitive to motor and motivational factors that are likely to be modified by chronic stress procedures, leading to possible critical issues for the interpretation of data. Consequently, it is essential to control distance and speed performances, to confirm that the observed reduction in latencies across trials or days is correlated to a spatial learning alteration and not to motor, exploratory or motivational factors related to an anxiety-depression phenotype. Table 6 summarizes spatial learning and memory performances in different anxiety-depression models. A first clear dissociation emerges in learning abilities across models depending on the period during which chronic stress models were applied. Most spatial studies report that chronic stress procedures performed in early-life (PNS, MS) do not compromise spatial acquisition abilities [109,115,121,132,141,179,180,195–201,203], whereas other adulthood anxiety-depressive phenotype inductions (LH, UCMS, CORT) result in an impairment of spatial learning [102,123,128,154,159,160,182,190,204,205,207–209].

Two hypotheses can be proposed concerning the lack of spatial learning detrimental effects following early-life stress. It is likely that specific spatial learning neuronal networks and pathways may not be influenced by these chronic stress procedures at this young age, inducing no damaging effects in adulthood either. However, spatial learning performances being tested at the adult age, brain maturation occurring during adolescent and young adult periods, during which stress procedures are no longer applied, could be sufficient to compensate cognitive learning dysfunctions that might have been induced during previous prenatal or perinatal period.

It is also remarkable that impairment in spatial learning phase after adulthood anxiety-depression procedures is almost systematically associated by impairment in retention performances, suggesting that all the spatial abilities were impacted here [102,123,128,154,159,160,182,190,204,205,208,209]. Increasing evidence has showed that stress-induced depressive and cognitive phenotypes in adulthood are associated with a reduction of hippocampal neuronal plasticity and neurogenesis, supporting harmful effects of UCMS and CORT methods in learning and memory performances [15,210].

Among studies that assessed spatial retention memory, consequences of anxiety-depression phenotypes are more ambiguous, so that categorization according to the type and the nature of stress models appears inconceivable. According to the Table 6, chronic stress procedures modeling MDD in rodents:

- Impaired probe trial performances in twenty-two studies [102,106,121,123,128,141,154,157,159,160,182,190,194–196,198,201,204,205,208,209,211],
- Had no effect in nine studies [121,122,132,141,146,179,199,200,211],
- Enhanced retention performances in five studies [109,115,162,199,203].

Overall, almost 2/3 of cited studies displayed impairment in spatial reference memory, highlighting the vulnerability of retention ability in a low mood pathological context, independently from the anxiety-depression model. In early-stress life procedures (PNS and MS), factors that contribute to the high variability in results are multiple and rather easy to identify. For example, in PNS studies, the period and duration of stress procedure can affect the behavioural performance. One study examined the effects of three separate prenatal stress periods on spatial learning and memory retention in male rats: (1) before pregnancy stress during 10 days, (2) early pregnancy stress (Gestational day 0 (GD) to GD10) and (3) late pregnancy stress (GD11-GD21) [195]. Preconception and early pregnancy stress manipulations strongly impaired learning and memory performances, whereas late pregnancy stress only altered retention memory in adult offsprings, suggesting that the intensity of detrimental effects are linked to the time point of stress application. The hypothesis that the nature of stressors (physical or psychological stressors) might induce different responses on spatial performances in PNS-animals has also been recently tested [196]. Obviously, many other factors, including the nature of the task (MWM/BM/RAWM), the age of animals at the time of the test, species-, strains- and

sex-specificity, contribute to the difficulty to find a distinct profile in cognitive spatial deficits in early-life stress protocols.

Interestingly, some of the studies in which spatial learning was not affected in anxio-depressed animals revealed impairments in retention trial [195,196,198,201]. Even though retention performances are intimately linked to learning processes, two distinct functioning circuits involving different brain structures and neuronal pathways may exist [212,213]. In these particular cases, it appears that learning process is more resistant to pre/perinatal chronic procedures than retention functioning.

Studies using chronic unpredictable restraint (CUR) stress provide other pieces of information. A recent study investigated whether a recovery period between chronic stress application and spatial tests may impact behavioral responses. For that purpose, one group of rats received behavioral testing immediately after a 21 days-CUR procedure, whereas another group of animals was given 21 days to recover from chronic stress before assessing spatial performances. Immediately-stressed rats displayed a learning spatial impairment, while there was no impact on spatial learning in rats that benefited from a recovery period [207]. Focusing on retention parameters, the same pattern of results with an improvement in spatial reference memory after a recovery period was found in rats [206], confirming the reversibility of CUR-induced deficits in this hippocampal-dependent spatial learning and reference memory task.

Interestingly, administration of CUR in female rodents failed to alter spatial learning and memory performances, whether they had a recovery period or not. This comment is in line with all the other studies that have been conducted in female mouse or rat, because they did not report any changes in spatial learning and retention behaviors following different chronic stress procedures [115,121,141,207]. The female resilience to cognitive spatial alterations observed in males in an anxiety-depression context is not an isolated phenomenon and was also apparent in other types of memory such as episodic-like memory [106,108,121] and reversal learning tasks [111,181]. Further studies are required to clearly differentiate sex-specificities of cognitive performances in a MDD context.

8. Associative Memory in Rodent Models of Anxiety/Depression

Up to now, the vast majority of clinical studies using neuropsychological tests in MDD focused on cognitive deficits such as executive functions, attention, processing speed, working memory, verbal and visual learning and memory or psychomotor performances. Clinical emotional assessment remains paradoxically one of the less documented type of memory studied in MDD. Only few reports concentrate their research specifically on cognitive biases associated with mood disorders [73]. However, anhedonia, one of the core symptoms in the DSM-V, is defined as the loss of interest in originally rewarding and enjoyable activities. Precisely, cognitive biases in depressed patients refer to dysfunctions in emotional memory including distorted information processing and cognitive bias favoring sad information and lower responsiveness to positive outcomes [20,214,215].

Emotional memory (associative memory in rodents) refers to a non-declarative type of memory, implicit memory, characterized by the impossibility to reach the memory through a conscious process. Emotional memory is necessary for individuals to learn to predict a danger and adapt their behavior accordingly. In their natural environment, rodents respond to danger in a species-specific manner by a typical freezing behavior triggered to avoid detection from possible predators. Freezing is defined as a cessation of all movements, except breathing. This instinctive behavioral response is used in preclinical studies to assess associative learning and memory. In anxiety-depression models, this type of memory is evaluated using classical conditioning tasks (contextual or cued fear conditioning; passive avoidance test), based on negative reinforcers, and being dependent on the hippocampal brain structure [216,217]. Basically, animals learn to associate a conditioned stimulus (context, light, tone) with an aversive and inescapable unconditioned stimulus (mild foot shock applied through the grid floor) during an acquisition session. During the test session, animals are re-introduced in the conditioned chamber and are submitted to either a cued fear conditioning (tone or light presentation in a modified context) or

a contextual fear conditioning (similar context without footshock) [91]. This situation theoretically increases the freezing behavior in animals with an intact associative memory.

Findings presented in Table 7 about fear conditioning and fear extinction behaviors in anxiety-depression models are conflicting. We separated pre/perinatal models from adulthood-induced models. Early-life chronic stress and perinatal manipulations induced either fear conditioning impairments [106,111,112,218] or no effects on associative memory [105,219,220], whereas chronic stress procedures performed in adulthood (social defeat, learned helplessness, unpredictable chronic mild stress or chronic corticosterone administration) improved in associative memory in comparison to control animals [146,149,221–227]. These latter results may actually be in line with the observation that patients suffering from MDD display a better memory for negative stimuli than neutral or positive ones.

Table 7. Associative memory performances through conditioning tasks in different models of anxiety/depression.

Type of Task	Model	Species	Age When Tested	Sex	Fear Conditioning	Fear Extinction	Reference
Contextual/cued associative task	PNS	Mouse	Weaning	♂/♀	No effect	No effect	[105]
			Juvenile		No effect	No effect	
		Rat	Adult		-	Impairment	[208]
					Impairment	No effect	[106]
	MS	Mouse	Adult	♂/♀	Impairment	-	[111,112]
		Rat	Juvenile		Impairment	No effect	[218]
			Adult		Impairment	Impairment	
				♀	No effect	No effect	[219]
					No effect	-	[220]
	Social defeat	Mouse	Adult	♂	Improvement	-	[146,221,227]
	Learned Helplessness	Rat			Improvement	Impairment	[222]
	UCMS	Rat			Improvement	-	[149,223]
	CORT	Mouse			Impairment	-	[102]
		Rat			No effect	Impairment	[228]
					No effect	No effect	[155]
					Improvement	-	[224–226]

A recent study published in *Nature* showed that the activation of positive memory could reverse depression-like behavior. Optogenetic reactivation of dentate gyrus cells previously active during a positive experience can rescue stress-induced depression-related behaviors [229]. This work identified glutamatergic activity in the hippocampus-amygdala-nucleus-accumbens pathway as a candidate circuit supporting positive memory. Studies revealing a decrease in freezing duration compared to controls are also in accordance with an associative memory alteration since animals are unable to associate and distinguish an unsafe context from a safe one [230]. Here, opposite effects in the same task can be associated with behavioral associative impairment: an increase in freezing behavior may be interpreted as an enhancement in memory for negative stimuli, while a decrease in freezing behavior may be translated as a difficulty to associate a specific context with an aversive stimulus.

When the conditioned stimulus is repeatedly presented without the presence of the unconditioned stimulus, it corresponds to the extinction phase. Consequently, freezing behavior decreases over time because the context is no longer associated with the danger. In anxiety-depression models, assessment of extinction fear remains less investigated than fear conditioning. Among studies evaluating this aspect of associative memory, no consistent findings were described, suggesting in stressed-animals either conserved fear extinction performances [105,106,155,218,219] or the incapacity to adjust their behavioral response to a neutral stimulus [218,222,228]. Deficits in fear extinction are rather often

reported in post-traumatic stress disorders (PTSD) models in rodents, consistent with the incapacity of PTSD patients to distinguish the real traumatic situation they lived in the past from a neutral context situation in which an unconditioned stimulus related to the traumatic memory has been inserted. Although PTSD confers specific significant psychiatric disturbances and functional impairments, depression and PTSD are commonly co-morbid diseases. Research suggests that significant depressive symptomatology affects 30% to 50% of subjects diagnosed with PTSD [231]. Impairment in fear extinction could also be interpreted as a perseverative behavior [232]. Further studies are needed to identify the distinct role of fear extinction in MDD context.

The step-through passive avoidance test is a hippocampus-dependent memory task conducted in two sessions. The apparatus is divided in two chambers separated with a guillotine door: one illuminated, transparent "safe" compartment and one darkened, opaque "shock" compartment. During the first testing day, animals are put into the lighted compartment, in which they are expected to enter freely into the dark compartment. Once the animal has entered into the dark compartment, one inescapable foot shock is delivered. 24 h later, the animals are placed in the light compartment again for a retention test, with free access to the dark compartment without any shock. The latency for the subject to enter into the dark chamber is recorded [233]. The passive avoidance task offers a compelling complementarity to classical conditioned tasks previously presented. Instead of "simply" recognizing the environment in which they were previously shocked, animals need to inhibit their natural tendency to enter the dark chamber. This particular demand in avoidance behavior indicates the different neuronal pathways that regulate behavioral responses in this test compared to classical conditioned tasks. Involvement between the amygdala and other areas of the prefrontal cortex that mediate behavioral control are requested in avoidance mechanisms, whereas associative learning tasks is mediated by a hippocampus-amygdala circuitry [223].

Almost all the preclinical studies in the passive avoidance test found a significant decrease in latency to enter into the dark compartment in chronically-stressed animals compared to controls (Table 8), highlighting an overall deficit in associative memory conserved across anxiety-depression models [116,139,140,196,197,208,223,233–237]. This confirms, once again, the difficulty for depressed animals to associate the aversive stimuli with the context they met the day before and their incapacity to restrain their instinct. Similarly to classical conditioned tasks, passive avoidance measures can lead to a double-interpretation. According to studies, lower latencies to enter into the dark chamber can be interpreted as either a failure to associate the context with the aversive stimulus and the incapacity to inhibit their natural tendency to escape in a safer place, or an increase in an impulsive behavior [223].

Table 8. Associative memory performances in passive avoidance task in different rodent models of anxiety/depression.

Type of Task	Model	Species	Gender	Latency to Enter into the Dark Compartment Compared to Controls	Reference
Passive avoidance task	PNS	Rat	♂	Decreased	[139,197]
			♂/♀	Decreased	[140,196,233,234]
	MS	Rat	♂	Decreased	[116,235]
	Social defeat	Mouse	♂	No effect	[238]
	CMS	Rat	♂	Decreased	[223,236]
				No effect	[149]
	CORT		♂	Decreased	[208,237]

To conclude, despite some conflicting results across chronic stress models, preclinical studies appear to replicate some of the associative deficits observed in MDD patients, underlining a propensity for stressed-animals to remember negative stimuli and difficulties to associate aversive events to a specific context. Moreover, alterations in fear extinction remind symptoms observed in PTSD patients.

Further efforts are needed to identify measurable relevant parameters in associative memory behavioral paradigms that can lead to a greater interpretation.

9. Cognitive and Emotional Deficits in Rodent Models of Anxiety/Depression and Their Relationship with Hippocampus Function

9.1. Hippocampal Formation and Its Role in Cognitive/Emotional Deficits

Interestingly, some recent studies revealed that dorsal and ventral poles of the dentate gyrus of the hippocampus were functionally distinct [239,240]. A lesion study supported that the dorsal hippocampus was involved in learning and spatial memory, whereas the ventral hippocampal may regulate emotional and motivated behaviors [239]. Specifically, by using an optogenetic approach, another recent study demonstrated that dorsal granule cells might contribute to spatial and contextual learning (not memory retrieval), while ventral DG cells exert a major effect on anxiety-like behavior [240].

In humans, magnetic resonance imaging (MRI) studies revealed that first-episode MDD patients had smaller hippocampi than healthy subjects and these data correlated to neurocognitive alterations [53]. In addition, hippocampal volume reductions were associated to persistent multiple cognitive impairments at six months in MDD patients [241]. Moreover, MDD patients showed impaired spatial memory during a navigation task [242,243], a task previously shown to reflect hippocampal activation and particularly the neurogenesis process. Further investigations using specialized neuroimaging methods and studies are needed to better understand the crucial role of adult hippocampal neurogenesis in cognitive deficits of MDD context.

9.2. Impact of Neurogenesis on Cognitive and Emotional Function

Adult neurogenesis occurs in two regions of the mammalian brain: the subgranular zone of the dentate gyrus (DG) and the subventricular zone (SVZ). It is now well established that adult neurogenesis process is intimately involved in mood and cognition regulations as well as in antidepressant drug response. Although adult hippocampal neurogenesis was shown to be necessary for antidepressant action in chronic-stress animal models [15,244], altered neurogenesis integrity is not an etiological factor in the apparition of MDD pathology. On the contrary, reduction of adult neurogenesis in rodents can directly affect learning and memory functions. Many recent reviews focused on the role of adult neurogenesis in cognition [245] and particularly in hippocampus-dependent memory formation [246–249]. Despite the availability of a large amount of data, findings about the involvement of neurogenesis in learning and memory processes remain contradictory. For example, some studies demonstrated that adult hippocampal neurogenesis process was required in rodents to perform contextual fear memory [216,217,250,251], fear memory extinction [252] or spatial learning and memory tasks [253–256]. However, other evidences do not support this role of adult neurogenesis in contextual fear conditioning [252,253,257], spatial navigation tasks [216,258–260] or working memory [250]. One possibility to explain such conflicting results could rely on differences in experimental designs impacting the level of difficulty of the training tasks. In other words, adult neurogenesis appears to be specifically required in more challenging and complex learning and memory tasks, such as in the pattern separation process [257,261,262]. Likewise, a study demonstrated that increasing adult hippocampal neurogenesis by enhancing survival of adult-born cells in the DG was sufficient to improve pattern separation performances in naïve mice without displaying any anxiolytic or antidepressant-like effects [263]. However, increasing neurogenesis by deleting the pro-apoptotic gene Bax from neural stem cells and their progeny was able to rescue the higher emotionality in CORT-treated mice [264].

More specifically, various approaches have allowed studying the role of the DG-CA3 circuit in pattern separation. Recently, a study showed that in animal models where neurogenesis is altered (social defeat model) or ablated (x-irradiation), a decrease in reactivation of CA3 but not of DG neurons

priory activated by fear exposure was observed in a time dependent manner [265]. These results are consistent with the proposal that adult-born dentate granule cells are responsible for reactivation of memory traces in CA3, and how mal-adaptive neurogenesis state may participate in fear-generalization and stress response [266]. Overall, these preclinical observation correlates with the impaired pattern separation observed in MDD subjects [267]. Hippocampal neurogenesis inducement in adulthood, by modifying DG-CA3 circuits, may also participate in forgetting and memory clearance, depending on the strength of the initial memory [268], potentially participating to the clinical remission of MDD subjects.

The presence of cognitive alterations in mood disorders, including MDD, strongly suggests that brain structures implicated in emotional and cognitive functions are linked. However, questions of how cognitive and depressive symptoms are related to each other and how to distinguish specific biological mechanisms attributable to cognitive or emotional functions in a MDD context remain opened. Moreover, the fact that most of antidepressant drug treatments failed to treat the cognitive dysfunctions observed in MDD, despite the remission of mood-related symptoms, confirms that independent neural circuitry underlying cognitive functions and depressive symptoms may be engaged.

10. Conclusions

It is now well accepted that patients with depression suffer from associated cognitive dysfunctions, such as negative or distorted thinking, difficulty concentrating, indecisiveness, reduced reaction time or memory loss. However, whether these cognitive deficits are specific to a clinical subtype of MDD in Humans (melancholic or atypical) or to a specific chronic stress procedure in animal models of the disease (pre/peri natal, adulthood or social chronic stress methods) remains to be further determined. The use of translational anxiety/depression animal models will substantially improve our understanding about these cognitive alterations.

In this review, we provided an overview of clinical findings regarding the nature of cognitive dysfunctions in depressed patients from the first episode to recurrence according to the subtype of MDD. Then, we have summarized available data indicating the type of cognitive functions (including attention, executive functions, working memory, spatial memory, episodic-like memory and associative memory) affected in different anxiety/depression animal models.

Clinical evidence indicates an overall cognitive decline in MDD patients persisting even after remission and during recurrent episodes. Among MDD subtypes, melancholic patients were identified to experience more pronounced cognitive deficits, suggesting a different pattern of cognitive impairment from those found in non-melancholic patients. Among all the cognitive alterations, executive and attentional functions deficits seem to be a constant hallmark in MDD patients, since it is present in all stages of the MDD course and in melancholic patients. Through the study of different anxiety/depression animal models, it appears that these executive and attentional deficits are conserved in rodents regardless of the chronic stress procedure. About the other types of memory, results are more heterogeneous according to the chronic stress methods used. This review points out the limit of rodent models, since heterogeneity in the results was observable within models and/or within a same type of memory. Numerous factors such as sex, protocol details, species, strain, environmental conditions, and the nature of the test promote this variability. Besides, animal models cannot fully replicate all of the cognitive deficits observed in human MDD, but rather only reflects different etiologies or aspects of the disease. Overall, among all the suggested models, adulthood-induced chronic stress models (the UCMS and the CORT model for example) seems to show greater similarities with the cognitive symptoms observed in MDD patients, key advantages compared to pre or perinatal chronic stress procedures.

Unfortunately, cognitive condition in MDD patients tends to receive less attention than other depressive-related symptoms, particularly when it comes to consider the therapeutic strategy. Indeed, finding therapeutic strategies that treat both depressive and cognitive-related symptoms remains one of the main challenges in this field. Up to now, clinical reports about the efficacy of

classical antidepressant drugs are relatively inconsistent [46,47,50,51,185]. Recent preclinical results are in line with clinical disparate findings using antidepressant drug therapies, specifically with selective serotonin reuptake inhibitors (see [192] for review). Further investigations, including non-antidepressant and innovative drugs strategies, are needed to determine which treatment could benefit to depression and comorbidities-associated signs.

Acknowledgments: This work was supported by the Brain & Behavior Research Foundation (formerly NARSAD) and the Pierre Deniker Foundation (D.J.D.), by the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche (MENESR, Paris, France) Fellowship (F.D.).

Author Contributions: All authors participated to the writing of the manuscript.

Conflicts of Interest: D.J.D. serves as a consultant for Lundbeck, Roche, and Servier. R.G. has received compensation as a member of the scientific advisory board of Janssen, Lundbeck, Roche, Takeda. He has served as consultant and/or speaker for Astra Zeneca, Pierre Fabre, Lilly, Otsuka, Sanofi, Servier and received compensation, and he has received research support from Servier. The remaining authors declare no conflict of interest.

References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed.; American Psychiatric Publishing: Arlington, VA, USA, 2013.
2. Porter, R.J.; Gallagher, P.; Thompson, J.M.; Young, A.H. Neurocognitive impairment in drug-free patients with major depressive disorder. *Br. J. Psychiatry* **2003**, *182*, 214–220. [CrossRef] [PubMed]
3. Hammar, A.; Ardal, G. Cognitive functioning in major depression—A summary. *Front. Hum. Neurosci.* **2009**, *3*, 26. [CrossRef] [PubMed]
4. Trivedi, M.H.; Greer, T.L. Cognitive dysfunction in unipolar depression: Implications for treatment. *J. Affect. Disord.* **2014**, *152–154*, 19–27. [CrossRef] [PubMed]
5. Hasselbalch, B.J.; Knorr, U.; Kessing, L.V. Cognitive impairment in the remitted state of unipolar depressive disorder: A systematic review. *J. Affect. Disord.* **2011**, *134*, 20–31. [CrossRef] [PubMed]
6. Snyder, H.R. Major depressive disorder is associated with broad impairments on neuropsychological measures of executive function: A meta-analysis and review. *Psychol. Bull.* **2013**, *139*, 81–132. [CrossRef] [PubMed]
7. Gonda, X.; Pompili, M.; Serafini, G.; Carvalho, A.F.; Rihmer, Z.; Dome, P. The role of cognitive dysfunction in the symptoms and remission from depression. *Ann. Gen. Psychiatry* **2015**, *14*, 27. [CrossRef] [PubMed]
8. Gershon, A.; Hayward, C.; Schraedley-Desmond, P.; Rudolph, K.D.; Booster, G.D.; Gotlib, I.H. Life stress and first onset of psychiatric disorders in daughters of depressed mothers. *J. Psychiatr. Res.* **2011**, *45*, 855–862. [CrossRef] [PubMed]
9. Hammen, C.; Hazel, N.A.; Brennan, P.A.; Najman, J. Intergenerational transmission and continuity of stress and depression: Depressed women and their offspring in 20 years of follow-up. *Psychol. Med.* **2012**, *42*, 931–942. [CrossRef] [PubMed]
10. Nishi, M.; Horii-Hayashi, N.; Sasagawa, T. Effects of early life adverse experiences on the brain: Implications from maternal separation models in rodents. *Front. Neurosci.* **2014**, *8*, 166. [CrossRef] [PubMed]
11. Richman, C.L.; Dember, W.N.; Kim, P. Spontaneous alternation behavior in animals: A review. *Curr. Psychol.* **1986**, *5*, 358–391. [CrossRef]
12. Blanchard, R.J.; McKittrick, C.R.; Blanchard, D.C. Animal models of social stress: Effects on behavior and brain neurochemical systems. *Physiol. Behav.* **2001**, *73*, 261–271. [CrossRef]
13. Chourbaji, S.; Zacher, C.; Sanchis-Segura, C.; Dormann, C.; Vollmayr, B.; Gass, P. Learned helplessness: Validity and reliability of depressive-like states in mice. *Brain Res. Brain Res. Protoc.* **2005**, *16*, 70–78. [CrossRef] [PubMed]
14. Willner, P. Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology* **2005**, *52*, 90–110. [CrossRef] [PubMed]
15. David, D.J.; Samuels, B.A.; Rainer, Q.; Wang, J.W.; Marsteller, D.; Mendez, I.; Drew, M.; Craig, D.A.; Guiard, B.P.; Guilloux, J.P.; *et al*. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* **2009**, *62*, 479–493. [CrossRef] [PubMed]

16. Belzung, C.; Lemoine, M. Criteria of validity for animal models of psychiatric disorders: Focus on anxiety disorders and depression. *Biol. Mood Anxiety Disord.* **2011**, *1*, 9. [CrossRef] [PubMed]
17. Willner, P. The validity of animal models of depression. *Psychopharmacology* **1984**, *83*, 1–16. [CrossRef] [PubMed]
18. Marazziti, D.; Consoli, G.; Picchetti, M.; Carlini, M.; Faravelli, L. Cognitive impairment in major depression. *Eur. J. Pharmacol.* **2010**, *626*, 83–86. [CrossRef] [PubMed]
19. Lee, R.S.; Hermens, D.F.; Porter, M.A.; Redoblado-Hodge, M.A. A meta-analysis of cognitive deficits in first-episode major depressive disorder. *J. Affect. Disord.* **2012**, *140*, 113–124. [CrossRef] [PubMed]
20. Murrough, J.W.; Iacoviello, B.; Neumeister, A.; Charney, D.S.; Iosifescu, D.V. Cognitive dysfunction in depression: Neurocircuitry and new therapeutic strategies. *Neurobiol. Learn. Mem.* **2011**, *96*, 553–563. [CrossRef] [PubMed]
21. Millan, M.J.; Agid, Y.; Brune, M.; Bullmore, E.T.; Carter, C.S.; Clayton, N.S.; Connor, R.; Davis, S.; Deakin, B.; DeRubeis, R.J.; *et al*. Cognitive dysfunction in psychiatric disorders: Characteristics, causes and the quest for improved therapy. *Nat. Rev. Drug Discov.* **2012**, *11*, 141–168. [CrossRef] [PubMed]
22. Papakostas, G.I. Cognitive symptoms in patients with major depressive disorder and their implications for clinical practice. *J. Clin. Psychiatry* **2014**, *75*, 8–14. [CrossRef] [PubMed]
23. Pardo, J.V.; Pardo, P.J.; Humes, S.W.; I Posner, M. Neurocognitive dysfunction in antidepressant-free, non-elderly patients with unipolar depression: Alerting and covert orienting of visuospatial attention. *J. Affect. Disord.* **2006**, *92*, 71–78. [CrossRef] [PubMed]
24. Godard, J.; Grondin, S.; Baruch, P.; Lafleur, M.F. Psychosocial and neurocognitive profiles in depressed patients with major depressive disorder and bipolar disorder. *Psychiatry Res.* **2011**, *190*, 244–252. [CrossRef] [PubMed]
25. Gualtieri, C.T. A practical approach to objective attention deficit/hyperactivity disorder diagnosis and management. *Psychiatry* **2005**, *2*, 16–25. [PubMed]
26. Fossati, P.; Ergis, A.M.; Allilaire, J.F. Executive functioning in unipolar depression: A review. *L'Encephale* **2002**, *28*, 97–107. [PubMed]
27. Gohier, B.; Ferracci, L.; Surguladze, S.A.; Lawrence, E.; El Hage, W.; Kefi, M.Z.; Allain, P.; Garre, J.B.; Le Gall, D. Cognitive inhibition and working memory in unipolar depression. *J. Affect. Disord.* **2009**, *116*, 100–105. [CrossRef] [PubMed]
28. Cella, M.; Dymond, S.; Cooper, A. Impaired flexible decision-making in major depressive disorder. *J. Affect. Disord.* **2010**, *124*, 207–210. [CrossRef] [PubMed]
29. Gruber, O.; Zilles, D.; Kennel, J.; Gruber, E.; Falkai, P. A systematic experimental neuropsychological investigation of the functional integrity of working memory circuits in major depression. *Eur. Arch. Psychiatry Clin. Neurosci.* **2011**, *261*, 179–184. [CrossRef] [PubMed]
30. Borbely-Ipkovich, E.; Janacsek, K.; Nemeth, D.; Gonda, X. The effect of negative mood and major depressive episode on working memory and implicit learning. *Neuropsychopharmacol. Hung.* **2014**, *16*, 29–42. [PubMed]
31. Schmid, M.; Hammar, A. A follow-up study of first episode major depressive disorder. Impairment in inhibition and semantic fluency-potential predictors for relapse? *Front. Psychol.* **2013**, *4*, 633. [CrossRef] [PubMed]
32. Foland-Ross, L.C.; Gotlib, I.H. Cognitive and neural aspects of information processing in major depressive disorder: An integrative perspective. *Front. Psychol.* **2012**, *3*, 489. [CrossRef] [PubMed]
33. Castaneda, A.E.; Tuulio-Henriksson, A.; Marttunen, M.; Suvisaari, J.; Lonnqvist, J. A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *J. Affect. Disord.* **2008**, *106*, 1–27. [CrossRef] [PubMed]
34. Best, J.R.; Miller, P.H.; Naglieri, J.A. Relations between executive function and academic achievement from ages 5 to 17 in a large, representative national sample. *Learn. Individ. Differ.* **2011**, *21*, 327–336. [CrossRef] [PubMed]
35. Vilgis, V.; Silk, T.J.; Vance, A. Executive function and attention in children and adolescents with depressive disorders: A systematic review. *Eur. Child Adolesc. Psychiatry* **2015**, *24*, 365–384. [CrossRef] [PubMed]
36. Baune, B.T.; Fuhr, M.; Air, T.; Hering, C. Neuropsychological functioning in adolescents and young adults with major depressive disorder—a review. *Psychiatry Res.* **2014**, *218*, 261–271. [CrossRef] [PubMed]

37. Nebes, R.D.; Butters, M.A.; Mulsant, B.H.; Pollock, B.G.; Zmuda, M.D.; Houck, P.R.; Reynolds, C.F., 3rd. Decreased working memory and processing speed mediate cognitive impairment in geriatric depression. *Psychol. Med.* **2000**, *30*, 679–691. [CrossRef] [PubMed]
38. Baudic, S.; Tzortzis, C.; Barba, G.D.; Traykov, L. Executive deficits in elderly patients with major unipolar depression. *J. Geriatr. Psychiatry Neurol.* **2004**, *17*, 195–201. [CrossRef] [PubMed]
39. Sheline, Y.I.; Barch, D.M.; Garcia, K.; Gersing, K.; Pieper, C.; Welsh-Bohmer, K.; Steffens, D.C.; Doraiswamy, P.M. Cognitive function in late life depression: Relationships to depression severity, cerebrovascular risk factors and processing speed. *Biol. Psychiatry* **2006**, *60*, 58–65. [CrossRef] [PubMed]
40. Koenig, A.M.; Butters, M.A. Cognition in late life depression: Treatment considerations. *Curr. Treat. Options Psychiatry* **2014**, *1*, 1–14. [CrossRef] [PubMed]
41. Russo, M.; Mahon, K.; Burdick, K.E. Measuring cognitive function in MDD: Emerging assessment tools. *Depression Anxiety* **2015**, *32*, 262–269. [CrossRef] [PubMed]
42. Papakostas, G.I. Antidepressants and their effect on cognition in major depressive disorder. *J. Clin. Psychiatry* **2015**, *76*, e1046. [CrossRef] [PubMed]
43. El Hammi, E.; Samp, J.; Remuzat, C.; Auray, J.P.; Lamure, M.; Aballea, S.; Kooli, A.; Akhras, K.; Toumi, M. Difference of perceptions and evaluation of cognitive dysfunction in major depressive disorder patients across psychiatrists internationally. *Ther. Adv. Psychopharmacol.* **2014**, *4*, 22–29. [CrossRef] [PubMed]
44. Bakkour, N.; Samp, J.; Akhras, K.; El Hammi, E.; Soussi, I.; Zahra, F.; Duru, G.; Kooli, A.; Toumi, M. Systematic review of appropriate cognitive assessment instruments used in clinical trials of schizophrenia, major depressive disorder and bipolar disorder. *Psychiatry Res.* **2014**, *216*, 291–302. [CrossRef] [PubMed]
45. Lim, J.; Oh, I.K.; Han, C.; Huh, Y.J.; Jung, I.K.; Patkar, A.A.; Steffens, D.C.; Jang, B.H. Sensitivity of cognitive tests in four cognitive domains in discriminating mdd patients from healthy controls: A meta-analysis. *Int. Psychogeriatr.* **2013**, *25*, 1543–1557. [CrossRef] [PubMed]
46. Gallassi, R.; Di Sarro, R.; Morreale, A.; Amore, M. Memory impairment in patients with late-onset major depression: The effect of antidepressant therapy. *J. Affect. Disord.* **2006**, *91*, 243–250. [CrossRef] [PubMed]
47. Herrera-Guzman, I.; Gudayol-Ferre, E.; Herrera-Abarca, J.E.; Herrera-Guzman, D.; Montelongo-Pedraza, P.; Padros Blazquez, F.; Pero-Cebollero, M.; Guardia-Olmos, J. Major depressive disorder in recovery and neuropsychological functioning: Effects of selective serotonin reuptake inhibitor and dual inhibitor depression treatments on residual cognitive deficits in patients with major depressive disorder in recovery. *J. Affect. Disord.* **2010**, *123*, 341–350. [CrossRef] [PubMed]
48. Diaconescu, A.O.; Kramer, E.; Hermann, C.; Ma, Y.; Dhawan, V.; Chaly, T.; Eidelberg, D.; McIntosh, A.R.; Smith, G.S. Distinct functional networks associated with improvement of affective symptoms and cognitive function during citalopram treatment in geriatric depression. *Hum. Brain Mapp.* **2011**, *32*, 1677–1691. [CrossRef] [PubMed]
49. Culang, M.E.; Sneed, J.R.; Keilp, J.G.; Rutherford, B.R.; Pelton, G.H.; Devanand, D.P.; Roose, S.P. Change in cognitive functioning following acute antidepressant treatment in late-life depression. *Am. J. Geriatr. Psychiatry* **2009**, *17*, 881–888. [CrossRef] [PubMed]
50. Ferguson, J.M.; Wesnes, K.A.; Schwartz, G.E. Reboxetine *versus* paroxetine *versus* placebo: Effects on cognitive functioning in depressed patients. *Int. Clin. Psychopharmacol.* **2003**, *18*, 9–14. [CrossRef] [PubMed]
51. Bastos, A.G.; Guimaraes, L.S.; Trentini, C.M. Neurocognitive changes in depressed patients in psychodynamic psychotherapy, therapy with fluoxetine and combination therapy. *J. Affect. Disord.* **2013**, *151*, 1066–1075. [CrossRef] [PubMed]
52. Gudayol-Ferre, E.; Guardia-Olmos, J.; Pero-Cebollero, M. Effects of remission speed and improvement of cognitive functions of depressed patients. *Psychiatry Res.* **2015**, *226*, 103–112. [CrossRef] [PubMed]
53. Kaymak, S.U.; Demir, B.; Senturk, S.; Tatar, I.; Aldur, M.M.; Ulug, B. Hippocampus, glucocorticoids and neurocognitive functions in patients with first-episode major depressive disorders. *Eur. Arch. Psychiatry Clin. Neurosci.* **2010**, *260*, 217–223. [CrossRef] [PubMed]
54. Hasselbalch, B.J. Cognition and neuroplasticity in the remitted state of unipolar depressive disorder. *Dan. Med. J.* **2015**, *62*, B5080. [PubMed]
55. Burcusa, S.L.; Iacono, W.G. Risk for recurrence in depression. *Clin. Psychol. Rev.* **2007**, *27*, 959–985. [CrossRef] [PubMed]
56. Galecki, P.; Talarowska, M.; Anderson, G.; Berk, M.; Maes, M. Mechanisms underlying neurocognitive dysfunctions in recurrent major depression. *Med. Sci. Monit.* **2015**, *21*, 1535–1547. [PubMed]

57. Lampe, I.K.; Sitskoorn, M.M.; Heeren, T.J. Effects of recurrent major depressive disorder on behavior and cognitive function in female depressed patients. *Psychiatry Res.* **2004**, *125*, 73–79. [CrossRef] [PubMed]
58. Talarowska, M.; Zajaczkowska, M.; Galecki, P. Cognitive functions in first-episode depression and recurrent depressive disorder. *Psychiatria Danubina* **2015**, *27*, 38–43. [PubMed]
59. Karabekiroglu, A.; Topcuoglu, V.; Gimzal Gonentur, A.; Karabekiroglu, K. Executive function differences between first episode and recurrent major depression patients. *Turk Psikiyatri Derg.* **2010**, *21*, 280–288. [PubMed]
60. Chen, J.; Ma, W.; Zhang, Y.; Wu, X.; Wei, D.; Liu, G.; Deng, Z.; Zhang, Z.; Yang, L. Distinct facial processing related negative cognitive bias in first-episode and recurrent major depression: Evidence from the n170 erp component. *PLoS ONE* **2014**, *9*, e109176. [CrossRef] [PubMed]
61. Caldieraro, M.A.; Baeza, F.L.; Pinheiro, D.O.; Ribeiro, M.R.; Parker, G.; Fleck, M.P. Clinical differences between melancholic and nonmelancholic depression as defined by the core system. *Compr. Psychiatry* **2013**, *54*, 11–15. [CrossRef] [PubMed]
62. Roca, M.; Monzon, S.; Vives, M.; Lopez-Navarro, E.; Garcia-Toro, M.; Vicens, C.; Garcia-Campayo, J.; Harrison, J.; Gili, M. Cognitive function after clinical remission in patients with melancholic and non-melancholic depression: A 6 month follow-up study. *J. Affect. Disord.* **2015**, *171*, 85–92. [CrossRef] [PubMed]
63. Lin, K.; Xu, G.; Lu, W.; Ouyang, H.; Dang, Y.; Lorenzo-Seva, U.; Guo, Y.; Bessonov, D.; Akiskal, H.S.; So, K.F.; *et al*. Neuropsychological performance in melancholic, atypical and undifferentiated major depression during depressed and remitted states: A prospective longitudinal study. *J. Affect. Disord.* **2014**, *168*, 184–191. [CrossRef] [PubMed]
64. Lamers, F.; Vogelzangs, N.; Merikangas, K.R.; de Jonge, P.; Beekman, A.T.; Penninx, B.W. Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic *versus* atypical depression. *Mol. Psychiatry* **2013**, *18*, 692–699. [CrossRef] [PubMed]
65. Antonijevic, I. HPA axis and sleep: Identifying subtypes of major depression. *Stress* **2008**, *11*, 15–27. [CrossRef] [PubMed]
66. Parker, G. Defining melancholia: The primacy of psychomotor disturbance. *Acta Psychiatr. Scand. Suppl.* **2007**, *115*, 21–30. [CrossRef] [PubMed]
67. Pier, M.P.; Hulstijn, W.; Sabbe, B.G. Differential patterns of psychomotor functioning in unmedicated melancholic and nonmelancholic depressed patients. *J. Psychiatr. Res.* **2004**, *38*, 425–435. [CrossRef] [PubMed]
68. Rogers, M.A.; Bellgrove, M.A.; Chiu, E.; Mileshkin, C.; Bradshaw, J.L. Response selection deficits in melancholic but not nonmelancholic unipolar major depression. *J. Clin. Exp. Neuropsychol.* **2004**, *26*, 169–179. [CrossRef] [PubMed]
69. Michopoulos, I.; Zervas, I.M.; Papakosta, V.M.; Tsaltas, E.; Papageorgiou, C.; Manessi, T.; Papakostas, Y.G.; Lykouras, L.; Soldatos, C.R. Set shifting deficits in melancholic *vs.* Non-melancholic depression: Preliminary findings. *Eur. Psychiatry* **2006**, *21*, 361–363. [CrossRef] [PubMed]
70. Michopoulos, I.; Zervas, I.M.; Pantelis, C.; Tsaltas, E.; Papakosta, V.M.; Boufidou, F.; Nikolaou, C.; Papageorgiou, C.; Soldatos, C.R.; Lykouras, L. Neuropsychological and hypothalamic-pituitary-axis function in female patients with melancholic and non-melancholic depression. *Eur. Arch. Psychiatry Clin. Neurosci.* **2008**, *258*, 217–225. [CrossRef] [PubMed]
71. Exner, C.; Lange, C.; Irle, E. Impaired implicit learning and reduced pre-supplementary motor cortex size in early-onset major depression with melancholic features. *J. Affect. Disord.* **2009**, *119*, 156–162. [CrossRef] [PubMed]
72. Withall, A.; Harris, L.M.; Cumming, S.R. A longitudinal study of cognitive function in melancholic and non-melancholic subtypes of major depressive disorder. *J. Affect. Disord.* **2010**, *123*, 150–157. [CrossRef] [PubMed]
73. Linden, S.C.; Jackson, M.C.; Subramanian, L.; Healy, D.; Linden, D.E. Sad benefit in face working memory: An emotional bias of melancholic depression. *J. Affect. Disord.* **2011**, *135*, 251–257. [CrossRef] [PubMed]
74. Quinn, C.R.; Harris, A.; Felmingham, K.; Boyce, P.; Kemp, A. The impact of depression heterogeneity on cognitive control in major depressive disorder. *Aust. N. Z. J. Psychiatry* **2012**, *46*, 1079–1088. [CrossRef] [PubMed]

75. Day, C.V.; Gatt, J.M.; Etkin, A.; DeBattista, C.; Schatzberg, A.F.; Williams, L.M. Cognitive and emotional biomarkers of melancholic depression: An ispot-d report. *J. Affect. Disord.* **2015**, *176*, 141–150. [CrossRef] [PubMed]
76. Austin, M.P.; Mitchell, P.; Hadzi-Pavlovic, D.; Hickie, I.; Parker, G.; Chan, J.; Eyers, K. Effect of apomorphine on motor and cognitive function in melancholic patients: A preliminary report. *Psychiatry Res.* **2000**, *97*, 207–215. [CrossRef]
77. Romero, N.; Sanchez, A.; Vazquez, C. Memory biases in remitted depression: The role of negative cognitions at explicit and automatic processing levels. *J. Behav. Ther. Exp. Psychiatry* **2014**, *45*, 128–135. [CrossRef] [PubMed]
78. Behnken, A.; Schoning, S.; Gerss, J.; Konrad, C.; de Jong-Meyer, R.; Zwanzger, P.; Arolt, V. Persistent non-verbal memory impairment in remitted major depression—Caused by encoding deficits? *J. Affect. Disord.* **2010**, *122*, 144–148. [CrossRef] [PubMed]
79. Munkler, P.; Rothkirch, M.; Dalati, Y.; Schmack, K.; Sterzer, P. Biased recognition of facial affect in patients with major depressive disorder reflects clinical state. *PLoS ONE* **2015**, *10*, e0129863. [CrossRef] [PubMed]
80. Arnow, B.A.; Blasey, C.; Williams, L.M.; Palmer, D.M.; Rekshan, W.; Schatzberg, A.F.; Etkin, A.; Kulkarni, J.; Luther, J.F.; Rush, A.J. Depression subtypes in predicting antidepressant response: A report from the iSPOT-D trial. *Am. J. Psychiatry* **2015**, *172*, 743–750. [CrossRef] [PubMed]
81. Hofmann, S.G. Toward a cognitive-behavioral classification system for mental disorders. *Behav. Ther.* **2014**, *45*, 576–587. [CrossRef] [PubMed]
82. Whitton, A.E.; Treadway, M.T.; Pizzagalli, D.A. Reward processing dysfunction in major depression, bipolar disorder and schizophrenia. *Curr. Opin. Psychiatry* **2015**, *28*, 7–12. [CrossRef] [PubMed]
83. Eshel, N.; Roiser, J.P. Reward and punishment processing in depression. *Biol. Psychiatry* **2010**, *68*, 118–124. [CrossRef] [PubMed]
84. Liu, W.H.; Wang, L.Z.; Shang, H.R.; Shen, Y.; Li, Z.; Cheung, E.F.; Chan, R.C. The influence of anhedonia on feedback negativity in major depressive disorder. *Neuropsychologia* **2014**, *53*, 213–220. [CrossRef] [PubMed]
85. Vrieze, E.; Pizzagalli, D.A.; Demyttenaere, K.; Hompes, T.; Sienaert, P.; de Boer, P.; Schmidt, M.; Claes, S. Reduced reward learning predicts outcome in major depressive disorder. *Biol. Psychiatry* **2013**, *73*, 639–645. [CrossRef] [PubMed]
86. Treadway, M.T.; Zald, D.H. Reconsidering anhedonia in depression: Lessons from translational neuroscience. *Neurosci. Biobehav. Rev.* **2011**, *35*, 537–555. [CrossRef] [PubMed]
87. Olausson, P.; Kiraly, D.D.; Gourley, S.L.; Taylor, J.R. Persistent effects of prior chronic exposure to corticosterone on reward-related learning and motivation in rodents. *Psychopharmacology* **2013**, *225*, 569–577. [CrossRef] [PubMed]
88. Insel, T.; Cuthbert, B.; Garvey, M.; Heinssen, R.; Pine, D.S.; Quinn, K.; Sanislow, C.; Wang, P. Research domain criteria (RDOC): Toward a new classification framework for research on mental disorders. *Am. J. Psychiatry* **2010**, *167*, 748–751. [CrossRef] [PubMed]
89. Moore, H.; Geyer, M.A.; Carter, C.S.; Barch, D.M. Harnessing cognitive neuroscience to develop new treatments for improving cognition in schizophrenia: Cntrics selected cognitive paradigms for animal models. *Neurosci. Biobehav. Rev.* **2013**, *37*, 2087–2091. [CrossRef] [PubMed]
90. Young, J.W.; Powell, S.B.; Risbrough, V.; Marston, H.M.; Geyer, M.A. Using the matrics to guide development of a preclinical cognitive test battery for research in schizophrenia. *Pharmacol. Ther.* **2009**, *122*, 150–202. [CrossRef] [PubMed]
91. Wallace, T.L.; Ballard, T.M.; Glavis-Bloom, C. Animal paradigms to assess cognition with translation to humans. *Handb. Exp. Pharmacol.* **2015**, *228*, 27–57. [PubMed]
92. Oomen, C.A.; Hvoslef-Eide, M.; Heath, C.J.; Mar, A.C.; Horner, A.E.; Bussey, T.J.; Saksida, L.M. The touchscreen operant platform for testing working memory and pattern separation in rats and mice. *Nat. Protoc.* **2013**, *8*, 2006–2021. [CrossRef] [PubMed]
93. Williams, J.M.; Barnhofer, T.; Crane, C.; Herman, D.; Raes, F.; Watkins, E.; Dalgleish, T. Autobiographical memory specificity and emotional disorder. *Psychol. Bull.* **2007**, *133*, 122–148. [CrossRef] [PubMed]
94. Soderlund, H.; Moscovitch, M.; Kumar, N.; Daskalakis, Z.J.; Flint, A.; Herrmann, N.; Levine, B. Autobiographical episodic memory in major depressive disorder. *J. Abnorm. Psychol.* **2014**, *123*, 51–60. [CrossRef] [PubMed]

95. Sumner, J.A.; Griffith, J.W.; Mineka, S. Overgeneral autobiographical memory as a predictor of the course of depression: A meta-analysis. *Behav. Res. Ther.* **2010**, *48*, 614–625. [CrossRef] [PubMed]
96. Nandrino, J.L.; Pezard, L.; Poste, A.; Reveillere, C.; Beaune, D. Autobiographical memory in major depression: A comparison between first-episode and recurrent patients. *Psychopathology* **2002**, *35*, 335–340. [CrossRef] [PubMed]
97. Young, K.D.; Bellgowan, P.S.; Bodurka, J.; Drevets, W.C. Neurophysiological correlates of autobiographical memory deficits in currently and formerly depressed subjects. *Psychol. Med.* **2014**, *44*, 2951–2963. [CrossRef] [PubMed]
98. Woody, M.L.; Burkhouse, K.L.; Gibb, B.E. Overgeneral autobiographical memory in children of depressed mothers. *Cogn. Emot.* **2015**, *29*, 130–137. [CrossRef] [PubMed]
99. Leger, M.; Quiedeville, A.; Bouet, V.; Haelewyn, B.; Boulouard, M.; Schumann-Bard, P.; Freret, T. Object recognition test in mice. *Nat. Protoc.* **2013**, *8*, 2531–2537. [CrossRef] [PubMed]
100. Gronli, J.; Murison, R.; Fiske, E.; Bjorvatn, B.; Sorensen, E.; Portas, C.M.; Ursin, R. Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiol. Behav.* **2005**, *84*, 571–577. [CrossRef] [PubMed]
101. Kleen, J.K.; Sitomer, M.T.; Killeen, P.R.; Conrad, C.D. Chronic stress impairs spatial memory and motivation for reward without disrupting motor ability and motivation to explore. *Behav. Neurosci.* **2006**, *120*, 842–851. [CrossRef] [PubMed]
102. Darcet, F.; Mendez-David, I.; Tritschler, L.; Gardier, A.M.; Guilloux, J.P.; David, D.J. Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Front. Behav. Neurosci.* **2014**, *8*, 136. [CrossRef] [PubMed]
103. Ennaceur, A.; Delacour, J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav. Brain Res.* **1988**, *31*, 47–59. [CrossRef]
104. Antunes, M.; Biala, G. The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cogn. Process* **2012**, *13*, 93–110. [CrossRef] [PubMed]
105. Abdul Aziz, N.H.; Kendall, D.A.; Pardon, M.C. Prenatal exposure to chronic mild stress increases corticosterone levels in the amniotic fluid and induces cognitive deficits in female offspring, improved by treatment with the antidepressant drug amitriptyline. *Behav. Brain Res.* **2012**, *231*, 29–39. [CrossRef] [PubMed]
106. Markham, J.A.; Taylor, A.R.; Taylor, S.B.; Bell, D.B.; Koenig, J.I. Characterization of the cognitive impairments induced by prenatal exposure to stress in the rat. *Front. Behav. Neurosci.* **2010**, *4*, 173. [CrossRef] [PubMed]
107. Ehrlich, D.E.; Neigh, G.N.; Bourke, C.H.; Nemeth, C.L.; Hazra, R.; Ryan, S.J.; Rowson, S.; Jairam, N.; Sholar, C.A.; Rainnie, D.G.; *et al*. Prenatal stress, regardless of concurrent escitalopram treatment, alters behavior and amygdala gene expression of adolescent female rats. *Neuropharmacology* **2015**, *97*, 251–258. [CrossRef] [PubMed]
108. Schulz, K.M.; Pearson, J.N.; Neeley, E.W.; Berger, R.; Leonard, S.; Adams, C.E.; Stevens, K.E. Maternal stress during pregnancy causes sex-specific alterations in offspring memory performance, social interactions, indices of anxiety, and body mass. *Physiol. Behav.* **2011**, *104*, 340–347. [CrossRef] [PubMed]
109. Wilson, C.A.; Terry, A.V., Jr. Variable maternal stress in rats alters locomotor activity, social behavior, and recognition memory in the adult offspring. *Pharmacol. Biochem. Behav.* **2013**, *104*, 47–61. [CrossRef] [PubMed]
110. Said, N.; Lakehayli, S.; El Khachibi, M.; El Ouahli, M.; Nadifi, S.; Hakkou, F.; Tazi, A. Effect of prenatal stress on memory, nicotine withdrawal and 5ht1a expression in raphe nuclei of adult rats. *Int. J. Dev. Neurosci.* **2015**, *43*, 92–98. [CrossRef] [PubMed]
111. Wang, L.; Jiao, J.; Dulawa, S.C. Infant maternal separation impairs adult cognitive performance in BALB/cJ mice. *Psychopharmacology* **2011**, *216*, 207–218. [CrossRef] [PubMed]
112. Niwa, M.; Matsumoto, Y.; Mouri, A.; Ozaki, N.; Nabeshima, T. Vulnerability in early life to changes in the rearing environment plays a crucial role in the aetiopathology of psychiatric disorders. *Int. J. Neuropsychopharmacol.* **2011**, *14*, 459–477. [CrossRef] [PubMed]
113. Aisa, B.; Tordera, R.; Lasheras, B.; Del Rio, J.; Ramirez, M.J. Cognitive impairment associated to hpa axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology* **2007**, *32*, 256–266. [CrossRef] [PubMed]

114. Salomon, S.; Bejar, C.; Schorer-Apelbaum, D.; Weinstock, M. Corticosterone mediates some but not other behavioural changes induced by prenatal stress in rats. *J. Neuroendocrinol.* **2011**, *23*, 118–128. [CrossRef] [PubMed]
115. Plescia, F.; Marino, R.A.; Navarra, M.; Gambino, G.; Brancato, A.; Sardo, P.; Cannizzaro, C. Early handling effect on female rat spatial and non-spatial learning and memory. *Behav. Process.* **2014**, *103*, 9–16. [CrossRef] [PubMed]
116. Vivinetto, A.L.; Suarez, M.M.; Rivarola, M.A. Neurobiological effects of neonatal maternal separation and post-weaning environmental enrichment. *Behav. Brain Res.* **2013**, *240*, 110–118. [CrossRef] [PubMed]
117. Makena, N.; Bugarith, K.; Russell, V.A. Maternal separation enhances object location memory and prevents exercise-induced mapk/erk signalling in adult sprague-dawley rats. *Metab. Brain Dis.* **2012**, *27*, 377–385. [CrossRef] [PubMed]
118. Hill, R.A.; Klug, M.; Kiss Von Soly, S.; Binder, M.D.; Hannan, A.J.; van den Buuse, M. Sex-specific disruptions in spatial memory and anhedonia in a "two hit" rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling. *Hippocampus* **2014**, *24*, 1197–1211. [CrossRef] [PubMed]
119. Marco, E.M.; Valero, M.; de la Serna, O.; Aisa, B.; Borcel, E.; Ramirez, M.J.; Viveros, M.P. Maternal deprivation effects on brain plasticity and recognition memory in adolescent male and female rats. *Neuropharmacology* **2013**, *68*, 223–231. [CrossRef] [PubMed]
120. Llorente-Berzal, A.; Mela, V.; Borcel, E.; Valero, M.; Lopez-Gallardo, M.; Viveros, M.P.; Marco, E.M. Neurobehavioral and metabolic long-term consequences of neonatal maternal deprivation stress and adolescent olanzapine treatment in male and female rats. *Neuropharmacology* **2012**, *62*, 1332–1341. [CrossRef] [PubMed]
121. Naninck, E.F.; Hoeijmakers, L.; Kakava-Georgiadou, N.; Meesters, A.; Lazic, S.E.; Lucassen, P.J.; Korosi, A. Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice. *Hippocampus* **2015**, *25*, 309–328. [CrossRef] [PubMed]
122. Jin, H.M.; Shrestha Muna, S.; Bagalkot, T.R.; Cui, Y.; Yadav, B.K.; Chung, Y.C. The effects of social defeat on behavior and dopaminergic markers in mice. *Neuroscience* **2015**, *288*, 167–177. [CrossRef] [PubMed]
123. Gumuslu, E.; Mutlu, O.; Sunnetci, D.; Ulak, G.; Celikyurt, I.K.; Cine, N.; Akar, F.; Savli, H.; Erden, F. The antidepressant agomelatine improves memory deterioration and upregulates creb and bdnf gene expression levels in unpredictable chronic mild stress (UCMS)-exposed mice. *Drug Target Insights* **2014**, *8*, 11–21. [PubMed]
124. Haridas, S.; Kumar, M.; Manda, K. Melatonin ameliorates chronic mild stress induced behavioral dysfunctions in mice. *Physiol. Behav.* **2013**, *119*, 201–207. [CrossRef] [PubMed]
125. Elizalde, N.; Gil-Bea, F.J.; Ramirez, M.J.; Aisa, B.; Lasheras, B.; Del Rio, J.; Tordera, R.M. Long-lasting behavioral effects and recognition memory deficit induced by chronic mild stress in mice: Effect of antidepressant treatment. *Psychopharmacology* **2008**, *199*, 1–14. [CrossRef] [PubMed]
126. Li, S.; Wang, C.; Wang, W.; Dong, H.; Hou, P.; Tang, Y. Chronic mild stress impairs cognition in mice: From brain homeostasis to behavior. *Life Sci.* **2008**, *82*, 934–942. [CrossRef] [PubMed]
127. Orsetti, M.; Colella, L.; Dellarole, A.; Canonico, P.L.; Ghi, P. Modification of spatial recognition memory and object discrimination after chronic administration of haloperidol, amitriptyline, sodium valproate or olanzapine in normal and anhedonic rats. *Int. J. Neuropsychopharmacol.* **2007**, *10*, 345–357. [PubMed]
128. Liu, D.; Wang, Z.; Gao, Z.; Xie, K.; Zhang, Q.; Jiang, H.; Pang, Q. Effects of curcumin on learning and memory deficits, bdnf, and erk protein expression in rats exposed to chronic unpredictable stress. *Behav. Brain Res.* **2014**, *271*, 116–121. [CrossRef] [PubMed]
129. Dobarro, M.; Orejana, L.; Aguirre, N.; Ramirez, M.J. Propranolol reduces cognitive deficits, amyloid beta levels, tau phosphorylation and insulin resistance in response to chronic corticosterone administration. *Int. J. Neuropsychopharmacol.* **2013**, *16*, 1351–1360. [CrossRef] [PubMed]
130. Solas, M.; Gerenu, G.; Gil-Bea, F.J.; Ramirez, M.J. Mineralocorticoid receptor activation induces insulin resistance through c-Jun *n*-terminal kinases in response to chronic corticosterone: Cognitive implications. *J. Neuroendocrinol.* **2013**, *25*, 350–356. [CrossRef] [PubMed]
131. Muhammad, A.; Kolb, B. Mild prenatal stress-modulated behavior and neuronal spine density without affecting amphetamine sensitization. *Dev. Neurosci.* **2011**, *33*, 85–98. [CrossRef] [PubMed]

132. Baudin, A.; Blot, K.; Verney, C.; Estevez, L.; Santamaria, J.; Gressens, P.; Giros, B.; Otani, S.; Dauge, V.; Naudon, L. Maternal deprivation induces deficits in temporal memory and cognitive flexibility and exaggerates synaptic plasticity in the rat medial prefrontal cortex. *Neurobiol. Learn. Mem.* **2012**, *98*, 207–214. [CrossRef] [PubMed]
133. Grace, L.; Hescham, S.; Kellaway, L.A.; Bugarith, K.; Russell, V.A. Effect of exercise on learning and memory in a rat model of developmental stress. *Metab. Brain Dis.* **2009**, *24*, 643–657. [CrossRef] [PubMed]
134. Frankola, K.A.; Flora, A.L.; Torres, A.K.; Grissom, E.M.; Overstreet, S.; Dohanich, G.P. Effects of early rearing conditions on cognitive performance in prepubescent male and female rats. *Neurobiol. Learn. Mem.* **2010**, *94*, 91–99. [CrossRef] [PubMed]
135. Macri, S.; Chiarotti, F.; Wurbel, H. Maternal separation and maternal care act independently on the development of hpa responses in male rats. *Behav. Brain Res.* **2008**, *191*, 227–234. [CrossRef] [PubMed]
136. Russo, S.J.; Murrough, J.W.; Han, M.H.; Charney, D.S.; Nestler, E.J. Neurobiology of resilience. *Nat. Neurosci.* **2012**, *15*, 1475–1484. [CrossRef] [PubMed]
137. Dudchenko, P.A. An overview of the tasks used to test working memory in rodents. *Neurosci. Biobehav. Rev.* **2004**, *28*, 699–709. [CrossRef] [PubMed]
138. Morris, R. Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* **1984**, *11*, 47–60. [CrossRef]
139. Gue, M.; Bravard, A.; Meunier, J.; Veyrier, R.; Gaillet, S.; Recasens, M.; Maurice, T. Sex differences in learning deficits induced by prenatal stress in juvenile rats. *Behav. Brain Res.* **2004**, *150*, 149–157. [CrossRef]
140. Meunier, J.; Gue, M.; Recasens, M.; Maurice, T. Attenuation by a sigma1 (δ1) receptor agonist of the learning and memory deficits induced by a prenatal restraint stress in juvenile rats. *Br. J. Pharmacol.* **2004**, *142*, 689–700. [CrossRef] [PubMed]
141. Zheng, A.; Li, H.; Cao, K.; Xu, J.; Zou, X.; Li, Y.; Chen, C.; Liu, J.; Feng, Z. Maternal hydroxytyrosol administration improves neurogenesis and cognitive function in prenatally stressed offspring. *J. Nutr. Biochem.* **2015**, *26*, 190–199. [CrossRef] [PubMed]
142. Choy, K.H.; de Visser, Y.; Nichols, N.R.; van den Buuse, M. Combined neonatal stress and young-adult glucocorticoid stimulation in rats reduce bdnf expression in hippocampus: Effects on learning and memory. *Hippocampus* **2008**, *18*, 655–667. [CrossRef] [PubMed]
143. Klug, M.; van den Buuse, M. Chronic cannabinoid treatment during young adulthood induces sex-specific behavioural deficits in maternally separated rats. *Behav. Brain Res.* **2012**, *233*, 305–313. [CrossRef] [PubMed]
144. Garner, B.; Wood, S.J.; Pantelis, C.; van den Buuse, M. Early maternal deprivation reduces prepulse inhibition and impairs spatial learning ability in adulthood: No further effect of post-pubertal chronic corticosterone treatment. *Behav. Brain Res.* **2007**, *176*, 323–332. [CrossRef] [PubMed]
145. Mehta, M.; Schmauss, C. Strain-specific cognitive deficits in adult mice exposed to early life stress. *Behav. Neurosci.* **2011**, *125*, 29–36. [CrossRef] [PubMed]
146. Yu, T.; Guo, M.; Garza, J.; Rendon, S.; Sun, X.L.; Zhang, W.; Lu, X.Y. Cognitive and neural correlates of depression-like behaviour in socially defeated mice: An animal model of depression with cognitive dysfunction. *Int. J. Neuropsychopharmacol.* **2011**, *14*, 303–317. [CrossRef] [PubMed]
147. Novick, A.M.; Miiller, L.C.; Forster, G.L.; Watt, M.J. Adolescent social defeat decreases spatial working memory performance in adulthood. *Behav. Brain Funct. BBF* **2013**, *9*, 39. [CrossRef] [PubMed]
148. Furuta, M.; Ninomiya-Baba, M.; Chiba, S.; Funabashi, T.; Akema, T.; Kunugi, H. Exposure to social defeat stress in adolescence improves the working memory and anxiety-like behavior of adult female rats with intrauterine growth restriction, independently of hippocampal neurogenesis. *Horm. Behav.* **2015**, *70*, 30–37. [CrossRef] [PubMed]
149. Henningsen, K.; Andreasen, J.T.; Bouzinova, E.V.; Jayatissa, M.N.; Jensen, M.S.; Redrobe, J.P.; Wiborg, O. Cognitive deficits in the rat chronic mild stress model for depression: Relation to anhedonic-like responses. *Behav. Brain Res.* **2009**, *198*, 136–141. [CrossRef] [PubMed]
150. Henningsen, K.; Woldbye, D.P.; Wiborg, O. Electroconvulsive stimulation reverses anhedonia and cognitive impairments in rats exposed to chronic mild stress. *Eur. Neuropsychopharmacol.* **2013**, *23*, 1789–1794. [CrossRef] [PubMed]
151. Andreasen, J.T.; Henningsen, K.; Bate, S.; Christiansen, S.; Wiborg, O. Nicotine reverses anhedonic-like response and cognitive impairment in the rat chronic mild stress model of depression: Comparison with sertraline. *J. Psychopharmacol.* **2011**, *25*, 1134–1141. [CrossRef] [PubMed]

152. Mizoguchi, K.; Yuzurihara, M.; Ishige, A.; Sasaki, H.; Chui, D.H.; Tabira, T. Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *J. Neurosci.* **2000**, *20*, 1568–1574. [PubMed]
153. Palumbo, M.L.; Canzobre, M.C.; Pascuan, C.G.; Rios, H.; Wald, M.; Genaro, A.M. Stress induced cognitive deficit is differentially modulated in BALB/c and C57Bl/6 mice: Correlation with Th1/Th2 balance after stress exposure. *J. Neuroimmunol.* **2010**, *218*, 12–20. [CrossRef] [PubMed]
154. Coburn-Litvak, P.S.; Pothakos, K.; Tata, D.A.; McCloskey, D.P.; Anderson, B.J. Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats. *Neurobiol. Learn. Mem.* **2003**, *80*, 11–23. [CrossRef]
155. Gururajan, A.; Hill, R.A.; van den Buuse, M. Brain-derived neurotrophic factor heterozygous mutant rats show selective cognitive changes and vulnerability to chronic corticosterone treatment. *Neuroscience* **2015**, *284*, 297–310. [CrossRef] [PubMed]
156. Tata, D.A.; Markostamou, I.; Ioannidis, A.; Gkioka, M.; Simeonidou, C.; Anogianakis, G.; Spandou, E. Effects of maternal separation on behavior and brain damage in adult rats exposed to neonatal hypoxia-ischemia. *Behav. Brain Res.* **2015**, *280*, 51–61. [CrossRef] [PubMed]
157. Patki, G.; Solanki, N.; Atrooz, F.; Allam, F.; Salim, S. Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. *Brain Res.* **2013**, *1539*, 73–86. [CrossRef] [PubMed]
158. Patki, G.; Solanki, N.; Atrooz, F.; Ansari, A.; Allam, F.; Jannise, B.; Maturi, J.; Salim, S. Novel mechanistic insights into treadmill exercise based rescue of social defeat-induced anxiety-like behavior and memory impairment in rats. *Physiol. Behav.* **2014**, *130*, 135–144. [CrossRef] [PubMed]
159. Tagliari, B.; Scherer, E.B.; Machado, F.R.; Ferreira, A.G.; Dalmaz, C.; Wyse, A.T. Antioxidants prevent memory deficits provoked by chronic variable stress in rats. *Neurochem. Res.* **2011**, *36*, 2373–2380. [CrossRef] [PubMed]
160. Trofimiuk, E.; Braszko, J.J. Ciproxifan differentially modifies cognitive impairment evoked by chronic stress and chronic corticosterone administration in rats. *Behav. Brain Res.* **2015**, *283*, 145–153. [CrossRef] [PubMed]
161. Cerqueira, J.J.; Pego, J.M.; Taipa, R.; Bessa, J.M.; Almeida, O.F.; Sousa, N. Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. *J. Neurosci.* **2005**, *25*, 7792–7800. [CrossRef] [PubMed]
162. Workman, J.L.; Chan, M.Y.; Galea, L.A. Prior high corticosterone exposure reduces activation of immature neurons in the ventral hippocampus in response to spatial and nonspatial memory. *Hippocampus* **2015**, *25*, 329–344. [CrossRef] [PubMed]
163. Bushnell, P.J.; Strupp, B.J. Assessing attention in rodents. In *Methods of Behavior Analysis in Neuroscience*, 2nd ed.; Buccafusco, J.J., Ed.; CRC Press: Boca Raton, FL, USA, 2009.
164. De Bruin, N.M.; Fransen, F.; Duytschaever, H.; Grantham, C.; Megens, A.A. Attentional performance of (C57Bl/6J × 129Sv)F2 mice in the five-choice serial reaction time task. *Physiol. Behav.* **2006**, *89*, 692–703. [CrossRef] [PubMed]
165. Sanchez-Roige, S.; Pena-Oliver, Y.; Stephens, D.N. Measuring impulsivity in mice: The five-choice serial reaction time task. *Psychopharmacology* **2012**, *219*, 253–270. [CrossRef] [PubMed]
166. Bari, A.; Dalley, J.W.; Robbins, T.W. The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nat. Protoc.* **2008**, *3*, 759–767. [CrossRef] [PubMed]
167. Van Gaalen, M.M.; Stenzel-Poore, M.; Holsboer, F.; Steckler, T. Reduced attention in mice overproducing corticotropin-releasing hormone. *Behav. Brain Res.* **2003**, *142*, 69–79. [CrossRef]
168. Wilson, C.A.; Schade, R.; Terry, A.V., Jr. Variable prenatal stress results in impairments of sustained attention and inhibitory response control in a 5-choice serial reaction time task in rats. *Neuroscience* **2012**, *218*, 126–137. [CrossRef] [PubMed]
169. Comeau, W.L.; Winstanley, C.A.; Weinberg, J. Prenatal alcohol exposure and adolescent stress—Unmasking persistent attentional deficits in rats. *Eur. J. Neurosci.* **2014**, *40*, 3078–3095. [CrossRef] [PubMed]
170. Torregrossa, M.M.; Xie, M.; Taylor, J.R. Chronic corticosterone exposure during adolescence reduces impulsive action but increases impulsive choice and sensitivity to yohimbine in male sprague-dawley rats. *Neuropsychopharmacology* **2012**, *37*, 1656–1670. [CrossRef] [PubMed]
171. Amitai, N.; Markou, A. Comparative effects of different test day challenges on performance in the 5-choice serial reaction time task. *Behav. Neurosci.* **2011**, *125*, 764–774. [CrossRef] [PubMed]

172. Bangasser, D.A.; Kawasumi, Y. Cognitive disruptions in stress-related psychiatric disorders: A role for corticotropin releasing factor (CRF). *Horm. Behav.* **2015**, *76*, 125–135. [CrossRef] [PubMed]
173. Ohmura, Y.; Yamaguchi, T.; Futami, Y.; Togashi, H.; Izumi, T.; Matsumoto, M.; Yoshida, T.; Yoshioka, M. Corticotropin releasing factor enhances attentional function as assessed by the five-choice serial reaction time task in rats. *Behav. Brain Res.* **2009**, *198*, 429–433. [CrossRef] [PubMed]
174. Van't Veer, A.; Yano, J.M.; Carroll, F.I.; Cohen, B.M.; Carlezon, W.A., Jr. Corticotropin-releasing factor (CRF)-induced disruption of attention in rats is blocked by the kappa-opioid receptor antagonist jdtic. *Neuropsychopharmacology* **2012**, *37*, 2809–2816. [CrossRef] [PubMed]
175. Bondi, C.O.; Rodriguez, G.; Gould, G.G.; Frazer, A.; Morilak, D.A. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. *Neuropsychopharmacology* **2008**, *33*, 320–331. [CrossRef] [PubMed]
176. Naegeli, K.J.; O'Connor, J.A.; Banerjee, P.; Morilak, D.A. Effects of milnacipran on cognitive flexibility following chronic stress in rats. *Eur. J. Pharmacol.* **2013**, *703*, 62–66. [CrossRef] [PubMed]
177. Nikiforuk, A.; Popik, P. Long-lasting cognitive deficit induced by stress is alleviated by acute administration of antidepressants. *Psychoneuroendocrinology* **2011**, *36*, 28–39. [CrossRef] [PubMed]
178. Wallace, J.; Marston, H.M.; McQuade, R.; Gartside, S.E. Evidence that aetiological risk factors for psychiatric disorders cause distinct patterns of cognitive deficits. *Eur. Neuropsychopharmacol.* **2014**, *24*, 879–889. [CrossRef] [PubMed]
179. Xue, X.; Shao, S.; Wang, W.; Shao, F. Maternal separation induces alterations in reversal learning and brain-derived neurotrophic factor expression in adult rats. *Neuropsychobiology* **2013**, *68*, 243–249. [CrossRef] [PubMed]
180. Wang, Q.; Li, M.; Du, W.; Shao, F.; Wang, W. The different effects of maternal separation on spatial learning and reversal learning in rats. *Behav. Brain Res.* **2015**, *280*, 16–23. [CrossRef] [PubMed]
181. Laredo, S.A.; Steinman, M.Q.; Robles, C.F.; Ferrer, E.; Ragen, B.J.; Trainor, B.C. Effects of defeat stress on behavioral flexibility in males and females: Modulation by the mu-opioid receptor. *Eur. J. Neurosci.* **2015**, *41*, 434–441. [CrossRef] [PubMed]
182. Bisaz, R.; Schachner, M.; Sandi, C. Causal evidence for the involvement of the neural cell adhesion molecule, NCAM, in chronic stress-induced cognitive impairments. *Hippocampus* **2011**, *21*, 56–71. [CrossRef] [PubMed]
183. Bessa, J.M.; Mesquita, A.R.; Oliveira, M.; Pego, J.M.; Cerqueira, J.J.; Palha, J.A.; Almeida, O.F.; Sousa, N. A trans-dimensional approach to the behavioral aspects of depression. *Front. Behav. Neurosci.* **2009**, *3*, 1. [CrossRef] [PubMed]
184. Hill, M.N.; Patel, S.; Carrier, E.J.; Rademacher, D.J.; Ormerod, B.K.; Hillard, C.J.; Gorzalka, B.B. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. *Neuropsychopharmacology* **2005**, *30*, 508–515. [CrossRef] [PubMed]
185. Cerqueira, J.J.; Mailliet, F.; Almeida, O.F.; Jay, T.M.; Sousa, N. The prefrontal cortex as a key target of the maladaptive response to stress. *J. Neurosci.* **2007**, *27*, 2781–2787. [CrossRef] [PubMed]
186. Llorente, R.; Miguel-Blanco, C.; Aisa, B.; Lachize, S.; Borcel, E.; Meijer, O.C.; Ramirez, M.J.; De Kloet, E.R.; Viveros, M.P. Long term sex-dependent psychoneuroendocrine effects of maternal deprivation and juvenile unpredictable stress in rats. *J. Neuroendocrinol.* **2011**, *23*, 329–344. [CrossRef] [PubMed]
187. Heisler, J.M.; Morales, J.; Donegan, J.J.; Jett, J.D.; Redus, L.; O'Connor, J.C. The attentional set shifting task: A measure of cognitive flexibility in mice. *J. Vis. Exp. JoVE* **2015**. [CrossRef] [PubMed]
188. Tait, D.S.; Chase, E.A.; Brown, V.J. Attentional set-shifting in rodents: A review of behavioural methods and pharmacological results. *Curr. Pharm. Des.* **2014**, *20*, 5046–5059. [CrossRef] [PubMed]
189. Conrad, C.D. A critical review of chronic stress effects on spatial learning and memory. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2010**, *34*, 742–755. [CrossRef] [PubMed]
190. Song, L.; Che, W.; Min-Wei, W.; Murakami, Y.; Matsumoto, K. Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacol. Biochem. Behav.* **2006**, *83*, 186–193. [CrossRef] [PubMed]
191. Vorhees, C.V.; Williams, M.T. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. *Nat. Protoc.* **2006**, *1*, 848–858. [CrossRef] [PubMed]
192. Sunyer, B.; Patil, S.; Höger, H.; Luber, G. Barnes maze, a useful task to assess spatial reference memory in the mice. *Protocol Exch.* **2007**, *390*, 10–38. [CrossRef]
193. Olton, D.S. Mazes, maps, and memory. *Am. Psychol.* **1979**, *34*, 583–596. [CrossRef] [PubMed]

194. Huang, Y.; Shi, X.; Xu, H.; Yang, H.; Chen, T.; Chen, S.; Chen, X. Chronic unpredictable stress before pregnancy reduce the expression of brain-derived neurotrophic factor and n-methyl-d-aspartate receptor in hippocampus of offspring rats associated with impairment of memory. *Neurochem. Res.* **2010**, *35*, 1038–1049. [CrossRef] [PubMed]
195. Modir, F.; Elahdadi Salmani, M.; Goudarzi, I.; Lashkarboluki, T.; Abrari, K. Prenatal stress decreases spatial learning and memory retrieval of the adult male offspring of rats. *Physiol. Behav.* **2014**, *129*, 104–109. [CrossRef] [PubMed]
196. Nazeri, M.; Shabani, M.; Ghotbi Ravandi, S.; Aghaei, I.; Nozari, M.; Mazhari, S. Psychological or physical prenatal stress differentially affects cognition behaviors. *Physiol. Behav.* **2015**, *142*, 155–160. [CrossRef] [PubMed]
197. Palacios-Garcia, I.; Lara-Vasquez, A.; Montiel, J.F.; Diaz-Veliz, G.F.; Sepulveda, H.; Utreras, E.; Montecino, M.; Gonzalez-Billault, C.; Aboitiz, F. Prenatal stress down-regulates reelin expression by methylation of its promoter and induces adult behavioral impairments in rats. *PLoS ONE* **2015**, *10*, e0117680.
198. Benoit, J.D.; Rakic, P.; Frick, K.M. Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. *Behav. Brain Res.* **2015**, *281*, 1–8. [CrossRef] [PubMed]
199. Negron-Oyarzo, I.; Neira, D.; Espinosa, N.; Fuentealba, P.; Aboitiz, F. Prenatal stress produces persistence of remote memory and disrupts functional connectivity in the hippocampal-prefrontal cortex axis. *Cereb. Cortex* **2015**, *25*, 3132–3143. [CrossRef] [PubMed]
200. Sun, X.M.; Tu, W.Q.; Shi, Y.W.; Xue, L.; Zhao, H. Female-dependent impaired fear memory of adult rats induced by maternal separation, and screening of possible related genes in the hippocampal ca1. *Behav. Brain Res.* **2014**, *267*, 111–118. [CrossRef] [PubMed]
201. Couto, F.S.; Batalha, V.L.; Valadas, J.S.; Data-Franca, J.; Ribeiro, J.A.; Lopes, L.V. Escitalopram improves memory deficits induced by maternal separation in the rat. *Eur. J. Pharmacol.* **2012**, *695*, 71–75. [CrossRef] [PubMed]
202. Mello, P.B.; Benetti, F.; Cammarota, M.; Izquierdo, I. Physical exercise can reverse the deficit in fear memory induced by maternal deprivation. *Neurobiol. Learn. Mem.* **2009**, *92*, 364–369. [CrossRef] [PubMed]
203. Zhang, X.; Wang, B.; Jin, J.; An, S.; Zeng, Q.; Duan, Y.; Yang, L.; Ma, J.; Cao, X. Early deprivation reduced anxiety and enhanced memory in adult male rats. *Brain Res. Bull.* **2014**, *108*, 44–50. [CrossRef] [PubMed]
204. Rinwa, P.; Kumar, A. Piperine potentiates the protective effects of curcumin against chronic unpredictable stress-induced cognitive impairment and oxidative damage in mice. *Brain Res.* **2012**, *1488*, 38–50. [CrossRef] [PubMed]
205. Liu, D.; Zhang, Q.; Gu, J.; Wang, X.; Xie, K.; Xian, X.; Wang, J.; Jiang, H.; Wang, Z. Resveratrol prevents impaired cognition induced by chronic unpredictable mild stress in rats. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2014**, *49*, 21–29. [CrossRef] [PubMed]
206. Hoffman, A.N.; Krigbaum, A.; Ortiz, J.B.; Mika, A.; Hutchinson, K.M.; Bimonte-Nelson, H.A.; Conrad, C.D. Recovery after chronic stress within spatial reference and working memory domains: Correspondence with hippocampal morphology. *Eur. J. Neurosci.* **2011**, *34*, 1023–1030. [CrossRef] [PubMed]
207. Ortiz, J.B.; Taylor, S.B.; Hoffman, A.N.; Campbell, A.N.; Lucas, L.R.; Conrad, C.D. Sex-specific impairment and recovery of spatial learning following the end of chronic unpredictable restraint stress: Potential relevance of limbic gad. *Behav. Brain Res.* **2015**, *282*, 176–184. [CrossRef] [PubMed]
208. Lee, B.; Sur, B.; Shim, I.; Lee, H.; Hahm, D.H. Baicalin improves chronic corticosterone-induced learning and memory deficits via the enhancement of impaired hippocampal brain-derived neurotrophic factor and camp response element-binding protein expression in the rat. *J. Nat. Med.* **2014**, *68*, 132–143. [CrossRef] [PubMed]
209. McLay, R.N.; Freeman, S.M.; Zadina, J.E. Chronic corticosterone impairs memory performance in the barnes maze. *Physiol. Behav.* **1998**, *63*, 933–937. [CrossRef]
210. Mineur, Y.S.; Belzung, C.; Crusio, W.E. Functional implications of decreases in neurogenesis following chronic mild stress in mice. *Neuroscience* **2007**, *150*, 251–259. [CrossRef] [PubMed]
211. Wang, Y.; Ma, Y.; Hu, J.; Cheng, W.; Jiang, H.; Zhang, X.; Li, M.; Ren, J.; Li, X. Prenatal chronic mild stress induces depression-like behavior and sex-specific changes in regional glutamate receptor expression patterns in adult rats. *Neuroscience* **2015**, *301*, 363–374. [CrossRef] [PubMed]
212. McDonald, R.J.; Hong, N.S. How does a specific learning and memory system in the mammalian brain gain control of behavior? *Hippocampus* **2013**, *23*, 1084–1102. [CrossRef] [PubMed]

213. Anderson, E.B.; Grossrubatscher, I.; Frank, L. Dynamic hippocampal circuits support learning- and memory-guided behaviors. *Cold Spring Harb. Symp. Quant. Biol.* **2014**, *79*, 51–58. [CrossRef] [PubMed]
214. Mathews, A.; MacLeod, C. Cognitive vulnerability to emotional disorders. *Ann. Rev. Clin. Psychol.* **2005**, *1*, 167–195. [CrossRef] [PubMed]
215. Gotlib, I.H.; Joormann, J. Cognition and depression: Current status and future directions. *Ann. Rev. Clin. Psychol.* **2010**, *6*, 285–312. [CrossRef] [PubMed]
216. Saxe, M.D.; Battaglia, F.; Wang, J.W.; Malleret, G.; David, D.J.; Monckton, J.E.; Garcia, A.D.; Sofroniew, M.V.; Kandel, E.R.; Santarelli, L.; *et al.* Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17501–17506. [CrossRef] [PubMed]
217. Imayoshi, I.; Sakamoto, M.; Ohtsuka, T.; Takao, K.; Miyakawa, T.; Yamaguchi, M.; Mori, K.; Ikeda, T.; Itohara, S.; Kageyama, R. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat. Neurosci.* **2008**, *11*, 1153–1161. [CrossRef] [PubMed]
218. Chocyk, A.; Przyborowska, A.; Makuch, W.; Majcher-Maslanka, I.; Dudys, D.; Wedzony, K. The effects of early-life adversity on fear memories in adolescent rats and their persistence into adulthood. *Behav. Brain Res.* **2014**, *264*, 161–172. [CrossRef] [PubMed]
219. Xiong, G.J.; Yang, Y.; Wang, L.P.; Xu, L.; Mao, R.R. Maternal separation exaggerates spontaneous recovery of extinguished contextual fear in adult female rats. *Behav. Brain Res.* **2014**, *269*, 75–80. [CrossRef] [PubMed]
220. Oomen, C.A.; Soeters, H.; Audureau, N.; Vermunt, L.; van Hasselt, F.N.; Manders, E.M.; Joels, M.; Krugers, H.; Lucassen, P.J. Early maternal deprivation affects dentate gyrus structure and emotional learning in adult female rats. *Psychopharmacology* **2011**, *214*, 249–260. [CrossRef] [PubMed]
221. Dulka, B.N.; Lynch, J.F., 3rd; Latsko, M.S.; Mulvany, J.L.; Jasnow, A.M. Phenotypic responses to social defeat are associated with differences in cued and contextual fear discrimination. *Behav. Process.* **2015**, *118*, 115–122. [CrossRef] [PubMed]
222. Shumake, J.; Barrett, D.; Gonzalez-Lima, F. Behavioral characteristics of rats predisposed to learned helplessness: Reduced reward sensitivity, increased novelty seeking, and persistent fear memories. *Behav. Brain Res.* **2005**, *164*, 222–230. [CrossRef] [PubMed]
223. Camp, R.M.; Johnson, J.D. Repeated stressor exposure enhances contextual fear memory in a beta-adrenergic receptor-dependent process and increases impulsivity in a non-beta receptor-dependent fashion. *Physiol. Behav.* **2015**, *150*, 64–68. [CrossRef] [PubMed]
224. Liao, Y.; Shi, Y.W.; Liu, Q.L.; Zhao, H. Glucocorticoid-induced enhancement of contextual fear memory consolidation in rats: Involvement of D1 receptor activity of hippocampal area CA1. *Brain Res.* **2013**, *1524*, 26–33. [CrossRef] [PubMed]
225. Marks, W.N.; Fenton, E.Y.; Guskjolen, A.J.; Kalynchuk, L.E. The effect of chronic corticosterone on fear learning and memory depends on dose and the testing protocol. *Neuroscience* **2015**, *289*, 324–333. [CrossRef] [PubMed]
226. Monsey, M.S.; Boyle, L.M.; Zhang, M.L.; Nguyen, C.P.; Kronman, H.G.; Ota, K.T.; Duman, R.S.; Taylor, J.R.; Schafe, G.E. Chronic corticosterone exposure persistently elevates the expression of memory-related genes in the lateral amygdala and enhances the consolidation of a pavlovian fear memory. *PLoS ONE* **2014**, *9*, e91530. [CrossRef] [PubMed]
227. Duclot, F.; Hollis, F.; Darcy, M.J.; Kabbaj, M. Individual differences in novelty-seeking behavior in rats as a model for psychosocial stress-related mood disorders. *Physiol. Behav.* **2011**, *104*, 296–305. [CrossRef] [PubMed]
228. Gourley, S.L.; Kedves, A.T.; Olausson, P.; Taylor, J.R. A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF. *Neuropsychopharmacology* **2009**, *34*, 707–716. [CrossRef] [PubMed]
229. Ramirez, S.; Liu, X.; MacDonald, C.J.; Moffa, A.; Zhou, J.; Redondo, R.L.; Tonegawa, S. Activating positive memory engrams suppresses depression-like behaviour. *Nature* **2015**, *522*, 335–339. [CrossRef] [PubMed]
230. Denny, C.A.; Burghardt, N.S.; Schachter, D.M.; Hen, R.; Drew, M.R. 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. *Hippocampus* **2012**, *22*, 1188–1201. [CrossRef] [PubMed]

231. Campbell, D.G.; Felker, B.L.; Liu, C.F.; Yano, E.M.; Kirchner, J.E.; Chan, D.; Rubenstein, L.V.; Chaney, E.F. Prevalence of depression-ptsd comorbidity: Implications for clinical practice guidelines and primary care-based interventions. *J. General Intern. Med.* **2007**, *22*, 711–718. [CrossRef] [PubMed]
232. Sotres-Bayon, F.; Cain, C.K.; LeDoux, J.E. Brain mechanisms of fear extinction: Historical perspectives on the contribution of prefrontal cortex. *Biol. Psychiatry* **2006**, *60*, 329–336. [CrossRef] [PubMed]
233. Cherian, S.B.; Bairy, K.L.; Rao, M.S. Chronic prenatal restraint stress induced memory impairment in passive avoidance task in post weaned male and female wistar rats. *Indian J. Exp. Biol.* **2009**, *47*, 893–899. [PubMed]
234. Wu, J.; Song, T.B.; Li, Y.J.; He, K.S.; Ge, L.; Wang, L.R. Prenatal restraint stress impairs learning and memory and hippocampal pkcbeta1 expression and translocation in offspring rats. *Brain Res.* **2007**, *1141*, 205–213. [CrossRef] [PubMed]
235. Sung, Y.H.; Shin, M.S.; Cho, S.; Baik, H.H.; Jin, B.K.; Chang, H.K.; Lee, E.K.; Kim, C.J. Depression-like state in maternal rats induced by repeated separation of pups is accompanied by a decrease of cell proliferation and an increase of apoptosis in the hippocampus. *Neuroscience Lett.* **2010**, *470*, 86–90. [CrossRef] [PubMed]
236. Radahmadi, M.; Alaei, H.; Sharifi, M.R.; Hosseini, N. Effects of different timing of stress on corticosterone, bdnf and memory in male rats. *Physiol. Behav.* **2015**, *139*, 459–467. [CrossRef] [PubMed]
237. Bisagno, V.; Ferrini, M.; Rios, H.; Zieher, L.M.; Wikinski, S.I. Chronic corticosterone impairs inhibitory avoidance in rats: Possible link with atrophy of hippocampal CA3 neurons. *Pharmacol. Biochem. Behav.* **2000**, *66*, 235–240. [CrossRef]
238. Costa-Nunes, J.; Zubareva, O.; Araujo-Correia, M.; Valenca, A.; Schroeter, C.A.; Pawluski, J.L.; Vignisse, J.; Steinbusch, H.; Hermes, D.; Phillipines, M.; *et al*. Altered emotionality, hippocampus-dependent performance and expression of nmda receptor subunit mrnas in chronically stressed mice. *Stress* **2014**, *17*, 108–116. [CrossRef] [PubMed]
239. Fanselow, M.S.; Dong, H.W. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* **2010**, *65*, 7–19. [CrossRef] [PubMed]
240. Kheirbek, M.A.; Drew, L.J.; Burghardt, N.S.; Costantini, D.O.; Tannenholz, L.; Ahmari, S.E.; Zeng, H.; Fenton, A.A.; Hen, R. Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. *Neuron* **2013**, *77*, 955–968. [CrossRef] [PubMed]
241. O'Brien, J.T.; Lloyd, A.; McKeith, I.; Gholkar, A.; Ferrier, N. A longitudinal study of hippocampal volume, cortisol levels, and cognition in older depressed subjects. *Am. J. Psychiatry* **2004**, *161*, 2081–2090. [CrossRef] [PubMed]
242. Cornwell, B.R.; Salvadore, G.; Colon-Rosario, V.; Latov, D.R.; Holroyd, T.; Carver, F.W.; Coppola, R.; Manji, H.K.; Zarate, C.A., Jr.; Grillon, C. Abnormal hippocampal functioning and impaired spatial navigation in depressed individuals: Evidence from whole-head magnetoencephalography. *Am. J. Psychiatry* **2010**, *167*, 836–844. [CrossRef] [PubMed]
243. Gould, N.F.; Holmes, M.K.; Fantie, B.D.; Luckenbaugh, D.A.; Pine, D.S.; Gould, T.D.; Burgess, N.; Manji, H.K.; Zarate, C.A., Jr. Performance on a virtual reality spatial memory navigation task in depressed patients. *Am. J. Psychiatry* **2007**, *164*, 516–519. [CrossRef] [PubMed]
244. Surget, A.; Tanti, A.; Leonardo, E.D.; Laugeray, A.; Rainer, Q.; Touma, C.; Palme, R.; Griebel, G.; Ibarguen-Vargas, Y.; Hen, R.; *et al*. Antidepressants recruit new neurons to improve stress response regulation. *Mol. Psychiatry* **2011**, *16*, 1177–1188. [CrossRef] [PubMed]
245. Oomen, C.A.; Bekinschtein, P.; Kent, B.A.; Saksida, L.M.; Bussey, T.J. Adult hippocampal neurogenesis and its role in cognition. *Wiley Interdiscip. Rev. Cogn. Sci.* **2014**, *5*, 573–587. [CrossRef] [PubMed]
246. Deng, W.; Aimone, J.B.; Gage, F.H. New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* **2010**, *11*, 339–350. [CrossRef] [PubMed]
247. Kim, W.R.; Christian, K.; Ming, G.L.; Song, H. Time-dependent involvement of adult-born dentate granule cells in behavior. *Behav. Brain Res.* **2012**, *227*, 470–479. [CrossRef] [PubMed]
248. Marin-Burgin, A.; Schinder, A.F. Requirement of adult-born neurons for hippocampus-dependent learning. *Behav. Brain Res.* **2012**, *227*, 391–399. [CrossRef] [PubMed]
249. Ming, G.L.; Song, H. Adult neurogenesis in the mammalian brain: Significant answers and significant questions. *Neuron* **2011**, *70*, 687–702. [CrossRef] [PubMed]

250. Hernandez-Rabaza, V.; Llorens-Martin, M.; Velazquez-Sanchez, C.; Ferragud, A.; Arcusa, A.; Gumus, H.G.; Gomez-Pinedo, U.; Perez-Villalba, A.; Rosello, J.; Trejo, J.L.; *et al*. Inhibition of adult hippocampal neurogenesis disrupts contextual learning but spares spatial working memory, long-term conditional rule retention and spatial reversal. *Neuroscience* **2009**, *159*, 59–68. [CrossRef] [PubMed]
251. Winocur, G.; Wojtowicz, J.M.; Sekeres, M.; Snyder, J.S.; Wang, S. Inhibition of neurogenesis interferes with hippocampus-dependent memory function. *Hippocampus* **2006**, *16*, 296–304. [CrossRef] [PubMed]
252. Deng, W.; Saxe, M.D.; Gallina, I.S.; Gage, F.H. Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. *J. Neurosci.* **2009**, *29*, 13532–13542. [CrossRef] [PubMed]
253. Dupret, D.; Revest, J.M.; Koehl, M.; Ichas, F.; De Giorgi, F.; Costet, P.; Abrous, D.N.; Piazza, P.V. Spatial relational memory requires hippocampal adult neurogenesis. *PLoS ONE* **2008**, *3*, e1959. [CrossRef] [PubMed]
254. Garthe, A.; Kempermann, G. An old test for new neurons: Refining the morris water maze to study the functional relevance of adult hippocampal neurogenesis. *Front. Neurosci.* **2013**, *7*, 63. [CrossRef] [PubMed]
255. Jessberger, S.; Clark, R.E.; Broadbent, N.J.; Clemenson, G.D., Jr.; Consiglio, A.; Lie, D.C.; Squire, L.R.; Gage, F.H. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn. Mem.* **2009**, *16*, 147–154. [CrossRef] [PubMed]
256. Clelland, C.D.; Choi, M.; Romberg, C.; Clemenson, G.D., Jr.; Fragniere, A.; Tyers, P.; Jessberger, S.; Saksida, L.M.; Barker, R.A.; Gage, F.H.; *et al*. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* **2009**, *325*, 210–213. [CrossRef] [PubMed]
257. Shors, T.J.; Townsend, D.A.; Zhao, M.; Kozorovitskiy, Y.; Gould, E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* **2002**, *12*, 578–584. [CrossRef] [PubMed]
258. Meshi, D.; Drew, M.R.; Saxe, M.; Ansorge, M.S.; David, D.; Santarelli, L.; Malapani, C.; Moore, H.; Hen, R. Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nat. Neurosci.* **2006**, *9*, 729–731. [CrossRef] [PubMed]
259. Groves, J.O.; Leslie, I.; Huang, G.J.; McHugh, S.B.; Taylor, A.; Mott, R.; Munafo, M.; Bannerman, D.M.; Flint, J. Ablating adult neurogenesis in the rat has no effect on spatial processing: Evidence from a novel pharmacogenetic model. *PLoS Genet.* **2013**, *9*, e1003718. [CrossRef] [PubMed]
260. Saxe, M.D.; Malleret, G.; Vronskaya, S.; Mendez, I.; Garcia, A.D.; Sofroniew, M.V.; Kandel, E.R.; Hen, R. Paradoxical influence of hippocampal neurogenesis on working memory. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4642–4646. [CrossRef] [PubMed]
261. Schmidt, B.; Marrone, D.F.; Markus, E.J. Disambiguating the similar: The dentate gyrus and pattern separation. *Behav. Brain Res.* **2012**, *226*, 56–65. [CrossRef] [PubMed]
262. Aimone, J.B.; Deng, W.; Gage, F.H. Resolving new memories: A critical look at the dentate gyrus, adult neurogenesis, and pattern separation. *Neuron* **2011**, *70*, 589–596. [CrossRef] [PubMed]
263. Sahay, A.; Scobie, K.N.; Hill, A.S.; O'Carroll, C.M.; Kheirbek, M.A.; Burghardt, N.S.; Fenton, A.A.; Dranovsky, A.; Hen, R. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* **2011**, *472*, 466–470. [CrossRef] [PubMed]
264. Hill, A.S.; Sahay, A.; Hen, R. Increasing adult hippocampal neurogenesis is sufficient to reduce anxiety and depression-like behaviors. *Neuropsychopharmacology* **2015**, *40*, 2368–2378. [CrossRef] [PubMed]
265. Denny, C.A.; Kheirbek, M.A.; Alba, E.L.; Tanaka, K.F.; Brachman, R.A.; Laughman, K.B.; Tomm, N.K.; Turi, G.F.; Losonczy, A.; Hen, R. Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. *Neuron* **2014**, *83*, 189–201. [CrossRef] [PubMed]
266. Besnard, A.; Sahay, A. Adult hippocampal neurogenesis, fear generalization, and stress. *Neuropsychopharmacology* **2016**, *41*, 24–44. [CrossRef] [PubMed]
267. Fujii, T.; Saito, D.N.; Yanaka, H.T.; Kosaka, H.; Okazawa, H. Depressive mood modulates the anterior lateral ca1 and dg/ca3 during a pattern separation task in cognitively intact individuals: A functional mri study. *Hippocampus* **2014**, *24*, 214–224. [CrossRef] [PubMed]
268. Akers, K.G.; Martinez-Canabal, A.; Restivo, L.; Yiu, A.P.; De Cristofaro, A.; Hsiang, H.L.; Wheeler, A.L.; Guskjolen, A.; Niibori, Y.; Shoji, H.; *et al*. Hippocampal neurogenesis regulates forgetting during adulthood and infancy. *Science* **2014**, *344*, 598–602. [CrossRef] [PubMed]

3 Le récepteur 5-HT$_4$ et son implication dans la dépression et les troubles cognitifs

L'action physiologique de la sérotonine est portée par une quinzaine de récepteurs sérotoninergiques regroupés en 7 familles (famille des récepteurs 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, 5-HT$_5$, 5-HT$_6$, 5-HT$_7$) (Barnes and Sharp, 1999) Ce travail de thèse se concentre sur le récepteur sérotoninergique de type 4 (5-HT$_4$). Avec les récepteurs 5-HT$_6$ et 5-HT$_7$, ce sont les seuls récepteurs métabotropiques couplés positivement à l'adénylate cyclase et stimulant donc la production de second messager, l'AMPc (Gerald et al., 1995).

3.1 Généralités sur le récepteur 5-HT$_4$

3.1.1 Découverte du récepteur sérotoninergique de type 4

Découvert en 1985 parmi tous les sous-types de récepteurs sérotoninergiques connus, le récepteur sérotoninergique de type 4 (5-HT$_4$) a été initialement apparenté au récepteur sérotoninergique de type 3 (5-HT$_3$). En effet, ces deux récepteurs présentaient un profil pharmacologique similaire, avec une forte affinité pour des ligands communs, notamment pour le tropisetron (Bockaert et al., 2004). En outre, les premiers ligands découverts pour le récepteur 5-HT$_4$ présentent une forte affinité pour le récepteur 5-HT$_3$ (Bockaert et al., 2004; Dumuis et al., 1988; Eglen et al., 1995). En 1992, le récepteur 5-HT$_4$ a été finalement classé comme un nouveau sous-type de récepteur sérotoninergique puisqu'insensible aux antagonistes connus des récepteurs sérotoninergiques de la famille des 5-HT$_1$, 5-HT$_2$ et 5-HT$_3$ (Dumuis et al., 1988), mais aussi grâce à la découverte de puissants agonistes sélectifs pour le récepteur 5-HT$_4$, comme le cisapride et le metoclopramide (Ki= 29,0 et 1080 nM, respectivement ; (Yoshikawa et al., 1998)). Ces molécules prokinétiques ont également permis de mettre en évidence chez la souris l'expression du récepteur 5-HT$_4$ à la périphérie, insensible aux antagonistes du récepteur 5-HT$_3$.

Par la suite, la caractérisation du gène codant pour le récepteur 5-HT$_4$ humain, exceptionnellement long (700 kb, 38 exons) a permis de confirmer que le récepteur 5-HT$_4$ pouvait être classé comme un nouveau type de récepteur sérotoninergique (Claeysen et al., 1999; Van den Wyngaert et al., 1997). Ce gène complexe, cloné dans deux espèces différentes, le Rat et la Souris (Claeysen et al., 1996; Gerald et al., 1995) conduit après épissage alternatif à plusieurs isoformes différentes aux propriétés fonctionnelles bien distinctes (Bockaert et al., 2004). Dans le cerveau et les tissus périphériques, les Humains expriment six variants du récepteur 5-HT$_4$ (a, b, c, g, i et n), contre

quatre chez la souris (a, b, e et f) (Claeysen et al., 1999). Ces isoformes peuvent différer dans leur activité constitutive, leur sensibilité face à un agoniste pour induire les phénomènes de désensibilisation ou d'internalisation ou encore dans leur affinité avec les ligands. Ces variants ne se distinguent qu'au niveau de la partie C-terminale, pourtant leur localisation et leurs propriétés fonctionnelles spécifiques impactent l'ensemble du couplage et de la régulation du récepteur 5-HT_4. et donc le potentiel du récepteur 5-HT_4 à être une cible thérapeutique (Marin et al., 2012).

3.1.2 Structure du récepteur sérotoninergique de type 4

Le récepteur sérotoninergique de type 4 (5-HT_4) est un récepteur à 7 domaines transmembranaires possédant trois boucles intracellulaires et trois boucles extracellulaires. Le domaine N-terminal est orienté vers le milieu extracellulaire tandis que le domaine C-terminal, couplé à une protéine Gs est orienté vers le cytoplasme (Figure 3).

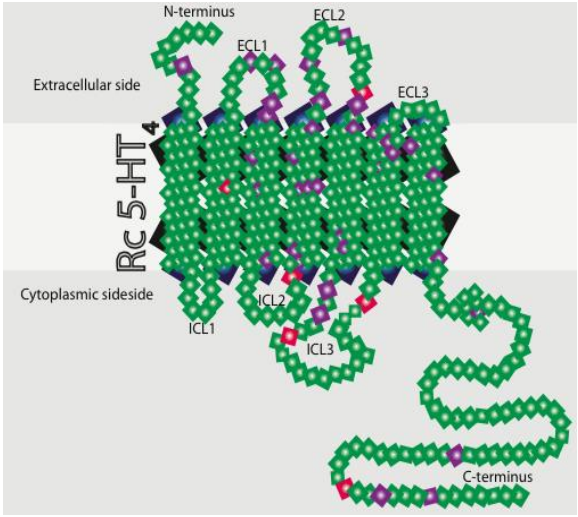


Figure 3: Représentation bidimensionnelle du récepteur 5-HT_4 (Samuels et al., 2016). Le récepteur 5-HT_4 est un récepteur métabotropique à 7 domaines transmembranaires de 3 boucles intracellulaires et 3 boucles extracellulaires dont le domaine N-terminal est intracellulaire et le domaine C-terminal extracellulaire

Son activation sélective, par un agoniste, entraine le recrutement de la protéine Gs qui stimule l'adénylate cyclase (AC), responsable de la production d'adénosine monophosphate cyclique (AMPc) (Dumuis et al., 1988). La protéine kinase A (PKA) ainsi activée par l'AMPc module différents courants ioniques et en particulier les courants potassiques dont l'inhibition génère une hyperexcitabilité neuronale (Ansanay et al., 1995). La PKA est également capable de phosphoryler la protéine liant l'élément de réponse de l'AMPc (CREB, cAMP response element binding protein), ce qui a pour

conséquence d'augmenter la transcription des gènes du facteur neurotrophique cérébral BDNF (brain-derived neurotrophic factor), impliqué dans la cognition, l'humeur et la survie cellulaire (Ahmad and Nirogi, 2011)(Figure 4).

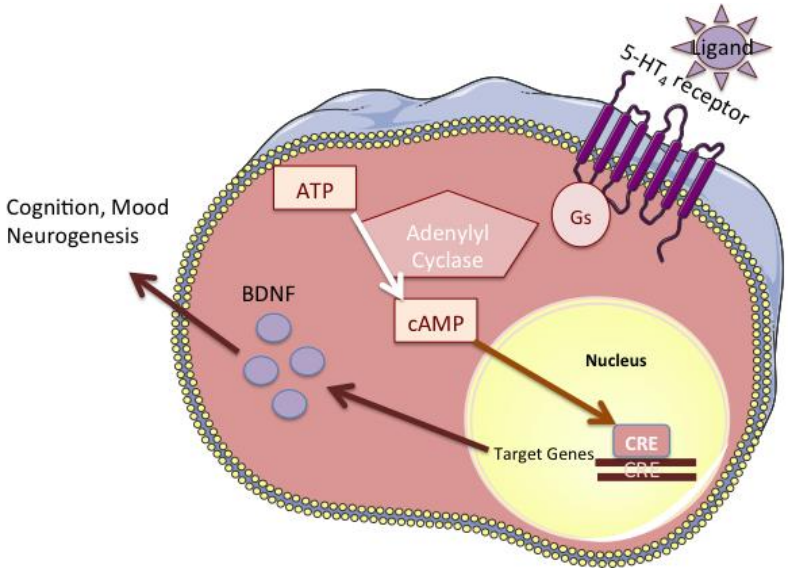


Figure 4: Activation du récepteur 5-HT4 menant à de nombreux effets cellulaires et moléculaires (adapté et modifié de Ahmad et Nirogi, 2011)**.** L'activation du récepteur 5-HT4 mène à une libération d'acétylcholine, couplée à la libération de BDNF, facteur favorisant la mémoire et l'humeur, mais aussi le processus de neurogenèse chez l'adulte.

3.2 Localisations et fonctionnalités du récepteur 5-HT$_4$

Le récepteur 5-HT$_4$ est exprimé aussi bien dans le SNC qu'à la périphérie chez les vertébrés.

3.2.1 Localisations et fonctionnalités du récepteur 5-HT$_4$ au niveau périphérique

A la périphérie, ce récepteur sérotoninergique est retrouvé dans l'œsophage, l'iléon, le colon, les cellules adréno-corticales, la vessie et le cœur (Eglen et al., 1995) (Figure 5). L'activation du récepteur 5-HT$_4$ au niveau des oreillettes induit une tachycardie et une augmentation de la contractilité du myocarde (effet inotrope positif observé chez plusieurs espèces, notamment chez l'Homme et le cochon) (Hegde and Eglen, 1996). Dans le tractus gastro-intestinal, l'activation du récepteur 5-HT$_4$ facilite la motilité intestinale induite par l'acétylcholine (Eglen et al., 1990; Kilbinger and Wolf, 1992), stimule le péristaltisme et la sécrétion d'électrolytes chez le cobaye (Craig and Clarke, 1991) et favorise les nausées et vomissements (Hegde and Eglen, 1996). Les récepteurs 5-HT$_4$

stimulent également les contractions des muscles lisses de la vessie induites par l'acétylcholine (Hegde and Eglen, 1996). Enfin, dans les surrénales, la sécrétion des corticostéroïdes et la régulation de la production d'aldostérone et de cortisol sont favorisées par l'activation du récepteur 5-HT_4 (Lefebvre et al., 1992).

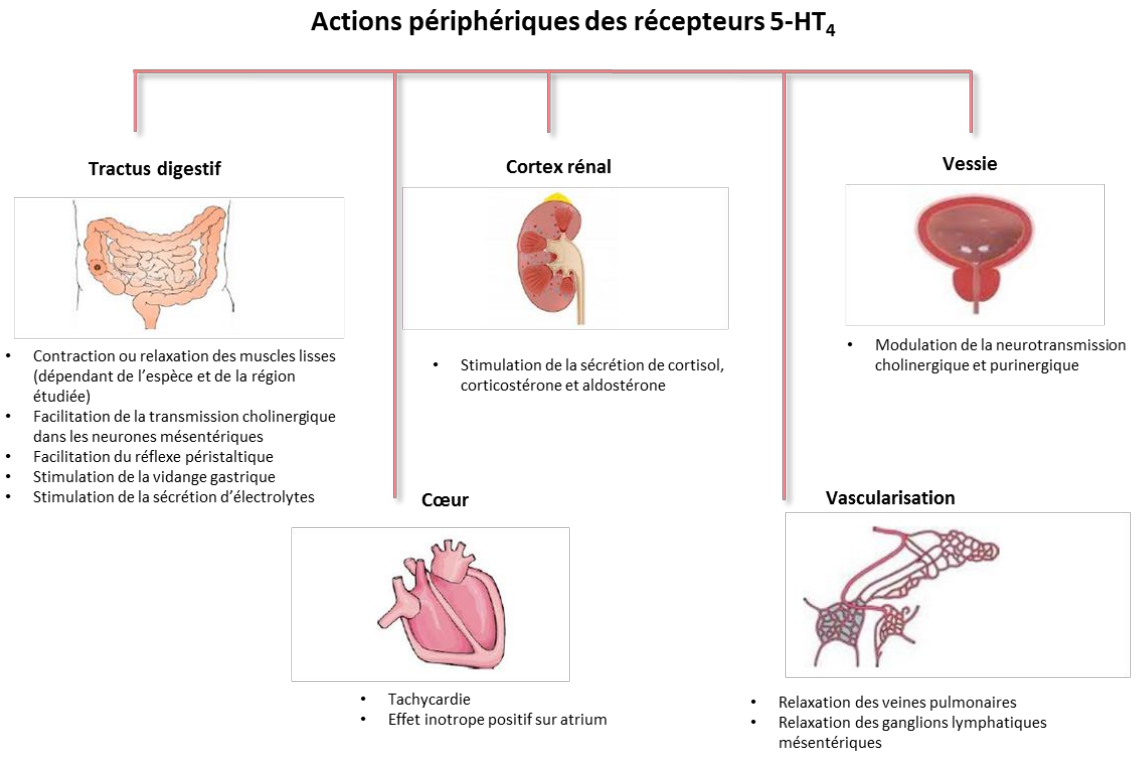


Figure 5 : Localisation et effets principaux du récepteur 5-HT_4 au niveau des organes périphériques (adapté de Hedge et Eglen, 1996)

3.2.2 Localisations et fonctionnalités du récepteur 5-HT_4 dans le SNC

Le développement en 1993 de deux nouveaux ligands radio-spécifiques du récepteur 5-HT_4, les antagonistes le [3H]-GR 113808 et le [125I]-SB 207710, a permis une détermination précise de la distribution régionale du récepteur 5-HT_4 dans le cerveau (Grossman et al., 1993). La grande majorité des récepteurs 5-HT_4 exprimée dans le cerveau chez l'Homme ou le rongeur, l'est dans l'hippocampe, l'hypothalamus, le noyau accumbens, le pallidum ventral, l'amygdale, les noyaux gris centraux, les bulbes olfactifs, le cortex frontal et la substance noire (Bockaert et al., 2004; Eglen et al., 1995; Vilaro et al., 1996; Vilaro et al., 2005; Waeber et al., 1993). La localisation de ces récepteurs dans le système limbique suggère donc un rôle dans le contrôle des émotions et de la cognition (Figure 6).

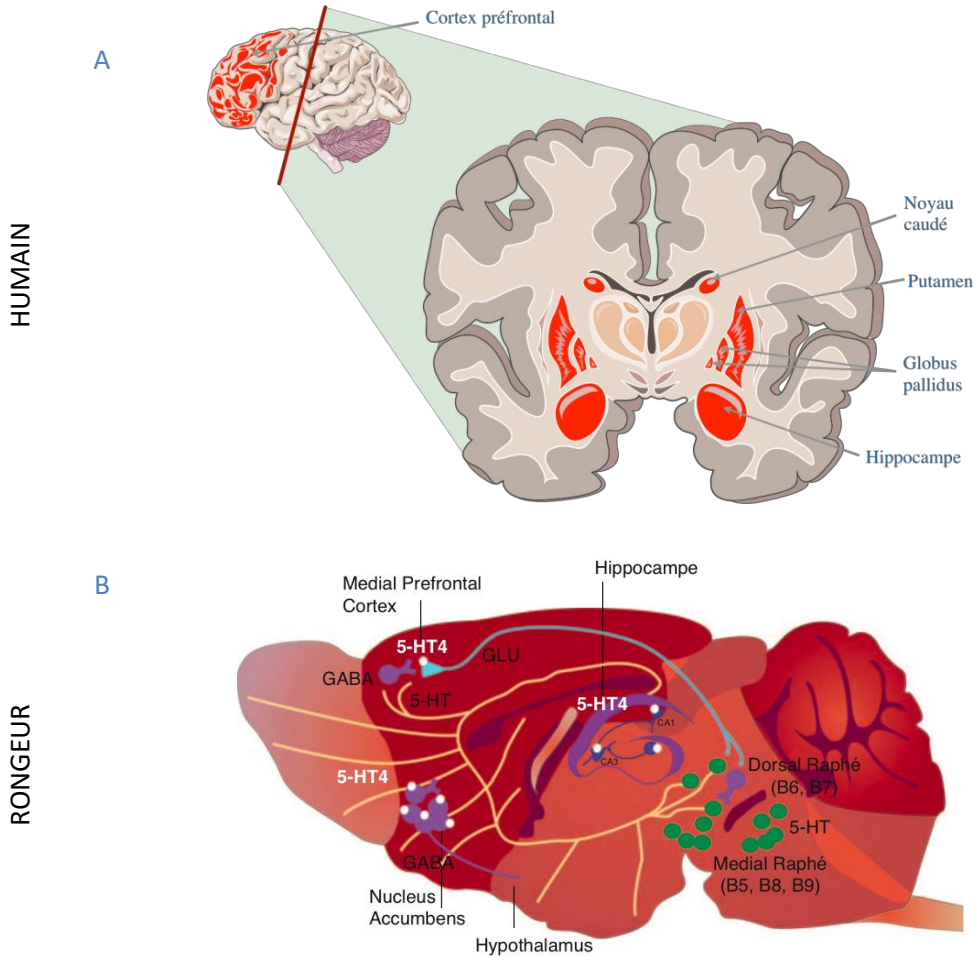


Figure 6: Localisation cérébrale du récepteur 5-HT$_4$ chez l'Humain (A) et chez le rongeur (B) (d'après Samuels et al., 2014 et Bockart et al., 2004). Le récepteur 5-HT$_4$ est principalement exprimé dans l'hippocampe, le cortex, le globus pallidus et le noyau caudé.

Grâce à des approches méthodologiques variées telles que l'étude de liaison du récepteur 5-HT$_4$ ou d'autoradiographie, il est possible de connaitre aujourd'hui la localisation cellulaire et subcellulaire du récepteur 5-HT$_4$ dans le cerveau (Bockaert et al., 2008; Bonaventure et al., 2000; Compan et al., 1996; Lucas, 2009; Waeber et al., 1993; Waeber et al., 1994). Le récepteur 5-HT$_4$ est en fait un hétérorécepteur, situé au niveau somatodendritique et au niveau des terminaisons axonales des neurones épineux efférents GABAergiques du striatum (Cai et al., 2002; Compan et al., 1996; King et al., 2008). Ils sont également exprimés dans les neurones pyramidaux glutamatergiques dans le cortex median préfrontal, des cornes d'Ammon de l'hippocampe (CA1, CA3) et dans les cellules granulaires du gyrus dentelé (Bockaert et al., 2004; King et al., 2008; Roychowdhury et al., 1994; Vilaro et al., 2005). Dans le cortex, l'hippocampe et l'amygdale, les récepteurs 5-HT$_4$ sont aussi localisés sur les neurones cholinergiques et glutamatergiques (pour revue : (Bockaert et al., 2004)). Par ailleurs, lorsque nous nous intéressons aux différentes isoformes du récepteur 5-HT$_4$, il apparaît

que les isoformes a et b sont exprimées de façon importante dans les régions du système limbique et notamment des structures qui sont responsables du contrôle des réponses de motivation et de plaisir et sont affectées dans la dépression comme le noyau accumbens, l'hippocampe et le striatum, (Bockaert et al., 2008; Marin et al., 2012; Ponimaskin et al., 2002; Qanbar and Bouvier, 2003; Vilaro et al., 2002). Ces isoformes ont fait l'objet de recherches comme cibles potentielles pour le développement d'un nouveau traitement antidépresseur d'action rapide (Lucas et al., 2007; Pascual-Brazo et al., 2012). Cependant, en raison du manque d'anticorps et de ligands spécifiques des isoformes, la distinction et la caractérisation des propriétés de chacune dans les fonctions de signalisation et de régulation restent difficiles.

Une revue de la littérature montre que le récepteur 5-HT_4 est une cible de choix dans la régulation des fonctions cérébrales telles que la mémoire et la cognition, les troubles de l'humeur et les désordres de la prise alimentaire (Ahmad and Nirogi, 2011; Bockaert et al., 2004, 2008; King et al., 2008; Lucas, 2009; Lucas et al., 2007; Pascual-Brazo et al., 2012). Le rôle du récepteur 5-HT_4 dans le contrôle de la prise alimentaire ne sera pas développé dans le cadre de ce travail. Une attention plus particulière sera donnée aux implications du récepteur 5-HT_4 dans les fonctions émotionnelles et cognitives.

3.1 Implication du récepteur 5-HT_4 et de ses ligands dans l'anxiété /dépression

3.1.1 Implication du récepteur 5-HT_4 chez l'Homme dans la dépression

Chez l'Homme, quatre études cliniques supportent le rôle du récepteur 5-HT_4 dans la dépression. L'une d'entre elle mentionne des polymorphismes sur la région variant d'épissage du gène codant pour le récepteur 5-HT_4, associés à la dépression unipolaire (Ohtsuki et al., 2002). Cette étude d'association menée sur un panel d'hommes et de femmes japonais âgés de 27 à 77 ans, souffrant de dépression majeure récurrente, a rapporté l'existence d'un lien entre les polymorphismes des variants issus de l'épissage alternatif du gène codant pour le récepteur 5-HT_4 et les dépressions unipolaires (83097C/T, 83159G/A, 83164(T)9-10 et 83198A/G) (Ohtsuki et al., 2002). En revanche, l'influence du polymorphisme du récepteur 5-HT_4 sur la réponse au traitement antidépresseur ou anxiolytique n'a pas enocore été étudiée à ce jour. D'autre part, l'association entre les génotypes du 5-HTTLPR (allèle S et/ou allèle L) et la liaison du récepteur 5-HT_4 a été mesurée en utilisant un radioligand ([^{11}C]SB207145) et une technique de tomographie par émission de positron *in vivo* (Fisher

et al., 2012). Il a ainsi été montré que la liaison de ce radioligand au récepteur 5-HT_4 dans le néocortex était plus faible chez les homozygotes pour l'allèle S de 5-HTT ou SERT ou les hétérozygotes que chez les homozygotes pour l'allèle L. Cette donnée est cohérente avec le fait que l'allèle S conduit à une diminution de la transcription du transporteur de la sérotonine (SERT) menant à une diminution de l'expression du récepteur 5-HT_4. Une autre étude récente, utilisant la même technique d'imagerie cérébrale, s'est quant à elle intéressée au lien entre le risque familial de développer un épisode dépressif majeur et l'expression du récepteur 5-HT_4 (Madsen et al., 2015). Ce travail montre qu'un individu dont un ou plusieurs parents au premier degré ont été traités pour un épisode dépressif présente une diminution de la liaison du radioligand ([^{11}C]SB207145) au récepteur 5-HT_4 dans le striatum ainsi que dans d'autres régions limbiques. Ces données confirment ainsi l'implication du récepteur 5-HT_4 dans le mécanisme neurobiologique qui sous-tend le risque familial de dépression et suggèrent qu'une densité plus faible du récepteur 5-HT_4 dans le striatum est associée à un risque accru de développer un épisode dépressif majeur. Enfin, une autre étude révèle des altérations des sites de liaison du récepteur 5-HT_4 et des concentrations d'AMPc dans plusieurs régions cérébrales (cortex frontal, hippocampe, noyau caudé et amygdale) chez des patients déprimés ayant commis un acte de suicide violent (Rosel et al., 2004). Enfin, le nombre de récepteurs 5-HT_4 et les taux d'AMPc sont augmentés dans le cortex frontal et le noyau caudé de patients suicidés sans changement d'affinité de liaison pour le récepteur 5-HT_4 (Haahr et al., 2013).

L'expression importante du récepteur 5-HT_4 dans le système limbique suggère un rôle prépondérant de ce récepteur dans la régulation de l'humeur. Dans le domaine préclinique, de récents travaux indiquent que les désordres psychologiques tels que l'anxiété et/ou la dépression majeure pourraient bénéficier d'une modulation de la signalisation du récepteur 5-HT_4 (Lucas, 2009; Lucas et al., 2007; Pascual-Brazo et al., 2012).

3.1.2 Implication du récepteur 5-HT_4 chez l'animal dans l'anxiété/dépression

Les premières observations impliquant le récepteur 5-HT_4 dans la régulation de la réponse anxiolytique/antidépressive reposent sur des données d'électrophysiologie. En effet, il semble qu'une administration unique par certains agonistes de ce récepteur (cisapride, prucalopride) chez le Rat augmente la fréquence de décharge des neurones sérotoninergiques des noyaux du raphé dorsal (Lucas and Debonnel, 2002). En revanche, lorsque les souris sont privées du récepteur 5-HT_4 (souris KO constitutive R.5-HT_4), cette fréquence moyenne de décharge est diminuée de moitié par rapport aux souris sauvages (Conductier et al., 2006). Par ailleurs, une étude de microdialyse intracérébrale a montré que l'activation du récepteur 5-HT_4 (RS67333, 1,5 mg/kg, i.v.) potentialisait les effets d'une

administration aiguë de paroxetine (0,5 mg/kg, i.v.), un ISRS, sur les taux de sérotonine extracellulaire dans l'hippocampe ventral de rat (Licht et al., 2010).

3.1.2.1 Implication du récepteur 5-HT$_4$ et de ses ligands au niveau phénotypique

La caractérisation phénotypique comportementale suite à l'administration unique ou chronique d'un ligand du récepteur 5-HT$_4$ a permis d'en préciser son rôle. Par exemple, dans le test de la double enceinte éclairée, un paradigme permettant de mesurer l'activité anxiolytique d'un ligand, les effets de type anxiolytiques induits par une administration unique de diazépam (0,16 mg/kg) chez la souris naïve « non-stressée » sont bloqués par des antagonistes du récepteur 5-HT$_4$ (GR 113808, SB 204070 et SDZ 205-557) et cela de façon dose-dépendante (Costall and Naylor, 1997). Ces données suggèrent de façon très surprenante que le récepteur 5-HT$_4$ est impliqué dans l'effet anxiolytique du diazépam. D'autres études montrent en revanche un effet anxiolytique propre de certains antagonistes du récepteur 5-HT$_4$ tels que le SB 204070, GR 113808 (Silvestre et al., 1996) et SB 207266A (Kennett et al., 1997; Silvestre et al., 1996) chez le rat dans le labyrinthe en croix surélevée (Tableau 3). Les rats traités avec le SB 204070 et le GR 113808 ont montré une augmentation du pourcentage de temps total passé dans les bras ouverts du labyrinthe. Cependant, il est difficile de conclure formellement que les antagonistes du récepteur 5-HT$_4$ présentent des effets de type anxiolytiques, puisque ces exemples issus de la littérature qui indiquent soit une absence d'effet dans la double enceinte éclairée, soit une réduction des effets anxieux dans le labyrinthe en croix surélevée, ont été réalisés chez des animaux naïfs.

De manière plus convaincante, il a été montré que chez le rat, l'administration d'agoniste du récepteur 5-HT$_4$ (RS67333) induit des effets similaires à ceux des ISRSs dans certains tests prédictifs d'une activité antidépressive, notamment dans le test de la nage forcée chez le Rat après 3 jours de traitement (Lucas et al., 2007). Ainsi, des agonistes des récepteur du 5-HT$_4$, tel que le RS67333 (1,5 mg/kg, i.p.) ou le prucalopride (2,5 mg/kg , i.p.) réduisent significativement le temps d'immobilité d'environ 50% comparé aux animaux contrôles, tandis que le citalopram (10 mg/kg , i.p.) ne le réduit que de 23% (Lucas et al., 2007) (Tableau 3 et 4). Dans cette même étude, Lucas et al., ont montré que les réponses

cellulaires et comportementales qui nécessitent 2 à 3 semaines de traitement antidépresseur chez le rongeur pourraient apparaitre après seulement 1 à 3 jours de traitement avec un agoniste sélectif des récepteur du 5-HT_4 comme le RS67333. Les mêmes auteurs ont montré en 2010, à nouveau grâce à des techniques d'électrophysiologie, que la co-administration d'un ISRSs avec un agoniste du récepteur 5-HT_4 potentialise l'effet de type antidépresseur rapide chez le Rat (Lucas et al., 2010). Très récemment, il a été mis en évidence qu'un traitement de 7 jours par le RS67333 induit un effet de type antidépresseur et augmente l'étape de prolifération cellulaire de la neurogenèse hippocampique ainsi que certains facteurs liés à la neuroplasticité (BDNF, CREB) (Pascual-Brazo et al., 2012). Ces effets sont d'autant plus remarquables qu'ils sont de même intensité qu'un traitement de 2 à 3 semaines avec des antidépresseurs classiques.

Tableau 3: Effets des ligands du récepteur 5-HT$_4$ sur l'anxiété/dépression – Partie 1

Référence	Nom	Propriété	Dose	Espèce	Test	Effet
Silvestre et al., 1996	**SB204070,** 8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride	Antagoniste	0,3-3 mg/kg, s.c, aigu	Rat	Labyrinthe en Croix surélevée	Anxiolytique
	GR 113808, 1-[2 methylsulphonylamino)ethyl]-4-piperidinyl]methyl-1-methyl-1Hindole-3-carboxylate maleate	Antagoniste	0,3-3 mg/kg, s.c, aigu			Anxiolytique
Kennet et al., 1997	**SB204070,** 8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride	Antagoniste	0,01 et 10 mg/kg, p.o 0,01 et 1 mg/kg s.c, aigu	Rat	Interaction sociale/Labyrinthe en Croix surélevée	Augmente le temps d'interaction sociale
	SB 207266A, 2H-(1,3)Oxazino(3,2-a)indole-10-carboxamide, N-((1-butyl-4-piperidinyl)methyl)-3,4-dihydro-, monohydrochloride	Antagoniste	0,01 et 10 mg/kg p.o ; 0,01 et 1 mg/kg, s.c, aigu		Interaction sociale/x-maze	Augmente le temps d'interaction sociale
Costall et Naylor, 1997	**SDZ205-557,** 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino ester)	Antagoniste	0,001–100 kg/kg, i.p, aigu	Souris	Double Enceinte éclairée	Pas d'effet par lui-même, réduit l'effet désinhibiteur du diazépam
	GR113808, 1-[2-methylsulphonylamino)ethyl]-4-piperidinyl]methyl-1-methyl-1Hindole-3-carboxylate maleate	Antagoniste	0,001–100 kg/kg, i.p, aigu			Pas d'effet par lui-même, réduit l'effet désinhibiteur du diazépam
	SB204070, 8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride	Antagoniste	0,001–100 kg/kg, i.p, aigu			Pas d'effet par lui-même, réduit l'effet désinhibiteur du diazépam
(Schreiber et al., 1998)	**GR 125487,** [1-[2-[(Methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl 5-fluoro-2-methoxy1Hindole-3-carboxylate	Antagoniste	3 mg/kg, s.c., aigu	Rat	Vocalisation ultrasonique	Pas d'effet

Tableau 4: Effets des ligands du récepteur 5-HT$_4$ sur l'anxiété/dépression – Partie 2

Référence	Nom	Propriété	Dose	Espèce	Test	Effet
Cryan et Lucki, 2000	**SB204070,** 8-amino-7-chloro-(N-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride	Antagoniste	3 mg/kg, s.c., aigu	Rat	FST	Pas d'effet
Lucas et al., 2007	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste	1,5 mg/kg, i.p. pendant 3 jours	Rat	FST	Diminue la durée d'immobilité et augmente la durée d'escalade
		Agoniste	1,5 mg/kg, i.p. pendant 3 et 14 jours	Rat OBX	Activité locomotrice	Corrige la hausse de l'activité induite par OBX après 14 jours
		Agoniste	1,5 mg/kg, i.p. pendant 3 et 14 jours	Rat CMS	Consommation sucrose	Corrige la diminution de la consommation de sucrose induite par le CMS après 14 jours
Pascual-Brazo et al., 2012	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste	1,5 mg/kg, i.p. pendant 3/7 jours 1,5 mg/kg, i.p. pendant 3/7 jours	Rat	FST/NSF	Diminue le temps d'immobilité à 3/7 jours dans le FST et diminue la latence à se nourrir à 7 jours
		Agoniste	1,5 mg/kg, i.p. pendant 3/7 jours 1,5 mg/kg, i.p. pendant 3/7 jours	Rat traité CORT	Consommation sucrose	Augmente la consommation de sucrose
Gomez-Lazaro et al., 2012	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste	1,5 mg/kg, i.p. pendant 5 jours	Rat avec stress social	FST	Augmente le temps de nage

De plus, des molécules de signalisation qui interagissent avec le récepteur 5-HT_4 comme la protéine p11 (S100A10) (Egeland et al., 2011; Warner-Schmidt et al., 2009) pourraient représenter de nouvelles cibles pour des traitements anxiolytique et/ou antidépresseur à action rapide. De nombreux éléments récents montrent que les neurones corticaux qui co-expriment p11 et les récepteurs du 5-HT_4 impliqués dans les effets comportementaux des IRSSs (Schmidt et al., 2012) et qu'un traitement chronique par la fluoxetine induit une augmentation de l'expression des récepteur du 5-HT_4 dans les neurones corticaux. Enfin, dans des tests comportementaux tels que le FST ou le test de suspension caudale, l'activité antidépressive du RS67333 est absente chez les souris privées du gène codant pour p11 comparées à leur contrôles WT (Warner-Schmidt et al., 2009).

3.1.2.2 Implication des ligands du récepteur 5-HT_4 sur la plasticité cérébrale et la neurogenèse hippocampique chez l'adulte

En plus des données comportementales, de nombreux changements au niveau de la plasticité neuronale et notamment au niveau de la neurogenèse hippocampique interviennent avec l'administration chronique d'antidépresseur monoaminergique (Santarelli et al., 2003; Wang et al., 2008). Il en est de même avec certains agonistes du récepteur 5-HT_4 puisqu'une administration chronique de RS67333 chez le rongeur favorise l'étape de survie de la neurogenèse hippocampique (Lucas et al.,2007 ;2009). D'autre part, la stimulation *in vitro* des récepteurs 5-HT_4 par deux autres agonistes du récepteur 5-HT_4 (RS67506 et tegaserod) favorise également la survie neuronale entérique (Liu et al., 2009). En complément des résultats comportementaux, une étude récente réalisée chez des rats naïfs a confirmé que 3 jours de traitement avec le RS67333, augmentent de façon significative la neurogenèse dans la zone sous granulaire du gyrus dentelé de l'hippocampe, un effet connu pour apparaitre après minimum 2 semaines de traitement aux antidépresseurs classiques comme les IRSSs (Pascual-Brazo et al., 2012). Ces résultats sont déterminants dans la compréhension du lien entre les données comportementales et les changements dans la neurogenèse hippocampique adulte en réponse à un traitement par un antidépresseur. La neurogenèse hippocampique adulte est impliquée dans certains des effets comportementaux des antidépresseurs chez le rongeur adulte (David et al., 2009; Santarelli et al., 2003). Cependant, aucune preuve directe n'a été trouvée pour relier les effets de type antidépresseur de la stimulation du récepteur 5-HT_4 et ses effets neurogéniques.

D'autres données basées sur l'analyse du signal de transduction suite à l'activation du récepteur 5-HT_4 pourraient expliquer l'effet de type antidépresseur des agonistes 5-HT_4. Les

récepteurs 5-HT_4 sont couplés à une protéine G(s) qui active l'adénylate cyclase, augmentant alors la production d'AMPc (Dumuis et al., 1989; Torres et al., 1995) qui à son tour active la protéine kinase A (PKA) qui phosphoryle le facteur de transcription CREB. De manière intéressante, il a été démontré qu'un traitement chronique par un antidépresseur activait cette même voie de signalisation (Nibuya et al., 1995). Il semblerait donc que l'activation de CREB en CREB phosphorylé constitue une étape clé de la facilitation de la neurogenèse hippocampique, une des autres propriétés caractéristiques des antidépresseurs (Duman et al., 2001; Malberg et al., 2000).

Le facteur neurotrophique BDNF a également un rôle dans la croissance neuronale et la plasticité synaptique (McAllister, 1999). Plusieurs études ont montré que les antidépresseurs régulaient à la hausse la production de BDNF hippocampique (Duman and Monteggia, 2006). Alors que certains auteurs ont reporté une induction de l'expression de BDNF après un traitement sub-chronique aux antidépresseurs (Larsen et al., 2008; Musazzi et al., 2009), d'autres études ne montrent une augmentation du BDNF qu'après un traitement chronique aux antidépresseurs (De Foubert et al., 2004; Nibuya et al., 1995). L'effet à long-terme des antidépresseurs modulant le comportement et la survie des nouveaux neurones est médié au moins en partie par le BDNF. De plus, dans la mesure où l'activation du récepteur 5-HT_4 peut favoriser l'expression de BDNF, son activation pourrait mener à des effets de type antidépresseurs BDNF-dépendants, sur le comportement et la neuroplasticité. Une étude récente a démontré une augmentation de l'expression du BDNF après 3 jours de traitement avec le RS67333 et une accumulation significative de la protéine du BDNF dans l'ensemble de la structure de l'hippocampe après un traitement de 7 jours (Pascual-Brazo et al., 2012).

D'autre part, il a été fortement suggéré que le second messager AMPc était impliqué dans le mécanisme d'action des antidépresseurs (Donati and Rasenick, 2003). Une diminution significative de la production d'APMc dépendante du récepteur 5-HT_4 a été rapporté après un traitement chronique de 3 semaines par de la fluoxetine, de la venlafaxine (Vidal et al., 2010) ou de l'imipramine (Reierson et al., 2009), probablement reflétant le processus de désensibilisation, dû à un couplage aux protéines moins efficaces. Une étude a également reporté qu'un traitement de 7 jours par une stimulation du récepteur 5-HT_4 est nécessaire pour désensibiliser entièrement la voie de signalisation post-récepteur associée aux récepteurs 5-HT_4, en accord avec les résultats obtenus dans les tests comportementaux prédictifs d'une réponse chronique (Pascual-Brazo et al., 2012).

3.2 Implications des ligands du récepteur 5-HT$_4$ dans la cognition

Ainsi, il a été montré que le récepteur 5-HT$_4$ modulait la potentialisation à long terme ou la dépression à long terme (respectivement PLT et DLT) dans le gyrus dentelé chez le Rat adulte (Kulla and Manahan-Vaughan, 2002), deux processus physiologiques jouant un rôle essentiel dans les processus de mémorisation (Kemp and Manahan-Vaughan, 2004; Neves et al., 2008). Ainsi, une injection locale de RS67333 dans le GD de rat, diminuerait la DLT. Des effets similaires ont été observés chez des rats anesthésiés en utilisant un autre agoniste sélective du récepteur 5-HT$_4$ appelé SC 53116 (Matsumoto et al., 2001). L'injection intracérébroventriculaire de SC 53116 corrige non seulement les déficits cognitifs induits par un pré-traitement de scopolamaine dans le test de l'évitement passif, mais augmente surtout la PLT. Le récepteur 5-HT$_4$ serait donc une cible clé dans la régulation des fonctions cognitives.

Ainsi, dans différents modèles animaux, les agonistes partiels ou entier du récepteur 5-HT$_4$ se sont révélés très efficaces pour favoriser l'apprentissage et la mémoire dans plusieurs tests comportementaux (Bockaert et al., 2004, 2008; Marchetti et al., 2008) (Tableaux 5 à 8). Des études pharmacologiques *in vivo* réalisées chez l'animal ont permis de confirmer le rôle supposé du récepteur 5-HT$_4$ dans la mémoire et l'apprentissage. En effet, ces agonistes du récepteur 5-HT$_4$, BIMU1 et BIMU8, se sont montrés capables d'augmenter la phase d'acquisition de l'apprentissage mais d'altérer la consolidation de l'information apprise dans un test chez le rat (Meneses and Hong, 1997). De plus, certains travaux montrent que les agonistes du récepteur 5-HT$_4$ augmentent la navigation spatiale chez le rat. Par exemple, dans le test de la piscine de Morris, la stimulation aigüe du récepteur 5-HT$_4$ par le Zacopride a révélé une diminution du temps de latence durant l'apprentissage et une restauration des déficits induits par l'atropine (Fontana et al., 1996). Dans le labyrinthe en Y, une administration aigüe de RS67333 dans le noyau basal magnocellulaire est capable d'améliorer la reconnaissance de place, reflétée par une augmentation du nombre de visites dans le nouveau bras du labyrinthe (Orsetti et al., 2003) (Tableaux 5 à 8). Beaucoup plus récemment, une équipe française a montré que l'administration simultanée du RS67333 avec le donepezil (inhibiteur d'acétylcholinestérase) provoquait un effet synergique sur les performances de mémoire de type épisodique chez le rat (Freret et al., 2012). Ces mêmes auteurs ont alors développé une nouvelle molécule, le donecopride, associant les propriétés pharmacologiques d'un agoniste du récepteur 5-HT$_4$ (RS67333) et celles d'un inhibiteur de l'acétylcholinestérase (donepezil). L'administration aigue de ce nouveau composé améliore les performances de mémoire épisodique en augmentant la capacité de discrimination des animaux aux différentes doses testées dans le test de reconnaissance d'objet (Tableau 8) (Lecoutey et al., 2014). Par ailleurs, l'administration chronique

de RS67333 aux doses de 0,3 et de 1 mg/kg pendant 14 jours, dans le test de reconnaissance d'objet a mis en évidence une amélioration de l'indice de discrimination chez la souris (Quiedeville et al., 2015).

Enfin, chez des macaques jeunes et âgés, l'agoniste RS17107 a induit des améliorations de la mémoire associative dans un tâche cognitive « delayed-matching-to-sample task » dans lequel les singes devaient apprendre à associer correctement des couleurs pour obtenir une récompense alimentaire (Terry et al., 1998). L'efficacité des agonistes du récepteur 5-HT_4 chez les singes âgés supporte l'utilisation potentielle de ces composés dans le traitement des désordres cognitifs.

Tableau 5: Effets des ligands du récepteur 5-HT_4 sur les performances cognitives - Partie 1

Référence	Molécule	Propriété	Dose	Espèce	Test	Effet
Fontana et al. 1996	**(R)-Zacopride,** 4-amino-5-chloro-2-methoxy-N-(quinuclidin-3-yl)benzamide	Agoniste (aussi antagoniste de R.5HT_2)	0,001-100 µg/kg, i.p, aigu	Rat traités à atropine	Piscine de Morris	Pas d'effet
	(S)-Zacopride, 4-amino-5-chloro-2-methoxy-N-(quinuclidin-3-yl)benzamide		0,001-100 µg/kg, i.p, aigu			Diminue le déficit cognitif
Fontana et al 1997	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste	0,1, 10 et 1000 µg/kg, i.p, aigu	Rat	Piscine de Morris	Diminue le déficit cognitif
	RS 67506, (1-(4-amino- 5-chloro-2-methoxyphenyl)-3-[1-[2-[(methylsulfonyl)amino]ethyl]-4- piperidinyl]-1-propanone)					Pas d'effet
	RS 67532 1-(4-amino-5-chloro-2-(3, 5- dimethoxy benzyloxyphenyl)-5-(1-piperidinyl)-1-pentanone)	Antagoniste				Pas d'effet
Meneses and Hong, 1997	**BIMU 1,** (3-ethyl-2,3-dihydro-N-[endo-8-methyl- 8-azabicyclo (3.2.1)-oct-3-yl]-2-oxo-1H) benzimidazole-1-carboxamide hydrochloride	Agoniste	5-20 mg/kg, i.p, aigu	Rat	Conditionnement à un stimulus	Augmente la réponse conditionnée à 10 and 20mg/kg
	BIMU8, 2,3-Dihydro-*N*-[(3-*endo*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl]-3-(1-methylethyl)-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride	Agoniste	10-30 mg/kg, i.p, aigu			Augmente la réponse conditionnée à 20 mg/kg et réduit à 5 mg/kg
	SDZ 205557, 2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino ester)	Antagoniste	1,0-10.0 mg/Kg, i.p, aigu			Pas d'effet
	GR 125487D, [1-[2-[(Methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate	Antagoniste	0,39-1,56 mg/kg, i.p, aigu			Pas d'effet

Tableau 6: Effets des ligands du récepteur 5-HT$_4$ sur les performances cognitives - Partie 2

Référence	Molécule	Propriété	Dose	Espèce	Test	Effet
Galeotti et al., 1998	**BIMU 1,** (3-ethyl-2,3-dihydro-N-[endo-8-methyl-8-azabicyclo (3.2.1)-oct-3-yl]-2-oxo-1H) benzimidazole-1-carboxamide hydrochloride	Agoniste	10-25 mg/kg, i.p, aigu	Souris	Test de l'évitement passif chez des souris traitées par la scopolamine (SCO) ou dicyclomine (DICY)	Prévient l'amnésie induite par la SCO ou DICY à 10 mg/kg
	BIMU8, 2,3-Dihydro-*N*-[(3-*endo*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl]-3-(1-methylethyl)-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride	Agoniste	10-30 mg/kg, i.p, aigu			Prévient l'amnésie induite par la SCO ou DICY à 30 mg/kg
	GR 125487, [1-[2-[(Methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate	Antagoniste	5-30 mg/kg, i.p, aigu		Test de l'évitement passif	Induit une amnésie à 10 mg/kg
	SDZ 205557, 2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino ester)	Antagoniste	5-30 mg/kg, i.p, aigu			Induit une amnésie à 10 mg/kg
Terry et al., 1998	**RS 17017,** 1-(4-amino-5-chloro-2-methoxyphenyl)-5-(piperidin-1-yl)-1-pentanone hydrochloride	Agoniste	0,3, 3,0, 30, 300, 3000, et 10 000 µg/kg, p.o	Macaques jeunes et âgés	Test d'appariement différé	Améliore l'appariement chez singes jeunes et âgés
Marchetti et al., 2000	**RS 17017,** 1-(4-amino-5-chloro-2-methoxyphenyl)-5-(piperidin-1-yl)-1-pentanone hydrochloride	Agoniste	1 mg/kg, i.p, aigu	Rat	Discrimination associative olfactive	Améliore la mémoire associative
	RS67333, (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel				Améliore la mémoire associative
	RS 67532 1-(4-amino-5-chloro-2-(3, 5-dimethoxy benzyloxyphenyl)-5-(1-piperidinyl)-1-pentanone)	Antagoniste				Induit un déficit de la mémoire associative
Orsetti et al., 2003	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4	Agoniste partiel	40-500 ng/0.5µl dans le noyau basal magnocellulaire, aigu	Rat	Labyrinthe en Y	Améliore l'acquisition (200-500 ng/0,5 µL) et la consolidation (40-200 ng/0.5 µL)
	RS 39604, (1-[4-Amino-5-chloro-2-(3,5-dimethoxyphenyl}methyloxy]-3-[1-{2-methylsul-phonylamino]ethyl)piperidin-4-yl]propan-1-one)	Antagoniste	300 ng/0.5µl dans noyau basal magnocellulaire, aigu			Pas d'effet

Tableau 7: Effets des ligands du récepteur 5-HT$_4$ sur les performances cognitives - Partie 3

Référence	Molécule	Propriété	Dose	Espèce	Test	Effet
Lelong et al., 2003	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel	0,25, 0,5, 1 mg/kg, i.p, aigu	Souris	Labyrinthe en Y	Prévient le déficit de l'alternation induit par la SCO
	BIMU 1, (3-ethyl-2,3-dihydro-N-[endo-8-methyl-8-azabicyclo (3.2.1)-oct-3-yl]-2-oxo-1H) benzimidazole-1-carboxamide hydrochloride	Agoniste	1, 3, 10 mg/kg, i.p, aigu			Prévient le déficit de l'alternation induit par la SCO
Lamirault et al., 2003	**RS67333**, (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel	0,01, 1, 10 mg/kg i.p, aigu	Rat jeune ou âgé	Reconnaissance d'objet/place	Améliore la discrimination à tout âge
Mohler et al., 2007	**PRX 03140,** (formerly VRX-03011), 4-hydroxy-7-isopropyl-6-oxo-N-[3-(1-piperidinyl)propyl]-6,7-dihydrothieno[2,3-b]pyridine-5-carboxamide	Agoniste	0,1, 1, 5, or 10 mg/kg, i.p, aigu	Rat	Alternation spontanée	Améliore l'alternation à 1, 5 and 10 mg/kg
Sunyer et al., 2008	**RS67333, (**1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel	1 mg/kg, i.p, aigu	Souris	Piscine de Morris	Pas d'effet
Hille et al, 2008	**SL65.0155,** 5-(5-amino-6-chloro-2,3-dihydro-1,4-benzodioxin-8-yl)-3-(1-phenethyl- 4-piperidyl)-1,3,4-oxadiazol-2-one hydrochloride	Agoniste	0,1 ou 1 mg/kg s.c, aigu	Rat	Réactions en série à 5 choix	Augmente le pourcentage d'essais corrects
Marchetti et al., 2008	**SL65.0155,** 5-(5-amino-6-chloro-2,3-dihydro-1,4-benzodioxin-8-yl)-3-(1-phenethyl- 4-piperidyl)-1,3,4-oxadiazol-2-one hydrochloride	Agoniste	0,01 mg/kg, i.p., aigu	Rat traité à la colchicine	Discrimination associative olfactive	Récupération complète de la discrimination chez les rats lésés
Levallet et al., 2009	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel	1 mg/kg, i.p, aigu	Rat	Reconnaissance d'objet	Augmente le temps d'exploration du nouvel objet
Restivo et al., 2008	**SL65.0155,** 5-(5-amino-6-chloro-2,3-dihydro-1,4-benzodioxin-8-yl)-3-(1-phenethyl- 4-piperidyl)-1,3,4-oxadiazol-2-one hydrochloride	Agoniste	0,01 mg/kg, i.p., aigu	Souris	Discrimination associative olfactive	Augmente la discrimination olfactive spontanée
	RS 39604, (1-[4-Amino-5-chloro-2-(3,5-dimethoxyphenyl}methyloxy]-3-[1-{2-methylsul-phonylamino]ethyl)piperidin-4-yl]propan-1-one)	Antagoniste	0,5 mg/kg, i.p., aigu	Souris	Piscine de Morris	Pas d'effet

Tableau 8: Effets des ligands du récepteur 5-HT$_4$ sur les performances cognitives - Partie 4

Référence	Molécule	Propriété	Dose	Espèce	Test	Effet
Cachard-Chastel et al., 2008	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel	1 mg/kg, i.p., aigu	Souris traitée à la scopolamine	Piscine de Morris	Réduit le déficit cognitif
Marchetti et al., 2011	**SL65.0155,** 5-(5-amino-6-chloro-2,3-dihydro-1,4-benzodioxin-8-yl)-3-(1-phenethyl-4-piperidyl)-1,3,4-oxadiazol-2-one hydrochloride	Agoniste	0,1 mg/kg, i.p., aigu	Rat âgé	Discrimination associative olfactive	Améliore la discrimination associative
Freret et al., 2012	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel	0,1, 0,3 et 1 mg/kg, i.p, aigu	Rat	Reconnaissance d'objet	Améliore la discrimination à 0,3 et 1 mg/kg
Hotte et al., 2012	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel	1 mg/kg, i.p, aigu	Rat	Reconnaissance d'objet	Améliore la discrimination
Lecoutey et al., 2014	**Donecopride**, -(4-amino-5-chloro-2-methoxyphenyl)-3-[1-(cyclohexylmethyl)-4-piperidinyl] propan-1-one	Agoniste partiel (et inhibiteur acétylcholinestérase)	0,1, 0,3, 1 et 3 mg/kg, i.p, aigu	Rat	Reconnaissance d'objet	Améliore la discrimination à 0,3 et 1 mg/kg
Lo et al., 2014	**SSP-002392,** - (4-amino-5-chloro-2,3-dihydro-benzofuran-7-carboxylic acid [3-hydroxy-1-(3-methoxy-propyl)-piperidin-4ylmethyl]-amide	Agoniste	0,3, 1,5 et 7,5 mg/kg, p.o, aigu	Souris traitée à la SCO	Test évitement passif	Prévient l'amnésie induite par la SCO à la dose de 7,5 mg/kg
					Piscine de Morris	Prévient l'amnésie induite par la SCO à la dose de 7,5 mg/kg
Quiedeville et al., 2015	**RS67333,** (1-[4-Amino-5-chloro-2-methoxyphenyl]-3-[1-butyl-4-piperidinyl]-1-propanone)	Agoniste partiel	0,1, 0,3 et 1 mg/kg, i.p, 14 jours	Souris	Reconnaissance d'objet	Améliore la discrimination à 0,3 et 1 mg/kg à 14 jours

Par ailleurs, il a été montré que l'activation de la protéine CREB, cible de la voie de signalisation du récepteur 5-HT_4, est un important médiateur de la formation de la mémoire et des effets pro-cognitifs (Bockaert et al., 2004, 2008). L'activation du récepteur 5-HT_4 améliore favorise la libération d'acétylcholine. Ainsi, l'équipe de Consolo a été la première à démontrer par des études de microdialyse *in vivo* chez le rat, que les agonistes du récepteur 5-HT_4, BIMU1 et BIMU8, étaient capable d'augmenter les concentrations d'acétylcholine dans les dialysats (Consolo et al., 1994). Par la suite, d'autres études ont confirmé ces résultats, et précisèrent que les agonistes du récepteur 5-HT_4 facilitaient la libération d'acétylcholine (Letty et al., 1997; Orsetti et al., 2007; Yamaguchi et al., 1997). Ces travaux montrent que les récepteurs 5-HT_4 peuvent donc améliorer la formation de l'apprentissage et de la mémoire en favorisant la libération synaptique d'acétylcholine dans le cerveau (Bockaert et al., 2008; King et al., 2008; Lucas, 2009).

Enfin, les agonistes du récepteur 5-HT_4 se sont révélés être de très intéressantes cibles dans la recherche de stratégies visant à traiter la maladie d'Alzheimer, caractérisée par la présence de troubles cognitifs variés. Des études *in vitro* évaluant le potentiel du RS67333 montrent que l'activation sélective du récepteur 5-HT_4 inhibe la sécrétion des peptides β-amyloïdes et augmente la survie neuronale dans des cultures de neurones corticaux de souris transgéniques Tg2576 (Cho and Hu, 2007). D'autre part, les récepteurs 5-HT_4 activent également le facteur d'échange Epac, qui stimule l'activité de l'α-sécrétase et la libération de la protéine précurseur amyloide soluble (sAPP), ayant des propriétés neuroprotectrices et pro-cognitives (King et al., 2008; Marin et al., 2012). Récemment, l'évaluation préclinique *in vivo* du donécopride, une molécule inhibitrice de l'acétylcholinestérase (donépézil) et agoniste du récepteur 5-HT_4 (RS67333) a démontré son grand potentiel dans le traitement de la maladie d'Alzheimer, révélé par une amélioration significative des performances cognitives dans le test de reconnaissance d'objets aux deux doses étudiées (Lecoutey et al., 2014). De nouvelles molécules dérivées des agonistes du récepteur 5-HT_4 sont en cours de synthèse et d'évaluation et pourraient représenter une grande avancée dans la mise en place de nouvelles thérapies dans la maladie d'Alzheimer (Brodney et al., 2012; Nirogi et al., 2015; Rochais et al., 2015).

Ces résultats obtenus par des études précliniques sont à relier à une étude clinique réalisée en *postmortem* chez des patients atteints de la maladie d'Alzheimer (Tsang et al., 2010), démontrant une diminution de la densité de récepteurs 5-HT_4 dans les régions corticales du cerveau. Ces données chez l'Homme confirment le rôle prometteur des agonistes du récepteur 5-HT_4 dans le traitement de la pathologie d'Alzheimer.

Au final, si les études précliniques montrent un certain attrait pour l'utilisation des agonistes du récepteur 5-HT_4 dans le traitement des épisodes dépressifs, la quasi majorité de ces travaux a été réalisée chez des animaux naïfs. Il est donc nécessaire de procéder à une caractérisation complète des conséquences d'un traitement chronique par un agoniste du récepteur 5-HT_4 dans un modèle d'anxiété/dépression. Par ailleurs, en plus des données de comportement, il a été proposé qu'une courte période de traitement avec des agonistes du récepteur 5-HT_4 augmentait l'une des étapes de la neurogenèse hippocampique adulte, la prolifération de cellules progénitrices dans GD (Pascual-Brazo et al., 2012). Si certains des effets comportementaux des antidépresseurs monoaminergiques ont été associés à l'augmentation de la neurogenèse hippocampique, aucune donnée à ce jour ne relie les effets antidépresseurs induits par l'activation du récepteur 5-HT_4 et ses effets neurogéniques.

4 La protéine β-arrestine 1

Conformément à l'idée que les antidépresseurs exercent des effets dans de multiples régions cérébrales, il a été démontré qu'un traitement par de la fluoxetine chez des animaux sous un régime de corticostérone, provoquait des effets sur l'expression de gènes, non seulement dans l'hippocampe, mais aussi dans l'hypothalamus et l'amygdale chez la souris adulte (David et al., 2009). Parmi les gènes étudiés, ceux codant pour les β-arrestines 1 et 2 montraient des changements d'expression dans ces régions cérébrales. Les protéines β-arrestines 1 et 2 pourraient donc être des déterminants moléculaires importants impliqués dans la réponse thérapeutique dans le cadre d'un épisode de dépression majeure. Dans cette thèse, seule la β-arrestine 1 sera développée.

4.1 Généralités sur les protéines β-arrestines

Les arrestines constituent une famille de protéines qui sont capables d'interagir avec les récepteurs couplés aux protéines G (RCPG) suite à l'activation de ceux-ci par un agoniste (DeWire et al., 2007). Quatre membres de la famille des arrestines ont été identifiés :

- Arrestine 1 ou arrestine visuelle codée par le gène *SAG*
- Arrestine 2 ou β-arrestine 1 codée par le gène *ARRB1*
- Arrestine 3 ou β-arrestine 2 codée par le gène *ARRB2*
- Arrestine 4 ou arrestine conale codée par le gène *ARR3*

Les arrestines 1 et 4 sont exprimées presque exclusivement dans la rétine, où elles régulent la fonction de photorécepteurs : la rhodopsine et les opsines colorées (Craft et al., 1994; Pfister et al., 1985). Au contraire, la β-arrestine 1 (ou Arrestine 2) et la β-arrestine 2 (ou Arrestine 3) sont des protéines ubiquitaires, c'est-à-dire qu'elles sont exprimées dans l'ensemble de l'organisme (Lohse et al., 1990; Parruti et al., 1993). De manière intéressante, on les trouve exprimée abondamment dans le cerveau mais aussi dans les leucocytes du sang périphérique (Parruti et al., 1993). D'autre part, comme leur nom l'indique, les β-arrestines ont tout d'abord été identifiées pour leur capacité à « arrêter » la signalisation du récepteur β_2 adrénergique stimulé par un agoniste. Elles sont donc initialement connues comme des régulateurs négatifs de la signalisation des RCPG.

4.2 Répartition cérébrale de la β-arrestine 1

Bien qu'elle soit présente dans l'ensemble des cellules de l'organisme, la β-arrestine 1 est exprimée en quantités importantes dans certaines régions cérébrales dédiées à des fonctions spécifiques. Des analyses d'hybridation *in situ* (Allen Brain Atlas), ont permis de mettre en évidence la présence de la β-arrestine 1 dans les bulbes olfactifs et l'hippocampe (Figure 7), deux régions cérébrales impliquées dans le processus de neurogenèse chez l'adulte.

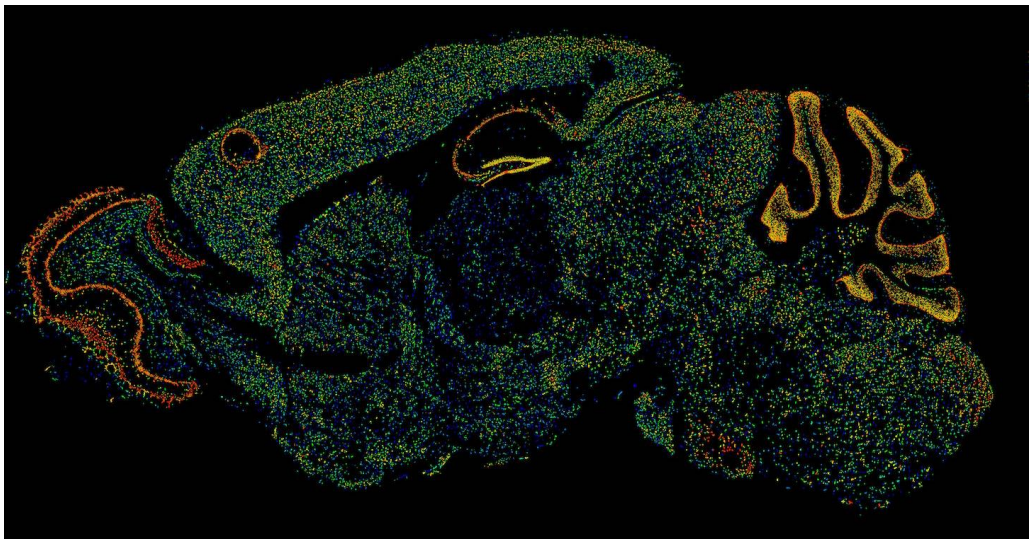


Figure 7 : Expression de la β-arrestine 1 dans le système nerveux central par une technique d'hybridation *in situ*. D'après Allen Brain Atlas.

4.3 Structure et mécanismes d'action de la protéine β-arrestine 1

La cristallographie a permis de décrire précisément la structure 3D de la protéine β-arrestine 1 ainsi que les changements conformationnels observés lors de son activation. Ainsi, la protéine β-arrestine 1 est une molécule de forme allongée possédant un noyau polaire central et sept boucles, incluant la « finger loop », la « middle loop » et la « lariat loop » encadrées de part et d'autre par des domaines N et C-terminaux qui permettent l'interaction avec les RCPG (Shukla et al., 2013) (Figure 8). Lors de son activation, la protéine β-arrestine 1 change de conformation au niveau de ces trois boucles, un changement qui applique une force de torsion sur les domaines N et C-terminaux de la protéine β-arrestine 1. Cette torsion est responsable d'une rotation du domaine C-terminal de 20° par rapport au domaine N-terminal.

Les régions N et C terminales constituent des domaines fonctionnels majeurs pour le mécanisme de la protéine β-arrestine 1 :

- le domaine **N** est responsable de la reconnaissance du récepteur à 7 domaines transmembranaires activé
- le domaine **C** est responsable de la reconnaissance secondaire du récepteur

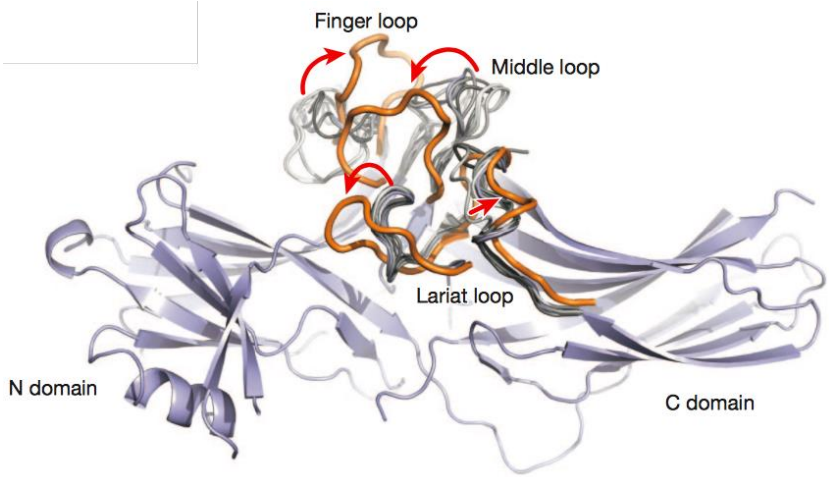


Figure 8 : Structure cristallographique de la protéine β-arrestine 1 (Shukla et al., 2013). La protéine β-arrestine 1 possède une grande flexibilité conformationnelle au niveau de ses boucles. La protéine β-arrestine 1 est inactive lorsque les boucles sont dans la conformation grise et active lorsqu'elles sont dans la conformation orange.

De nouveaux rôles ont été découverts pour les β-arrestines tels que le phénomène de désensibilisation et d'internalisation des récepteurs (Lefkowitz and Whalen, 2004) (Figure 9). Suite à leur activation par un agoniste, le RCPG est phosphorylé par des kinases RCPG appelées GRK (récepteur couplé à une kinase). Les β-arrestines, capables de reconnaître les sites de phosphorylation sur le RCPG activé, vont créer une association robuste avec le récepteur. Cette liaison β-arrestine/RCPG est d'une telle affinité qu'elle empêche le récepteur de se coupler avec sa protéine G apparentée, de telle sorte que malgré l'activation continue du récepteur par l'agoniste, il ne peut pas échanger le groupement GTP sur la sous-unité de la protéine G pour du GDP, causant alors une désensibilisation de la signalisation de la protéine G via les seconds messagers. Dans ce cas, les β-arrestines servent d'inhibiteurs de signal de transduction (Golan et al., 2009; Lefkowitz and Whalen, 2004).

En plus de leur rôle dans la désensibilisation des RCPG, les β-arrestines interagissent également avec des protéines de la machinerie endocytaire comme la clathrine et une protéine adaptatrice (AP-2) permettant ainsi l'internalisation des récepteurs via des vésicules couplées à la clathrine.

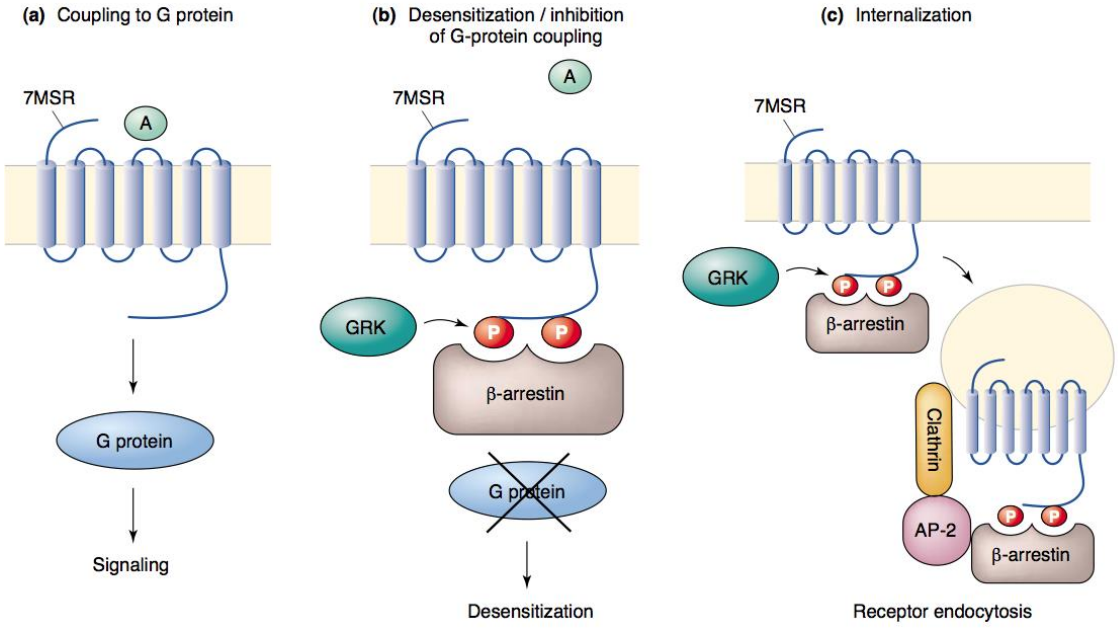


Figure 9 : Mécanisme d'action de la protéine β-arrestine

(a) les RCPG sont des récepteurs à 7 domaines transmembranaires (7 MSR) activés par un agoniste, activant ainsi un second messager et induisant le déclenchement des voies de signalisation ; **(b)** Le récepteur est d'abord phosphorylé par les enzymes kinases GRK qui reconnaissent le signal puis, la β-arrestine est recrutée. Elle se loge à la place de la protéine G, achevant le mécanisme de désensibilisation du récepteur et empêchant la transduction du signal ; **(c)** Une fois fixée au récepteur, la β-arrestine est capable de se lier à la machinerie d'endocytose (issu de Lefkowitz et al., 2004).

Bien que la plupart des recherches concernant les processus de désensibilisation et d'internalisation ait été réalisée avec des récepteurs β_2-adrénergiques comme modèle, ce processus régule la fonction de nombreux autres RCPG dont les récepteurs sérotoninergiques, dopaminergiques, muscariniques et cholinergiques (Schreiber et al., 2009).

4.4 Implication de la protéine β-arrestine 1 dans les troubles de l'humeur

De nombreuses études indiquent que les β-arrestines sont des protéines qui régulent le récepteur couplé au protéine G, jouant un rôle majeur dans la pathophysiologie des troubles de l'humeur et dans les mécanismes d'action des antidépresseurs (Alam et al., 2015; Avissar et al., 2004; Beaulieu et al., 2008; David et al., 2009; Golan et al., 2010; Matuzany-Ruban et al., 2005; Schreiber et al., 2009). La cascade de signalisation impliquant la β-arrestine présenterait un fort intérêt pour sa potentielle utilisation comme biomarqueur biochimique en pratique clinique pour la pathologie de la dépression et la prédiction de réponse aux antidépresseurs (pour revue, voir Schreiber et al., 2009).

En effet, l'équipe d'Avissar fut la première à mettre en évidence chez l'Homme un lien entre l'expression de la protéine β-arrestine 1 dans les leucocytes périphériques, les épisodes dépressifs majeurs et la réponse au traitement (Avissar et al., 2004). La protéine β-arrestine 1 a été mesurée dans des leucocytes mononucléaires de patients déprimés et il a ainsi été démontré que chez ces patients, l'expression de β-arrestine 1 est significativement diminuée en comparaison avec les patients sains (Figure 10A).

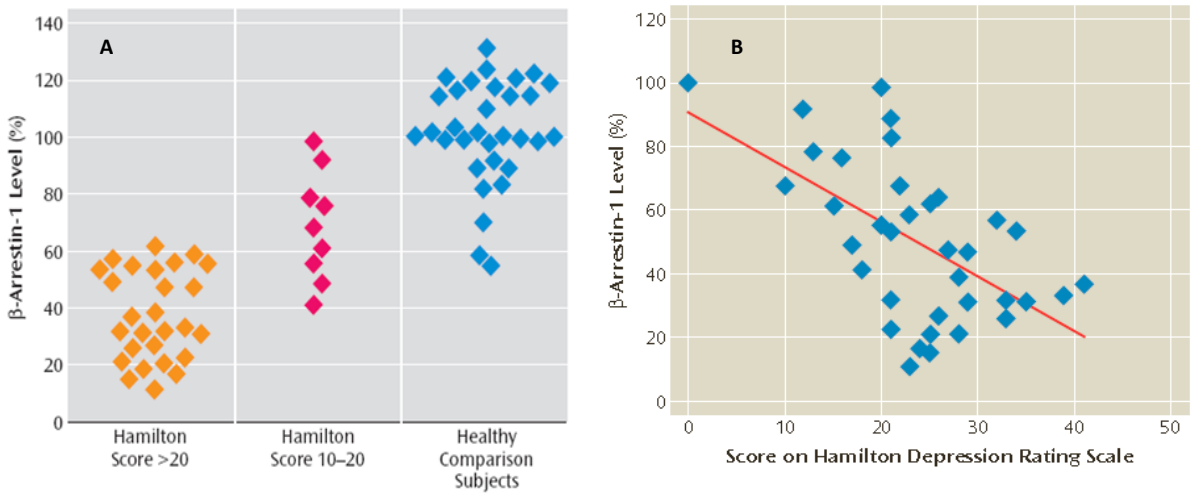


Figure 10 : **Expression de la β-arrestine 1 réduite dans les leucocytes de patients déprimés et corrélation entre les concentrations de β-arrestine 1 et la sévérité des symptômes dépressifs. (A)** L'expression de β-arrestine 1 est significativement réduite dans les cellules leucocytaires mononucléaires de patients souffrant d'épisode dépressif majeur selon l'échelle d'Hamilton en comparaison avec des volontaires sains ; **(B)** : le degré de la réduction des concentrations de β-arrestine 1 est corrélée de manière significative à la sévérité des épisodes dépressifs. D'après (Avissar et al., 2004).

Par ailleurs, les taux protéiques de β-arrestine 1 sont corrélés à la sévérité de la symptomatologie dépressive (Figure 10B) (Avissar et al., 2004; Schreiber et al., 2009). Dans la même étude, les auteurs ont montré que la diminution de l'ARNm codant pour la protéine β-arrestine 1 était corrigée par un traitement chronique par antidépresseurs (Figure 11A). Chez des rats naïfs, les IRSS, les IRSN et certains antidépresseurs non sélectifs de la recapture de la sérotonine (imipramine, desipramine) élèvent significativement les niveaux de β-arrestine au niveau du cortex et de l'hippocampe (Avissar et al., 2004; Beaulieu et al., 2008; David et al., 2009). De la même façon, l'expression de la β-arrestine a été trouvée diminuée dans l'hypothalamus et dans l'hippocampe chez des souris anxio-déprimés ayant été exposées à une administration chronique de corticostéroïdes. Un traitement chronique avec de la fluoxetine est capable de restaurer cette diminution (David et al., 2009). Enfin, la signalisation des β-arrestines 1 et 2 est impliquée dans la médiation de la réponse à la fluoxetine et au lithium (Beaulieu et al., 2008; David et al., 2009).

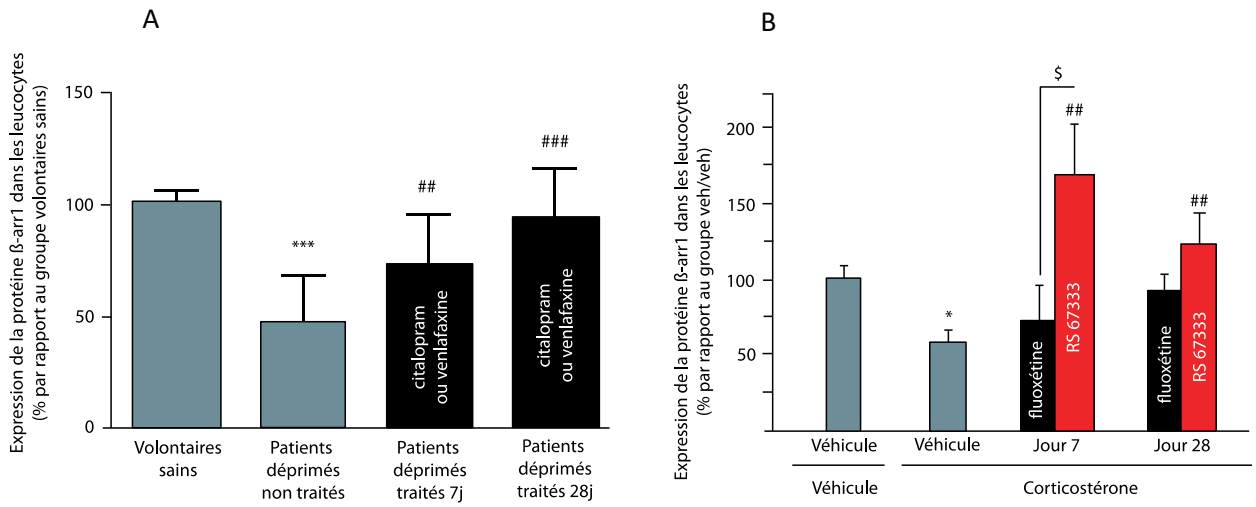


Figure 11: **Expression de la protéine ß-arrestine 1 dans les leucocytes de patients déprimés ou de souris anxio-déprimées non traité(e)s et après traitements antidépresseurs**. **(A)** Les patients déprimés montrent une diminution de l'expression de la β-arrestine 1 dans les leucocytes, rétablie par un traitement par le citalopram ou la venlafaxine. **(B)** Cette diminution, retrouvée chez les animaux présentant un phénotype de type anxio/dépressif est corrigée après un traitement chronique par la fluoxetine ou par le RS67333. ***p<0.001 *vs* volontaires sains ; ##p<0.01 et ###p<0.001 *vs* patients déprimés non traités.*p<0.05 vs Véhicule/Véhicule ; ##p<0.01 vs CORT/Véhicule ; $<0,05 vs CORT/Fluoxetine (Avissar et al., 2004 et (Mendez-David et al., 2015a)).

En se basant sur ces résultats précliniques, notre laboratoire a développé une technique permettant d'extraire et d'isoler les PBMCs du sang total d'un modèle de souris anxio-dépressives (Mendez-David et al., 2013). Dans un premier temps, les résultats cliniques ont été confirmés : les animaux traités par la CORT pendant 4 semaines affichant un phénotype anxio/dépressif, présentent une diminution de l'expression de la protéine β-arrestine 1 dans les PBMCs, une altération qui est

corrigée après 28 jours de traitement par la fluoxetine (18 mg/kg/j) (Mendez-David et al., 2013). D'autre part, cette expérience a été réitérée en comparant l'effet de la fluoxetine par rapport à celui du RS67333 sur l'expression de la protéine β-arrestine 1 dans les PBMCs. Il a été montré que la fluoxetine (18 mg/kg/j) et le RS67333 (1,5 mg/kg/j) corrigeaient tous les deux la diminution de l'expression de la protéine β-arrestine 1 périphérique induite par la corticostérone, mais que cette correction était observable après seulement 7 jours de traitement par l'agoniste du récepteur 5-HT_4 contre 28 jours pour la fluoxetine (Figure 11B). L'expression de la protéine β-arrestine 1 dans les PBMCs est donc un biomarqueur prédictif de la réponse thérapeutique utilisable chez le rongeur et chez l'Homme.

Bien que des interrogations subsistent encore sur l'identité du (des) récepteur(s) sérotoninergique(s) impliqués dans l'interaction avec la protéine β-arrestine 1, ces derniers résultats précliniques semblent indiquer que le récepteur 5-HT_4 serait un bon candidat pour participer aux effets comportementaux et neurogéniques induits par la fluoxetine, en interaction avec la protéine β-arrestine 1.

4.5 Les interactions entre le récepteur 5-HT_4 et la protéine β-arrestine 1

Les membres de la famille des arrestines sont connus pour participer à la transmission du signal intracellulaire mais aussi à la régulation de l'activité des récepteurs couplés aux protéines G (RCPG), dont fait partie le récepteur sérotoninergique de type 4 (5-HT_4) (Barthet et al., 2009). Plus précisément, la protéine β-arrestine 1 est une protéine cytosolique interagissant avec les RCPGs entrainant leur désensibilisation et leur internalisation suite à l'interaction avec leurs ligands (Vilaro et al., 2005) (Figure 12).

La désensibilisation du récepteur 5-HT_4 peut se faire de trois façons différentes (Mnie-Filali and Piñeyro, 2012) :

- La désensibilisation hétérologue affectant peu les récepteurs sérotoninergiques
- La désensibilisation homologue, phosphorylation-indépendante ou phosphorylation-dépendante médiée par une protéine de la famille des sérine/thréonine kinases, la GRK
- La désensibilisation ligand-spécifique

L'internalisation quant à elle, peut se faire selon trois processus (Mnie-Filali and Piñeyro, 2012) :

- L'internalisation constitutive du récepteur
- L'internalisation du récepteur 5-HT$_4$, médiée par GRK2, isoforme-spécifique
- L'internalisation ligand-spécifique

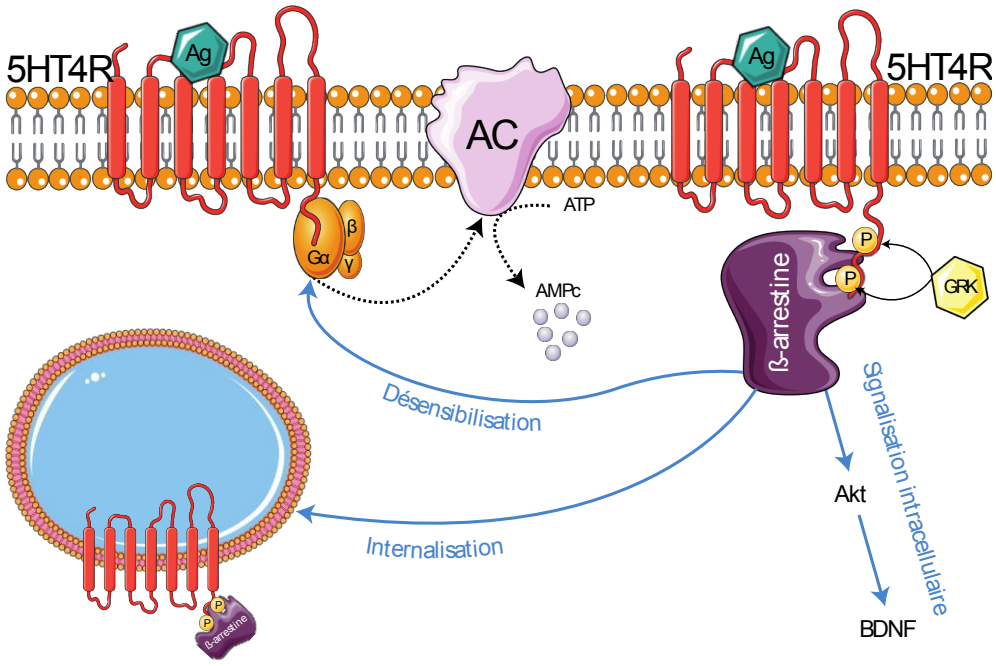


Figure 12: Interactions entre la protéine ß-arrestine 1 et le récepteur 5-HT$_4$. La voie d'activation classique du récepteur 5-HT$_4$ implique le recrutement d'une protéine Gs, qui active l'adénylate cyclase, responsable d'une production accrue d'AMPc. Par ailleurs, il existe une seconde voie impliquant le recrutement de la protéine ß-arrestine 1. Cette protéine est responsable de la désensibilisation et de l'internalisation du récepteur 5-HT$_4$, mais aussi dans la transmission du signal intracellulaire. Abréviation : **5-HT4R** : récepteur sérotoninergique de type 4 ; **AC** : adénylate cyclase ; **Ag** : agoniste ; **AMPc** : adénosine monophosphate cyclique ; **ATP** : adénosine triphosphate ; **BDNF** : brain-derived neurotrophic factor ; **GRK** : G-protein receptor kinase ; **P** : sites de phosphorylation (d'après Mnie-Filali & Piñeyro, 2012)

En 2005, la kinase GRK2 a été identifiée comme la kinase responsable de la désensibilisation du récepteur 5-HT$_4$ médiée par la signalisation G(s) (Barthet et al., 2005; Bockaert et al., 2008). A l'aide d'études *in vitro* utilisant des neurones primaires issus de *colliculi* ou des cellules HEK293, ces auteurs ont démontré que le découplage du récepteur 5-HT$_4$ de sa protéine G(s) et l'endocytose « β-arrestine-dépendante » du récepteur sont des évènements moléculaire distincts impliquant différemment les GRKs (Barthet et al., 2009; Bohn and Schmid, 2010; Marin et al., 2012). Par exemple, le découplage du récepteur 5-HT$_4$ des protéines Gαs nécessite la présence de GRK2 mais ne

dépend pas de son activité kinase (Barthet et al., 2005; Dumuis et al., 1988). En revanche, le recrutement des β-arrestines, permettant l'internalisation du récepteur, nécessite la phosphorylation du récepteur par GRK2 au niveau d'un groupement de sérines et de thréonines situées dans le domaine C-terminal du récepteur. Il semblerait que cette phosphorylation du domaine C-terminal du récepteur 5-HT_4 et ses groupes Ser/Thr soit indispensable à l'implication de la β-arrestine.

Une caractérisation plus complète a montré une interaction constitutive du récepteur 5-HT_4 avec la GRK5 et affirme que les effets de cette interaction contribuent à l'activité basale constitutive du récepteur comme précédemment reportée (Barthet et al., 2009; Claeysen et al., 1999). Ces études sont basées sur le rôle de la GRK5 dans la désensibilisation de la voie indépendante de la protéine G et ont identifié la β-arrestine 1 comme la protéine responsable de la régulation négative du récepteur 5-HT_4 médiée par la voie Src/ERK. Similairement, ce procédé de désensibilisation nécessite 2 complexes moléculaires dont le recrutement de la β-arrestine 1 aux groupements phosphorylés sérine/thréonine situé sur le domaine C-terminal du récepteur (Figure 13A) et une phosphorylation directe (sur la sérine 412) de la β-arrestine 1 par la GRK5 pour permettre l'inhibition de la désensibilisation du récepteur 5-HT_4 opérée par la voie Src/ERK (Figure 13B). Ces études ont démontré le rôle d'un membre de la famille GRK (GRK5) dans la désensibilisation de la voie indépendante de la protéine G et ont identifié la β-arrestine 1 comme un substrat de GRK5 en plus de ces substrats classiques que sont les RCPG. Ces travaux ont aussi montré que l'engagement de la voie ERK 1,2 (impliquée dans la modulation des fonctions cognitives) par les récepteur 5-HT_4 et sa régulation est dépendante d'un complexe « réceptosome » incluant au moins Src, GRK5, et la β-arrestine 1 (Barthet et al., 2009; Marin et al., 2012).

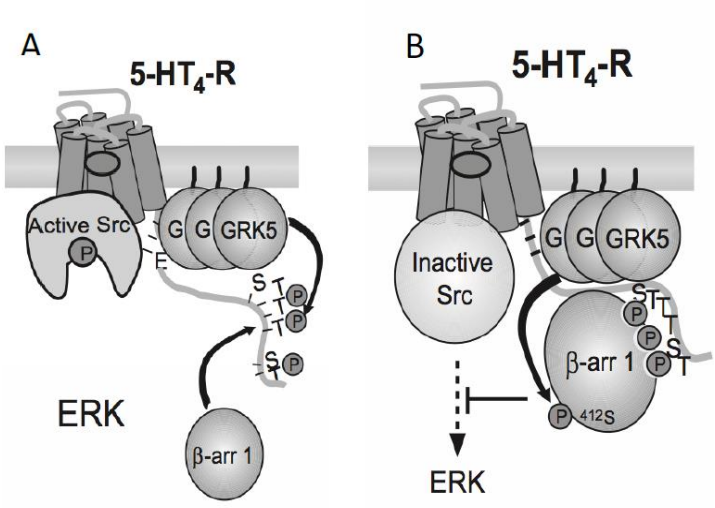


Figure 13: Modèle de désensibilisation par GRK5 du récepteur 5-HT_4 opéré par la signalisation ERK (Bockaert et al., 2008)

Puisque l'expression de GRK5 et de la β-arrestine 1 augmente considérablement pendant le développement (Gurevich et al., 2004), il est probable que la régulation négative du récepteur 5-HT_4 médiée par l'activation de la voie Src/ERK indépendante de la protéine G soit plus forte chez les adultes. De plus, GRK5 et la β-arrestine 1 jouent un rôle critique dans la détermination de la voie de signalisation (Gs ou ERK) recrutée via l'activation du récepteur 5-HT_4. Ce rôle pourrait être déterminant dans les phénomènes de plasticité synaptique dépendante du 5-HT_4 et de consolidation de la mémoire (Kemp and Manahan-Vaughan, 2005; Marchetti et al., 2004; Micale et al., 2006).

Deux études précliniques menées chez le rat ont montré que l'administration chronique de fluoxetine (Vidal et al., 2009) ou de venlafaxine (Vidal et al., 2010) étaient responsables d'une désensibilisation du récepteur 5-HT_4 et/ou de l'activation du rétrocontrôle négatif induit par le récepteur 5-HT_4. En effet, ces deux études ont montré une diminution de la densité de récepteurs 5-HT_4, notamment dans les ganglions de la base et dans l'hippocampe, une diminution de l'activité de l'AC induite par l'activation du récepteur 5-HT_4 et une diminution de l'excitabilité neuronale 5-HT_4-dépendante dans la zone CA1 de l'hippocampe. Ces altérations conduisent à une diminution de l'intensité de la réponse cellulaire malgré l'administration continue d'antidépresseur. Fait surprenant, le RS67333 à la dose de 1,5 mg/kg/j n'induit pas de tolérance puisque ses effets de type anxio/dépressif persistent plusieurs jours après l'administration (Lucas et al., 2007). Cette observation est pour le moins inattendue puisque les récepteurs 5-HT_4 sont connus pour se désensibiliser très rapidement après exposition à un agoniste (Ansanay et al., 1992).

Objectifs de la thèse

Différents troubles cognitifs (difficulté à se concentrer, perception négative de stimuli normalement perçus comme positifs) figurent parmi les critères de diagnostic d'un épisode de dépression majeure selon le DSM-5. Malheureusement, la prise en charge actuelle des patients dépressifs ne prend pas systématiquement en compte l'altération des fonctions cognitives dans la mise en place du traitement de première attention. En outre, l'efficacité d'une prise en charge spécifique des troubles de l'apprentissage et de la mémoire dans le contexte de la dépression par des antidépresseurs reste à ce jour non résolue.

Le premier objectif de ce travail a été de **caractériser les troubles cognitifs suspectés dans un modèle d'anxiété/dépression basé sur l'administration chronique de corticostérone : le modèle CORT (Article 1)**. Ces travaux s'inscrivent dans la caractérisation phénotypique complète de ce modèle animal d'anxiété/dépression faite au sein de l'UMRS 1178 *via* la description des troubles cognitifs en tant que facteurs de co-morbidité des troubles anxio/dépressifs.

Les antidépresseurs sont les molécules indiquées en première intention dans le traitement de la dépression majeure, mais la plupart de ces traitements sont aujourd'hui limités par plusieurs facteurs, tels que l'important nombre de patients non-répondeurs au traitement, le long délai d'action avant l'apparition des premiers effets thérapeutiques, les nombreux effets indésirables et l'absence d'efficacité contre les symptômes cognitifs étroitement liés aux symptômes dépressifs. De ce fait, le développement de nouvelles molécules plus efficaces, ayant des propriétés antidépressives rapides et traitant simultanément les altérations cognitives associées à la dépression, devient indispensable. **Le récepteur 5-HT_4 semble être une cible à privilégier car si son implication dans le traitement des troubles cognitifs chez des animaux naïfs est à ce jour bien décrit. L'efficacité thérapeutique des ligands du récepteur 5-HT_4 dans le traitement des troubles de l'humeur reste à confirmer.**

De nombreuses études se sont intéressées aux propriétés antidépresseurs des agonistes du récepteur 5-HT_4 (Lucas et al., 2007; Pascual-Brazo et al., 2012). Cependant, peu d'entre elles conduisent leurs expérimentations sur des animaux anxio-déprimés (**Article 2**) et moins encore se sont concentrées sur les propriétés pro-cognitives des agonistes du récepteur 5-HT_4 dans un contexte d'anxiété/dépression.

Le second objectif de ce travail a donc été de **corriger les troubles cognitifs observés chez les animaux anxio/dépressifs (modèle CORT) à l'aide de différentes stratégies thérapeutiques**. Ainsi, les effets d'une administration chronique d'un antidépresseur monoaminergique classique

(fluoxetine) ou d'un agoniste du récepteur 5-HT_4 (RS67333) chez les animaux anxio-déprimés ont été mesurés dans plusieurs tests d'apprentissage et de la mémoire, reflétant les différents types de mémoire existantes (**Article 3**).

Récemment, la protéine β-arrestine 1 a été signalée comme étant un acteur important de la physiopathologie de la dépression ainsi que dans la prédiction de la réponse aux antidépresseurs (Avissar et al., 2004; Matuzany-Ruban et al., 2005). Des données précliniques ont validé son implication dans un modèle d'anxiété/dépression basé sur l'administration chronique de corticostérone dans différentes régions cérébrales (David et al., 2009) ainsi qu'au niveau de marqueurs périphériques (Mendez-David et al., 2013; Mendez-David et al., 2015b). Ainsi, ces travaux rapportent une diminution de l'expression de la β-arrestine 1 dans les régions de l'hippocampe et de l'hypothalamus chez des animaux anxio-déprimés (restaurée après un traitement chronique par de la fluoxetine ou un traitement sub-chronique de RS67333) et ont confirmé l'utilisation de la protéine β-arrestine 1 comme un biomarqueur potentiel de l'état dépressif et de la réponse aux antidépresseurs. Ces résultats évoquent la possibilité que l'expression de la β-arrestine 1 dans le cerveau pourrait être un substrat potentiel pour les effets comportementaux et neurogéniques des antidépresseurs.

Le 3ème objectif de cette thèse a été de **caractériser les conséquences comportementales d'une délétion sélective de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé de l'hippocampe** dans plusieurs tests prédictifs d'une activité anxiolytique et/ou antidépressive en présence ou non d'un traitement chronique à la fluoxetine. Les conséquences neurogéniques de cette délétion sélective ont également été étudiées (**Article 4**).

Enfin, les conséquences comportementales d'une délétion sélective de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé de l'hippocampe dans différents tests de cognition (mémoire de type épisodique, spatiale et associative) ont été évaluées. Au vue de la relation fonctionnelle entre le récepteur 5-HT_4 et la protéine β-arrestine 1, le dernier objectif de ce travail a été **d'étudier les effets d'un traitement chronique par un agoniste du récepteur 5-HT_4 sur les performances cognitives d'animaux dont la β-arrestine 1 a été spécifiquement supprimée**, en comparaison avec un traitement antidépresseur classique de référence (fluoxetine) (**Résultats complémentaires**).

Après une partie consacrée aux méthodes utilisées pour réaliser ces travaux de thèse, les articles expérimentaux seront insérés en anglais dans leur format de publication, précédés d'un paragraphe en français précisant la question posée et introduisant l'étude et suivis de commentaires résumant les principaux effets découverts.

Matériel et Méthodes

1 Animaux

1.1 Choix du modèle animal d'anxiété/dépression

Le développement de modèles animaux est nécessaire pour saisir non seulement les divers aspects de la pathologie humaine, comme les changements physiologiques ou comportementaux, mais aussi pour comprendre la dynamique des effets thérapeutiques et leur mécanisme d'action. La création et la validation de modèles animaux en psychiatrie restent difficiles en raison de la difficulté à modéliser certains symptômes (pensées négatives, suicide). Willner entre autres, a proposé certains critères à l'établissement d'un bon modèle animal de pathologie (Willner, 1984) : les modèles doivent répondre à une validité de face (isomorphisme), une validité prédictive (corrélations pharmacologiques) et une validité de construction (homologie et similitude dans les mécanismes neurobiologiques). Un petit nombre de modèles animaux d'anxiété et de dépression sont fréquemment utilisés pour cribler de nouveaux composés ayant un potentiel antidépresseur. Les différentes approches de modélisation animale en neuropsychologie peuvent être regroupées par approche génétique, pharmacologique, comportementale ou par lésion (Nestler and Hyman, 2010). Le Tableau 9 ci-après présente différents protocoles de stress chronique fréquemment utilisés dans les études précliniques en neuropharmacologie. Dans la suite de ce chapitre, seul le stress induit par une administration chronique de corticostérone exogène (modèle CORT) sera détaillé.

Tableau 9: Modèles chroniques d'anxiété/dépression chez le rongeur.

Type de stress	Nom du modèle	Durée du stress	Forces	Faiblesses	Références
Stress environnementaux	Stress prénatal	1 à 4 semaines	-étude de l'impact du stress pendant période de gestation sur individu adulte	-validité prédictive et de construction	(Maccari and Morley-Fletcher, 2007)
	Résignation apprise	≈10 jours	-validité prédictive, faciale et de construction	-grande variabilité d'exécution -disparition du phénotype après arrêt des chocs	(Chourbaji et al., 2005; Hajszan et al., 2009)
	Stress imprédictible modéré chronique	3 à 4 semaines	-stress légers et aléatoires -conservation des altérations induites après l'arrêt du protocole -validité prédictive, faciale et de construction	-manque de reproductibilité -protocole contraignant	(Mineur et al., 2003; Pothion et al., 2004; Willner, 2005)
Interaction sociale	Défaite sociale	Au moins 3 semaines	-validité faciale et prédictive	-protocole non réalisable chez les femelles	(Buwalda et al., 2005; Krishnan et al., 2007)
	Séparation maternelle	24h au 9ème jour ou 3-4h par jour, plusieurs jours consécutifs	-étude de l'impact des évènements stressants en début de vie sur individu adulte	-validité de construction	(Finamore and Port, 2000)
Pharmacologique	CORT	28 jours	-facilité de mise en place du protocole -bonne reproductibilité -validité faciale et prédictive	-validité de construction	(David et al., 2009; Gourley et al., 2009)

1.1.1 Modèle d'administration chronique à la corticostérone

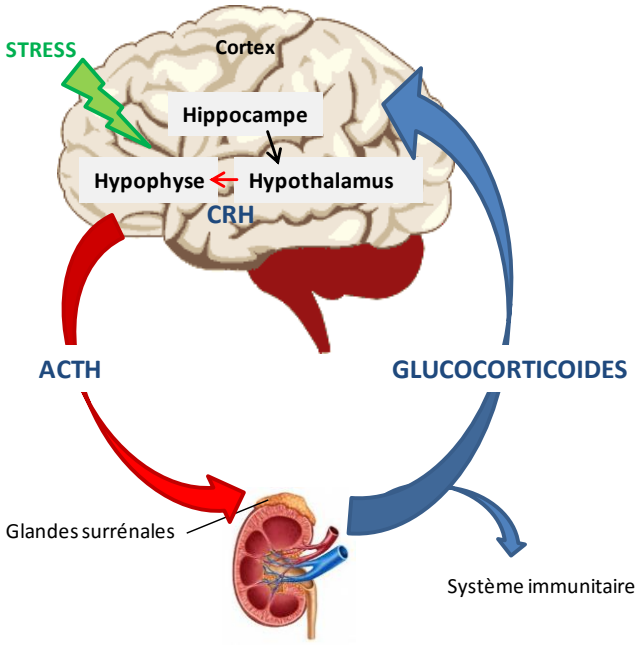


Figure 14 : Système de réponse au stress via l'axe hypothalamo-hypophyso-surrénalien.

Les glandes surrénales produisent du cortisol chez l'Homme et de la corticostérone chez la souris. **CRH** : corticotropin-releasing hormone ; **ACTH** : adrenocorticotropic hormone

Les stress sociaux ou le stress chronique induisent des perturbations de l'axe HPA chez le rongeur se matérialisant par une augmentation des concentrations plasmatiques en glucocorticoïdes (corticostérone pour les Rongeurs) (Grippo et al., 2006). Ainsi, le modèle développé à partir de l'augmentation des concentrations en glucocorticoïdes par l'apport de corticostérone exogène (CORT) soit par injection sous-cutanée, pompe osmotique ou plus simplement par l'intermédiaire de l'eau de boisson ou dans la nourriture, permet d'étudier directement l'influence des glucocorticoïdes sur le développement du phénotype anxio/dépressifs (Figure 14).

1.1.1.1 Conséquences phénotypiques comportementales d'un régime chronique de corticostérone chez le rongeur

1.1.1.1.1 Modifications du comportement d'anxiété et de dépression

L'administration répétée de CORT semble inhiber le comportement sexuel (Gorzalka et al., 2001), provoque une diminution de la prise de saccharose (Gourley and Taylor, 2009), mais également une diminution de la réponse à un renforcement alimentaire (Gourley et al., 2008) ainsi que du toilettage (David et al., 2009), tous ces paramètres étant indicateurs d'une anhédonie. En plus de l'anhédonie apparente, il apparaît que les animaux développent un phénotype anxio/dépressif à travers l'évaluation comportementale dans le labyrinthe en croix surélevée (Pego et al., 2008), la

double enceinte éclairée (Murray et al., 2008), le test d'odeur de prédateur (Kalynchuk et al., 2004), ou encore dans le test d'hypophagie induite par la faim, et le test du champ ouvert(David et al., 2009; Gregus et al., 2005). L'apparition d'un phénotype de dépression sans phénotype anxieux est rarement référencée chez les patients (Mineka et al., 1998), ce qui renforce les résultats obtenus par ce modèle. D'autres marqueurs physiologiques d'anxiété/dépression sont également altérés, notamment la dérégulation des fonctions et des concentrations des acteurs de l'axe HPA, la prise de poids et l'atrophie des glandes surrénales (Gourley et al., 2008; Murray et al., 2008). Cependant, de réelles données relatives aux constituants neurobiochimiques de l'axe HPA durant le protocole de stress restent à ce jour peu renseignées. Par ailleurs, la majorité des comportements induits par ce modèle est corrigée par l'administration chronique d'antidépresseurs (Ago et al., 2008; David et al., 2009; Rainer et al., 2012), supportant la validité prédictive de modèle préclinique de la dépression humaine.

1.1.1.1.2 Modifications comportementales des capacités cognitives

En plus des modifications comportementales modélisant les symptômes de la pathologie de la dépression, l'administration de CORT ainsi que de nombreux modèles d'anxiété/dépression provoquent également des changements au niveau de la plasticité synaptique, souvent révélés par des déficits comportementaux d'apprentissage et de mémoire. Les modifications comportementales induites par le modèle CORT sont présentées dans la **Revue** "**Cognitive dysfunction in major depressive disorder : A translational review in Animal models of the disease »** disponible dans la partie introductive de cette thèse et seront développées dans la partie Discussion.

1.1.1.2 Conséquences structurelles et moléculaires d'un régime chronique par de la corticostérone

Au-delà des modifications comportementales induites par l'administration chronique de CORT, ce modèle provoque également des modifications structurales liées à la plasticité et notamment la diminution du processus de prolifération cellulaire et de la neurogenèse dans l'hippocampe (David et al., 2009; Malberg et al., 2000). Plusieurs études ont pu mettre en évidence que les atteintes de la plasticité se localisent dans les régions cérébrales clefs impliquées dans la dépression. En effet, il a été observé un remodelage dendritique induit par la CORT dans

l'hippocampe (Magarinos et al., 1999; Southwick et al., 2005; Watanabe et al., 1992), l'amygdale (Mitra and Sapolsky, 2008), et le cortex préfrontal (Seib and Wellman, 2003). Ces résultats sont également retrouvés après des études *post mortem* de patients souffrant de dépression (Konarski et al., 2008). En particulier, il est connu que l'exposition prolongée à la CORT induit une atrophie dendritique des cellules pyramidales de l'hippocampe, et une perte de volume des dendrites apicaux des régions CA3 et CA1 (Magarinos et al., 1999). D'autre part, bien qu'étant également le siège de la neurogenèse chez le rongeur adulte, les effets des glucocorticoïdes dans le bulbe olfactif adulte restent moins étudiés. Néanmoins, une étude récente a mis en évidence la présence de déficits olfactifs chez les animaux traités chroniquement par de la corticostérone, partiellement restaurés par un traitement chronique par de la fluoxetine (Siopi et al., 2016).

L'administration de CORT chez l'Animal et chez l'Homme est associée à une réduction du facteur de transcription de l'élément de réponse liant l'AMPc appelé CREB (Pittenger and Duman, 2008). Fait intéressant, l'activation de CREB induit la neurogenèse alors que son inhibition la réduit (Nakagawa et al., 2002), faisant de CREB un acteur important de la stabilité neuronale associée au stress et à la dépression. Entre autres, CREB contrôle des gènes effecteurs, eux aussi impliqués dans la stabilité de la plasticité synaptique. L'un de ces gènes est celui codant pour le « brain derived neurotrophic factor » (BDNF), qui joue un rôle critique sur la plasticité dans le développement, dans la survie et la fonction des neurones (Pittenger and Duman, 2008). Chez l'animal, l'exposition aux glucocorticoïdes altère aussi bien CREB que le BDNF dans les différentes régions limbiques (Gourley et al., 2008; Jacobsen and Mork, 2006), également chez les patients atteints de dépression (Dwivedi et al., 2003; Sen et al., 2008).

1.2 Souris tissus-spécifiques Nestine-Cre^{ERT2}/FLOX ARRB1

1.2.1 Génération de la lignée

1.2.1.1 Caractéristique de la lignée FLOX ARRB1

Les souris FLOX β-Arrestine 1 (ARRB1) ont été générées initialement par le laboratoire du Professeur Jean-Martin BEAULIEU à l'Université de Laval, Québec, Canada. La lignée a ensuite été transférée à l'animalerie Centrale de la Faculté de Pharmacie. J'ai donc prise en charge la gestion de cette lignée au cours de ma thèse.

La lignée de souris FLOX ARRB1 a été créée en suivant la méthode de transgénèse classique par recombinaison homologue permettant l'invalidation du gène β-Arrestine 1 (construction génétique non publiée) (Figure 15).

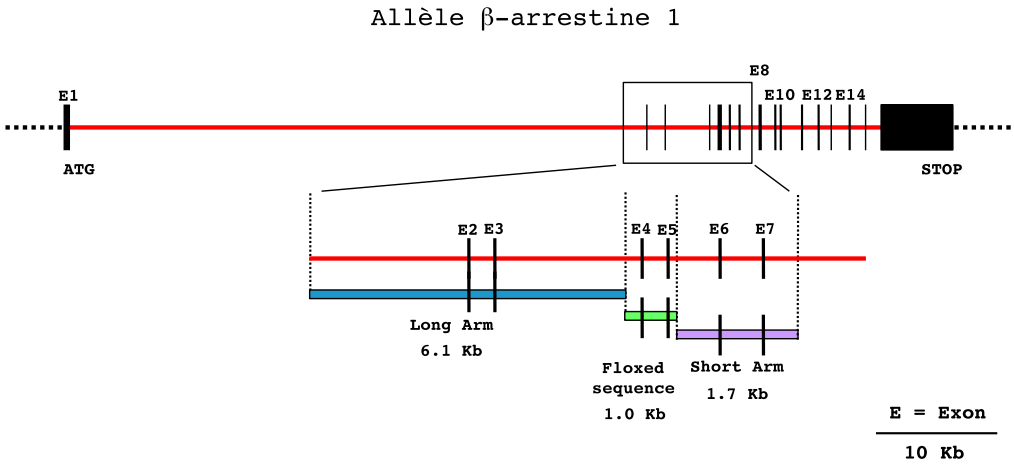


Figure 15: Schématisation du gène β-arrestine 1 de la souris. L'agrandissement de la région encadrée situe les trois fragments d'ADN utilisés pour la construction du vecteur de délétion pEZ-βarr1. Les fragments longs (bleu) et courts (violet) servent à provoquer une recombinaison homologue lorsque la construction est transfectée dans les cellules souches embryonnaires. Le fragment FLOX (trait vert) servira ultérieurement à produire la lignée de souris βarr1-FLOX. La taille respective de chacun des fragments utilisés est indiquée. Le trait rouge horizontal représente l'ADN intronique, les traits noirs horizontaux représentent l'ADN chromosomique situé à l'extérieur du gène β-arrestine 1, les traits noirs verticaux représentent les exons (E).

1.2.1.2 Caractéristiques de la lignée Nestine-Cre ERT2

La lignée de souris Nestine-CreER^{T2}, initialement créée par l'équipe du Professeur René Hen de l'Université de Columbia, New York, USA (Sahay et al., 2011) a été transférée à l'animalerie Centrale de la Faculté de Pharmacie. La recombinase Cre (pour « **C**auses **RE**combination ») est une enzyme tyrosine capable de catalyser la recombinaison homologue entre deux sites appelés loxP situés autour d'une séquence ADN spécifique. Pour permettre le contrôle temporel et spatial de l'expression de cette enzyme, la région promotrice Nestine est insérée dans la construction génétique. Elle permet l'expression spécifique dans les cellules souches neurales et les progéniteurs intermédiaires (Dranovsky and Hen, 2006). De plus, l'enzyme Cre a été fusionnée au domaine muté humain du récepteur aux oestrogènes (ERT2) donnant l'avantage à ce système d'être inductible dans le temps, contrairement aux modèles conditionnels classiques. Ainsi, la mutation n'est donc pas exprimée à la naissance des animaux mais est induite durant la période propice à l'étude. Afin de s'assurer que la recombinase Cre est active uniquement dans la région d'intérêt, les souris Nestine-

CreER^{T2} ont originellement été croisées avec des souris ROSA26R contenant une protéine fluorescente EYFP (Srinivas et al., 2001) (Figure 16).

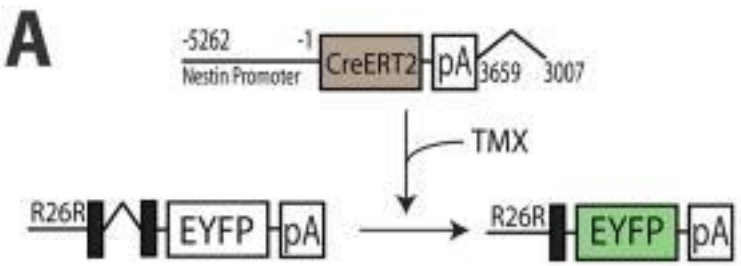


Figure 16: Insertion génétique du gène CreER^{T2} pour confirmer la spécificité de la construction génétique (Sahay et al., 2011)

1.2.1.3 Obtention des souris tissus-spécifiques Nestine-CreER^{T2} / ARRB1$^{-/-}$

Les souris Nestine-CreER^{T2}ont été croisées avec des souris FLOX β-Arrestine 1. Lorsqu'ils atteignent l'âge de se reproduire, les descendants de ce croisement sont ensuite croisés entre eux (souris β-Arrestine 1$^{FLOX/-}$/Nestine-CreER$^{T2-/+}$ avec souris β-Arrestine 1$^{FLOX/-}$/ Nestine-CreER$^{T2\ -/-}$) pour obtenir les animaux tissus-spécifiques à induire (Figure 17). Les souris Nestine-CreER^{T2} doivent impérativement être porteur du gène CRE à l'état hétérozygote pour éviter une hyper expression de la recombinase Cre et ainsi induire des délétions ectopiques (i.e., non tissus-spécifiques) (Sun et al., 2014). Dans toutes les expériences, les animaux FLOX β-Arrestine 1$^{-/-}$/ Nestine-CreER$^{T2\ -/+}$ sont utilisés comme contrôles.

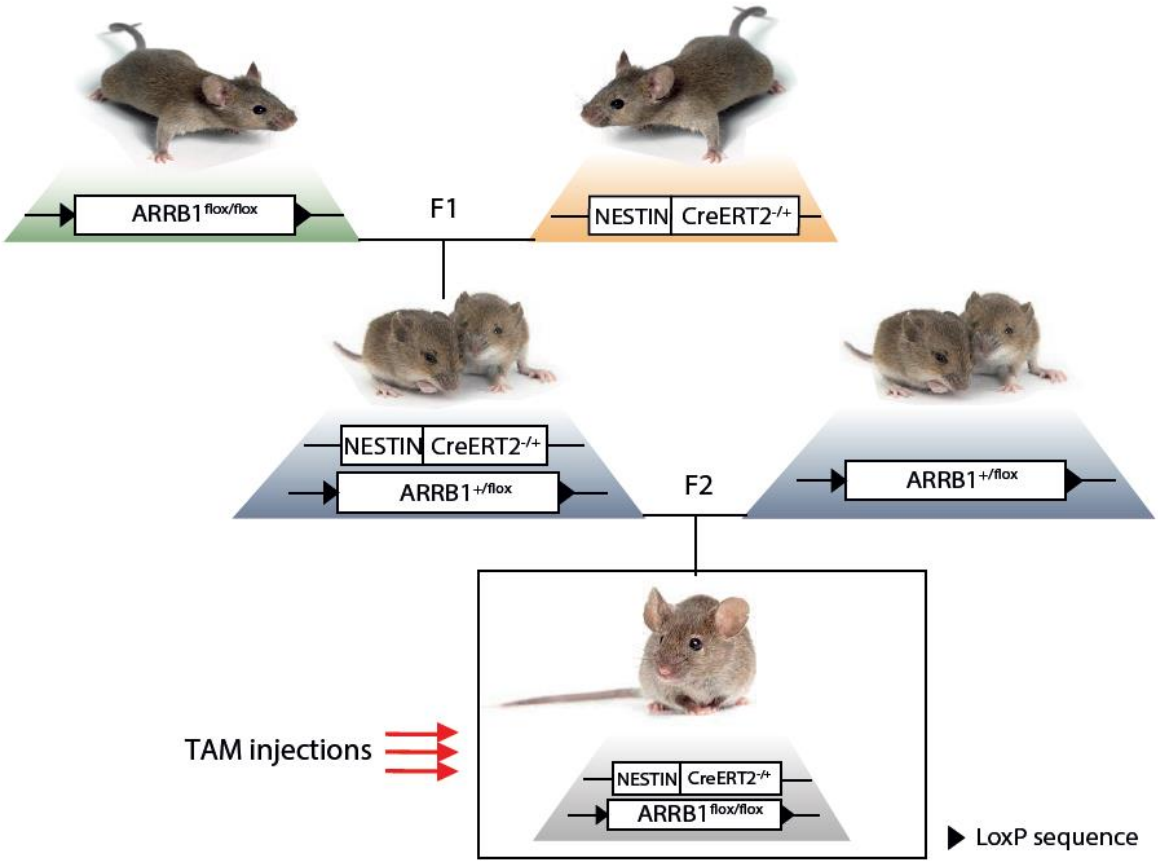


Figure 17: Schéma d'obtention des souris β-Arrestine 1$^{FLOX/FLOX}$/Nestine-CreER$^{T2-/+}$. Plusieurs croisements successifs sont nécessaires pour obtenir les souris tissus-spécifiques inductibles. Cinq jours d'injections par du tamoxifène à raison de 2 mg par jour permettent de provoquer la suppression génétique.

1.2.2 Induction de la délétion spécifique de la β-arrestine 1

1.2.2.1 Préparation du tamoxifène

La préparation et l'injection de tamoxifène (TAM) ont été réalisées comme décrit dans Sahay et al., 2011. Brièvement, les souris FLOX β-Arrestine 1$^{-/-}$/ Nestine-CreER$^{T2\ -/+}$ et FLOX β-Arrestine 1$^{+/+}$/ Nestine-CreER$^{T2\ -/+}$ âgées de 8 semaines ont reçu une injection par voie intra-péritonéale de 2 mg de tamoxifène (Sigma Aldrich, St Quentin Fallavier, France), une fois par jour pendant cinq jours consécutifs. Préalablement dilué dans de l'éthanol 100%, le tamoxifène est dissous de l'huile de maïs (Corn oil 90%/EthOH 10%) (Corn oil, Sigma Aldrich, St Quentin Fallavier, France) puis vortexé à vitesse maximale pendant 5 minutes. Pour que les bulles générées par le vortex disparaissent, la préparation est incubée à 37°C pendant 10 minutes. Celle-ci reste stable 4 à 5 jours à 4°C. Le

tamoxifène étant photosensible, la solution est conservée à l'abri de la lumière le plus possible. Après le schéma d'injection, la délétion requiert 5 à 6 jours pour être effective.

1.2.2.2 Mécanisme moléculaire de la délétion spécifique de la β-arrestine 1

En absence du ligand approprié, la recombinase Cre, fusionnée à ER^{T2}, est liée à la protéine HSP90 (Heat Shock protein) dans le cytoplasme, empêchant le complexe $CreER^{T2}$ de rentrer dans le noyau. Suite à l'administration du tamoxifène (antagoniste des récepteurs aux œstrogènes) et à sa liaison au récepteur à l'œstrogène muté ER^{T2}, HSP90 se dissocie du complexe $CreER^{T2}$ qui peut alors initier sa translocation dans le noyau sous forme activée. Une fois dans le noyau, $CreER^{T2}$ reconnait les sites loxP sur l'allèle conditionnel du gène de la β-Arrestine 1 et procède à la recombinaison (Figure 18).

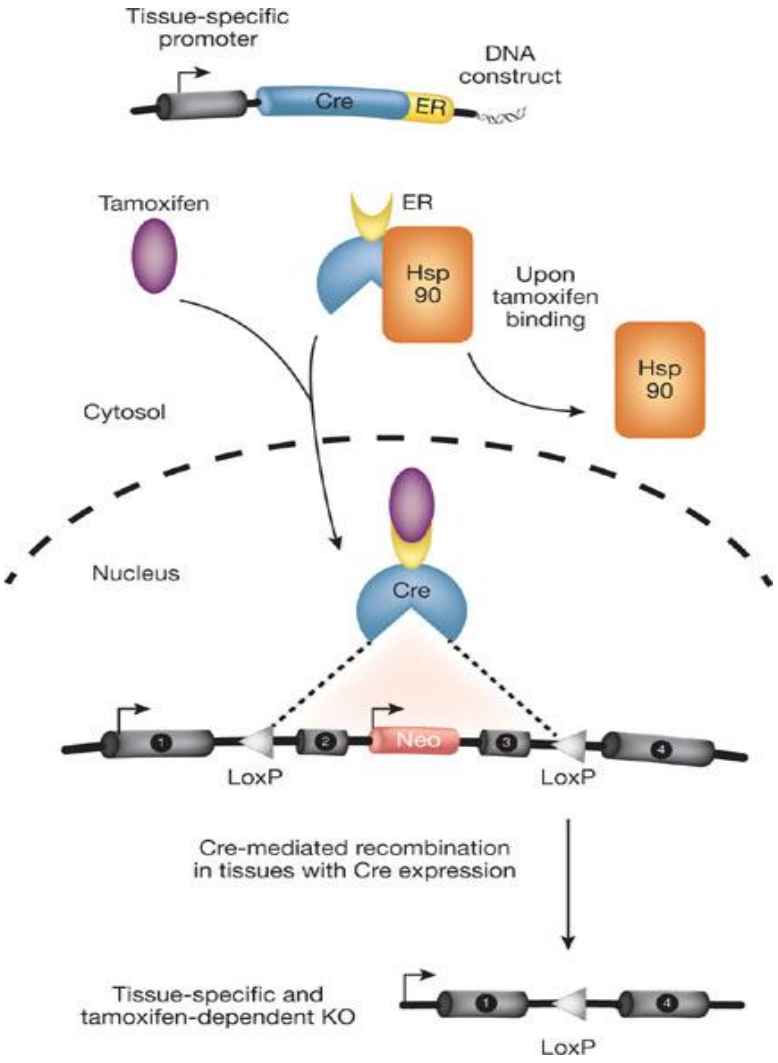


Figure 18: Système Cre-ER^{T2}-loxP inductible au tamoxifène (Gunschmann et al., 2014)

Ce mécanisme n'est possible que dans les cellules qui expriment Cre sous le promoteur Nestine, permettant ainsi une délétion tissu-spécifique de la protéine β-Arrestine 1 au niveau des cellules souches neurales du GD de l'hippocampe. Dans les autres types cellulaires dans lesquels le gène Cre n'est pas exprimé, le gène cible β-Arrestine 1 sera toujours présent dans le génome et fonctionnera donc normalement.

1.2.3 Génotypage des animaux

Le génotypage des différentes souris mutées a été réalisé grâce à la technique d'Amplification en Chaîne par Polymérase (PCR). Ce procédé permet d'amplifier *in vitro* une région spécifique de l'ADN afin d'en produire une quantité détectable. L'ADN génomique est extrait de fragments de queues des souris. Les animaux sont génotypés à partir du 18ème jour de vie et numérotés aux oreilles à l'aide d'un poinçon adapté. Les souriceaux ARRB1$^{+/+}$ et ARRB1$^{-/-}$ sont sevrés à 21 jours après leur naissance et sont répartis dans des cages selon leur sexe avant le début des expériences.

1.2.3.1 Extraction de l'ADN

Les queues sont d'abord digérées dans un tampon de lyse pendant 2 à 3 heures à 50°C. Le tampon de lyse est constitué de Tris pH8 à 1M, Sodium Dodécyl Sulfate 10% (SDS), d'acide éthylène diamine tétra-acétique (EDTA) 0,5M, Chlorure de Sodium (NaCl) 5M, de Protéinase K complété avec de l'eau distillée. Pour une digestion optimale, les fragments de queues doivent être recouverts de tampon. Une fois dans la cuve à 55°C, les tubes seront vortexés toutes les demi-heures jusqu'à digestion complète du tissu. L'ADN est précipité grâce à l'ajout de phénol (volume identique au tampon de lyse) qui permet de séparer l'ADN du reste des éléments de la queue. Les digestats sont ensuite centrifugés pendant 10 minutes à 15000 tours/min. Le surnageant contient l'ADN. Les tubes seront conservés à -20°C pour une longue conservation.

1.2.3.2 Amplification par Polymerase Chain Reaction (PCR)

Le mélange DNTP's et ADN polymérase est utilisé sous forme de petites billes lyophilisées appelées « Ready-to-Go PCR beads » (General Electric, USA). Il suffit alors d'ajouter 1,5 µL de l'ADN

que l'on veut amplifier et 24 µL d'un mélange d'amorces dans chaque tube. Les amorces utilisées pour l'amplification de la β-arrestine 1 et de Cre sont précisées dans le Tableau 11.

Tableau 11 : Descriptif des amorces utilisées en PCR pour le génotypage des souris NestineCre ER^{T2}/$ARRB1^{-/-}$

N°	Nom	Séquence (5'-3')	Taille	PM (g/mol)	Tm (°C)
1	βarr1	GAG GGG GTT TGT ATG CTA CC	20	6204	59,4
2	βarr1	GGA GCC TGT GAA TGA TTG CT	20	6188	57,3
3	CRE up	AAT GCT TCT GTC CGT TTG C	19	5761	53,8
4	CRE down	TAG CGC CGT AAA TCA ATC G	19	5797	52,8

La réaction débute par une étape de dénaturation de l'ADN de 90 sec à 94°C et se poursuit par un certain nombre de cycles d'amplification dont les caractéristiques diffèrent en fonction du gène à amplifier (Tableau 12). Enfin, une extension finale a lieu pendant 7 à 10 min entre 65 et 72 °C, selon le gène.

Tableau 12: Programmes PCR des gènes à amplifier

Gène	**ARRB1**		**CRE**	
Etape	Durée (sec)	Température (°C)	Durée (sec)	Température (°C)
Nombre de cycles	40		3, 3, 3, 3, 25	
Dénaturation	30	93	15	95
Hybridation	30	57	15	64, 62, 60, 58, 53
Elongation	180	65	45	72

1.2.3.3 Electrophorèse

Les produits PCR obtenus sont séparés par migration électrophorétique en gel d'agarose à 2% immergés dans un tampon de Tris Borate + Bromure d'éthidium (BET). 18 µL de produit PCR + 2 µL de tampon de charge sont déposés dans chaque puit du gel. Sous l'effet du champ électrique, les différents fragments d'ADN chargés négativement migrent vers la cathode et sont séparés en fonction de leur poids moléculaire. Leur coloration par le BET permet de visualiser chaque fraction sur une table à UV. Les fragments concernant le génotypage des souris mutées pour la β-arrestine 1 étant difficile à séparer, le schéma de migration se décompose en une phase lente de migration (60 V

pendant 1h) suivie d'une phase de migration plus rapide (100 V pendant 1h). En se basant sur le poids de chaque fragment, le génotype exact de la souris peut être déterminé (Figure 19).

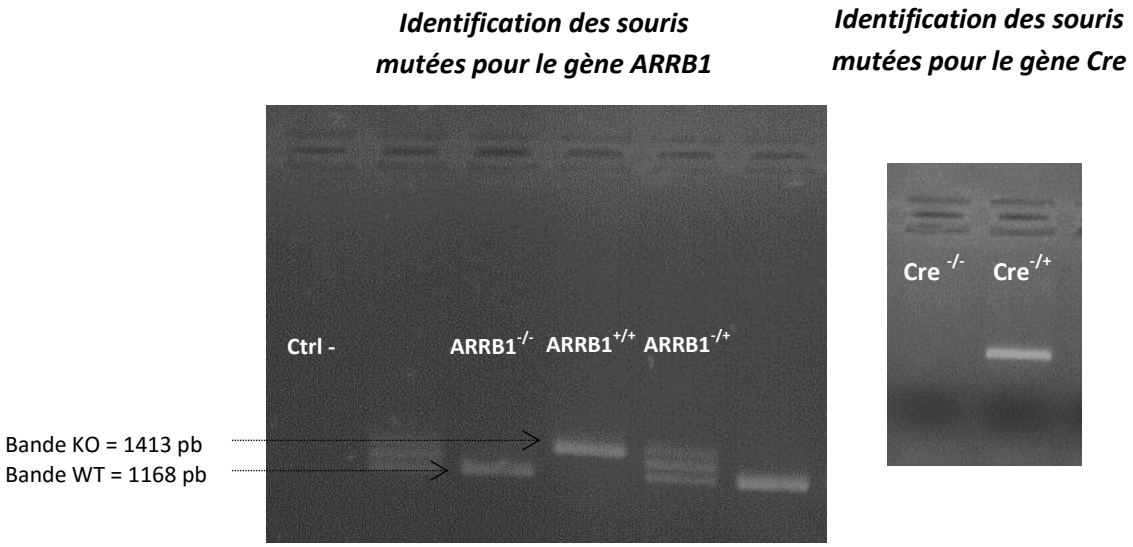


Figure 19: Génotypage des souris Nestine-CreER^{T2}/FLOX ARRB1. Les animaux porteurs des 2 séquences FLOX sont dits homozygotes (bande à 1413 paire de bases), les animaux porteurs d'une seule séquence FLOX sont dits hétérozygotes, et les animaux non porteurs de séquence FLOX sont dits Wild-Type (WT) (bande à 1168 paires de bases).

Dans le reste de ce travail, les différents génotypes des animaux seront définis comme suit :

- Les animaux ARRB1, Flox$^-$/Flox$^-$, Cre$^+$/Cre$^-$ seront notés ARRB1$^{+/+}$ ou WT
- Les animaux ARRB1, Flox$^+$/Flox$^-$, Cre$^+$/Cre$^-$ seront notés ARRB1$^{-/+}$ ou Hétérozygotes (Hets)
- Les animaux ARRB1, Flox$^+$/Flox$^+$, Cre$^+$/Cre$^-$ seront notés ARRB1$^{-/-}$ ou KO

2 Manipulations in vivo

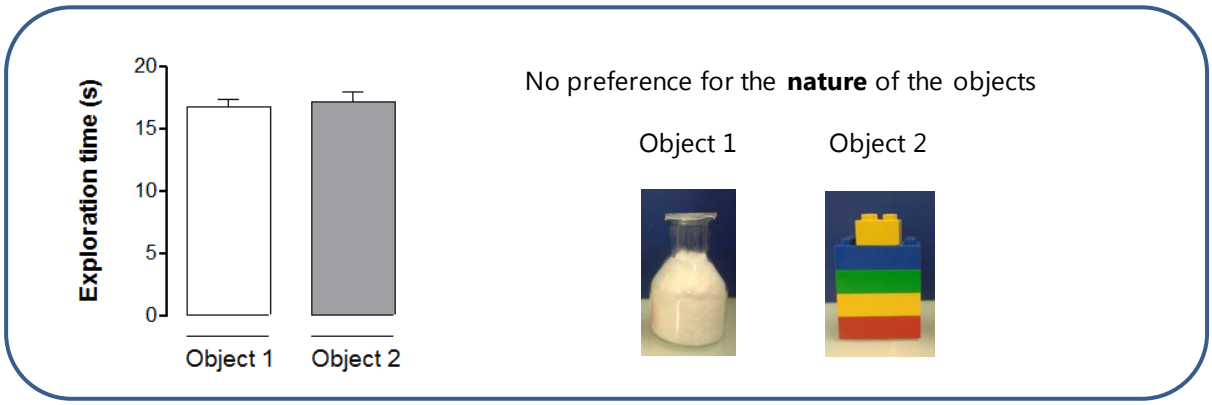
2.1 Etudes des performances mnésiques

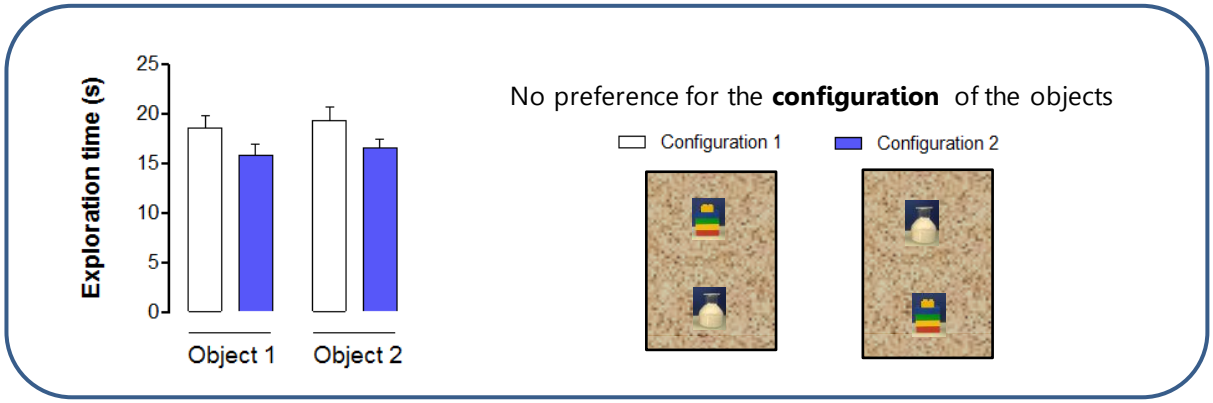
1. Fiche méthode : le test de reconnaissance d'objet

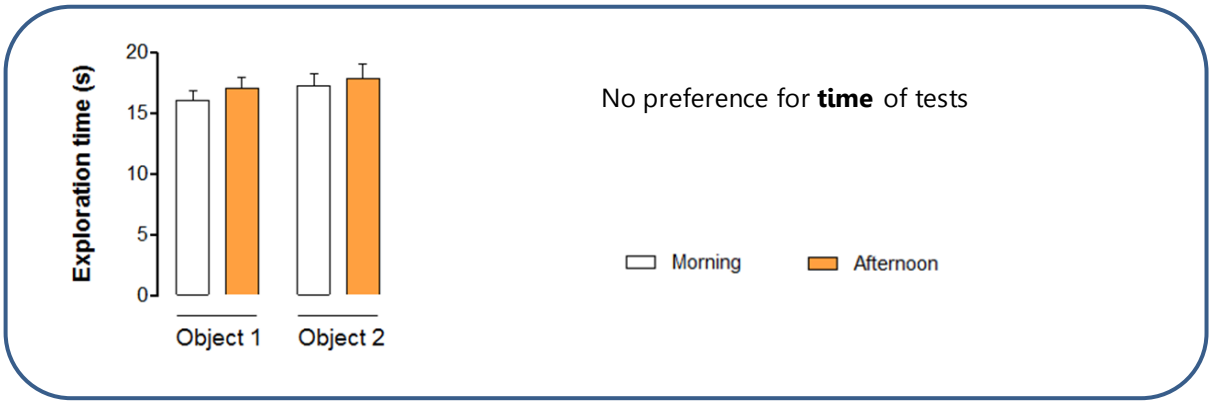
MATERIALS:

- ANY-maze software
- Camera recording + tripod above testing area
- Halogen light (enough to detect the animal with the camera) (near the pool, light pointed on the wall)
- 2 boxes (28×41×18 cm) recovered in black so that mice could not contact or see one another during the exposures.
- Sawdust (≈0.5–1 cm thickness)
- 2 different objects (4 of each objects are needed to alternate the nature of the novel object and to test 2 animals simultaneously)
    - Lego® rectangular structure (7×3×9 cm)
    - Cylindrical glassware (Ø:3 cm, height: 8 cm) filled with white cotton

CONTROLS:







Adapted from Sahay et al., 2011

1. Mice are transported into the testing room 30 minutes before the start of the experiment

2. See details below in Page 6 to prepare software settings.

3. Prepare the 2 boxes with sawdust and 2 identical objects in each box. Objects must be placed symmetrically in the box and ~ 4-5 cm away from the walls. Place and nature of the novel object are changed to avoid any preference.

4. Procedure

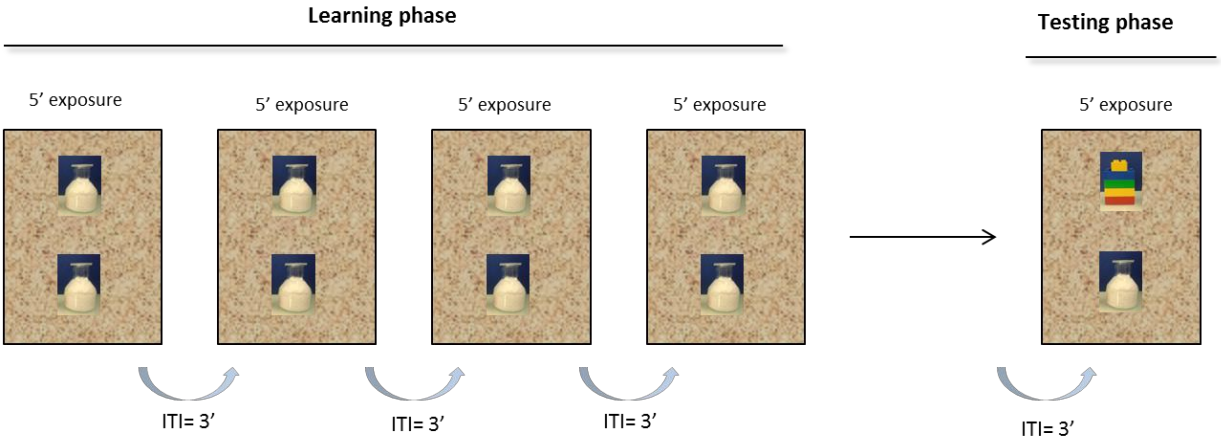
    a. The test is divided in 4 learning sessions (5 minutes each) with a 3-minutes inter-trial interval (ITI) and 1 retention session (5 minutes).

    b. Place animals in the middle of the box (between the 2 objects) and immediately start recording (by clicking simultaneously on F1 and F2). Recording is occurring when mice are tracked (orange point on the body) and time passes.

c. When the session is finished, take mice back in their home cage during ITI. Between each session, change sawdust in each cage, clean boxes and objects with 70% ethanol.

d. Repeat Steps **b** and **c** to perform 4 learning sessions.

e. For the retention session, remove one of the familiar objects from boxes and replace it with a novel object. Assess memory by scoring novel object exploration time during 5 minutes.



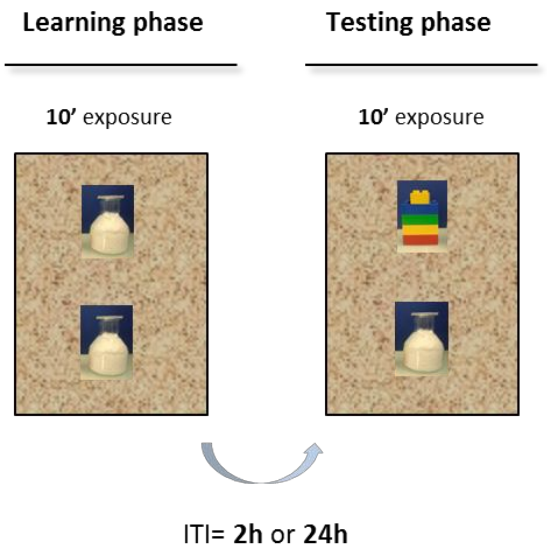
Modified protocol : exploration behavior different between strains.

1. Same as C57BL/6J mice protocol but only one learning session (10 minutes) and one retention session (10 minutes).

2. Load **"One-session learning protocol"** (File Desktop/Memory protocols/NORT/1-session learning protocol)

3. Perform the same procedure

4. According to the type of memory to assess, use a 2h or a 24h inter-trial interval between learning and retention sessions.



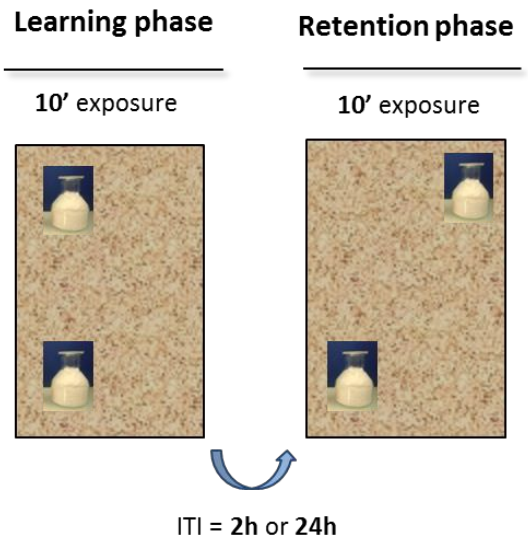
In this type of episodic memory, the ability to discriminate an object that was moved is tested. A control mouse will significantly explore more the object moved between the 2 sessions than the object that remained at the same place.

1. Load **"One-session NOLT"** (File Desktop/Memory protocols/NORT/1-session learning protocol)

2. During the training session (10'), place the 2 identical objects on the same side of the box.

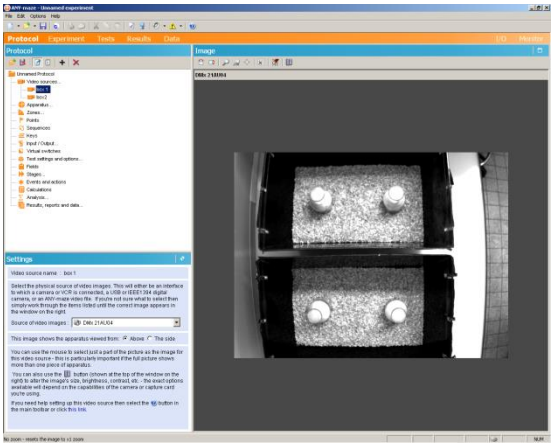
3. In the retention session, move one of the identical objects on the other side of the box. Make sure you drop animal exactly in the same place the learning session.

4. According to the type of memory to assess, use a 2h or a 24h inter-trial interval between learning and retention sessions.

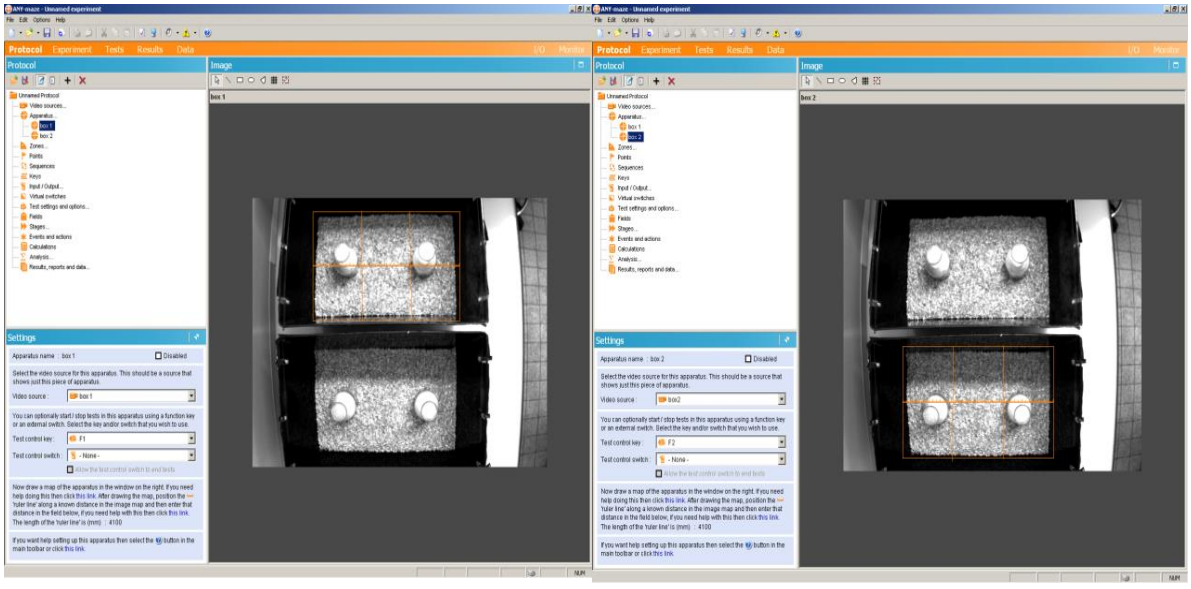


1. Open ANY-maze software icon on the desktop
2. Create a new experiment (File/New experiment)
3. Load a protocol (File Desktop/Memory protocols/NORT)
4. Select Protocol tab to control the settings

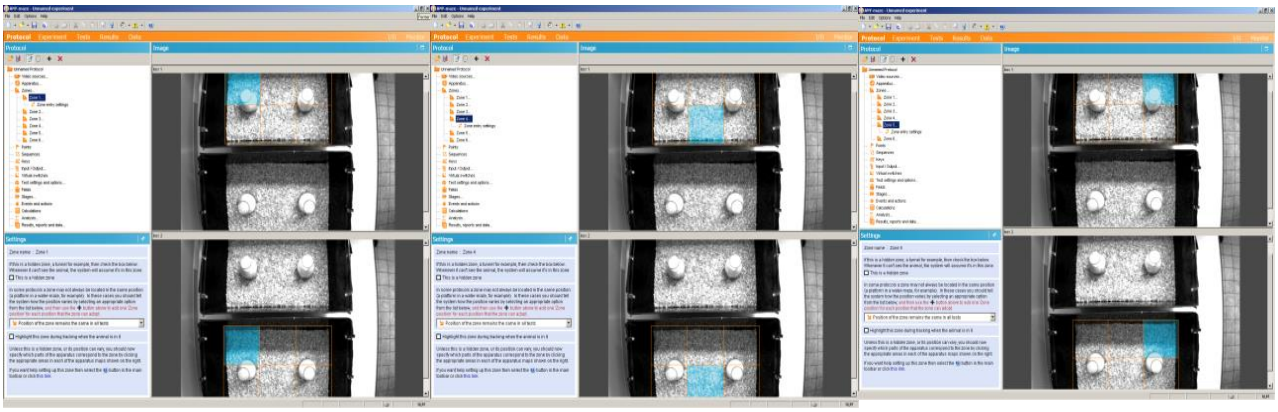
a. Check that the video camera is connected



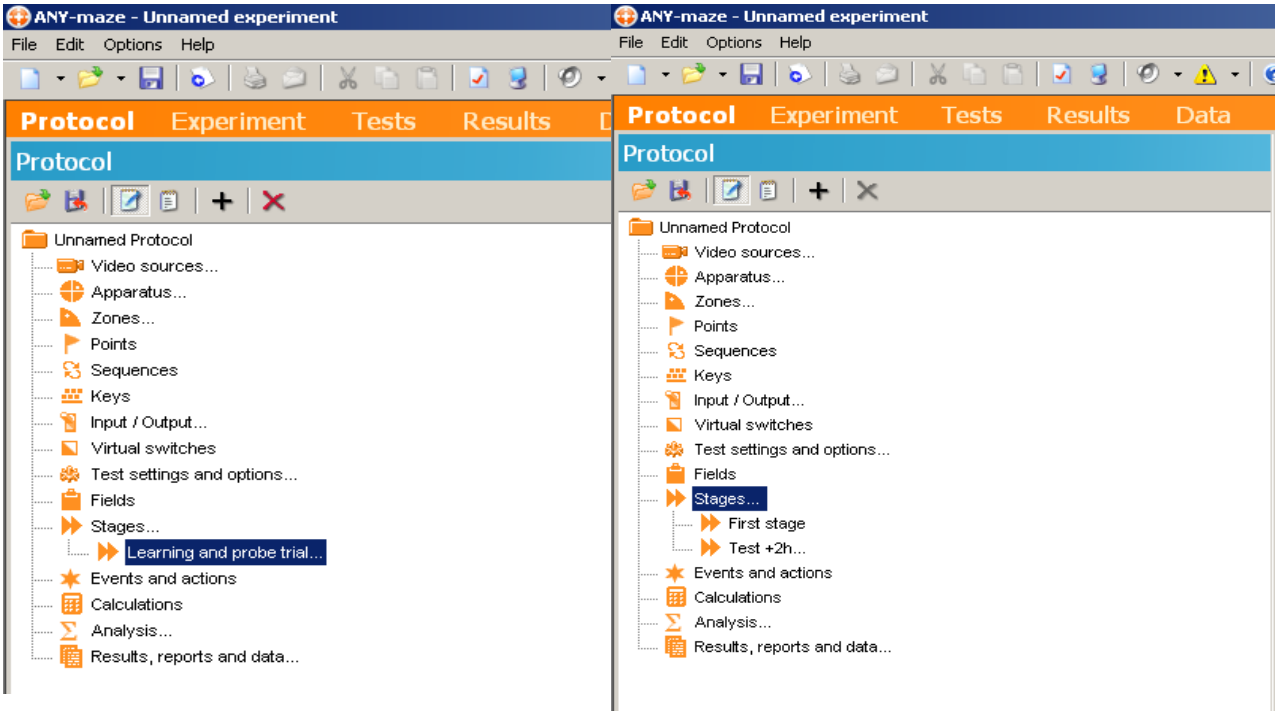
b. Adjust the position of Box 1 and Box 2 so they fit in the pre-defined shape

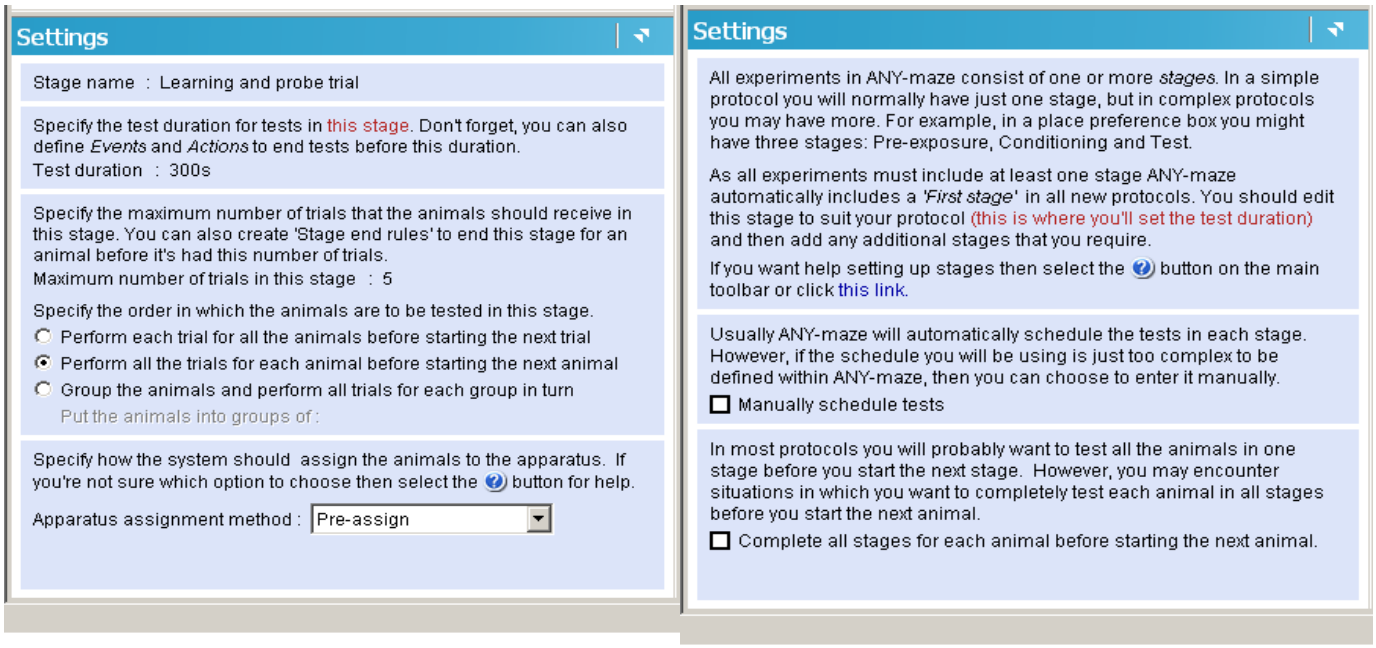


c. Check that each zone is well delimited for Box 1 and Box 2. If 2 zones are sharing, fix it by drawing the right shape in Apparatus. The 6 zones only use as a locomotor control.



d. Check that stages match with the protocol you want to do.

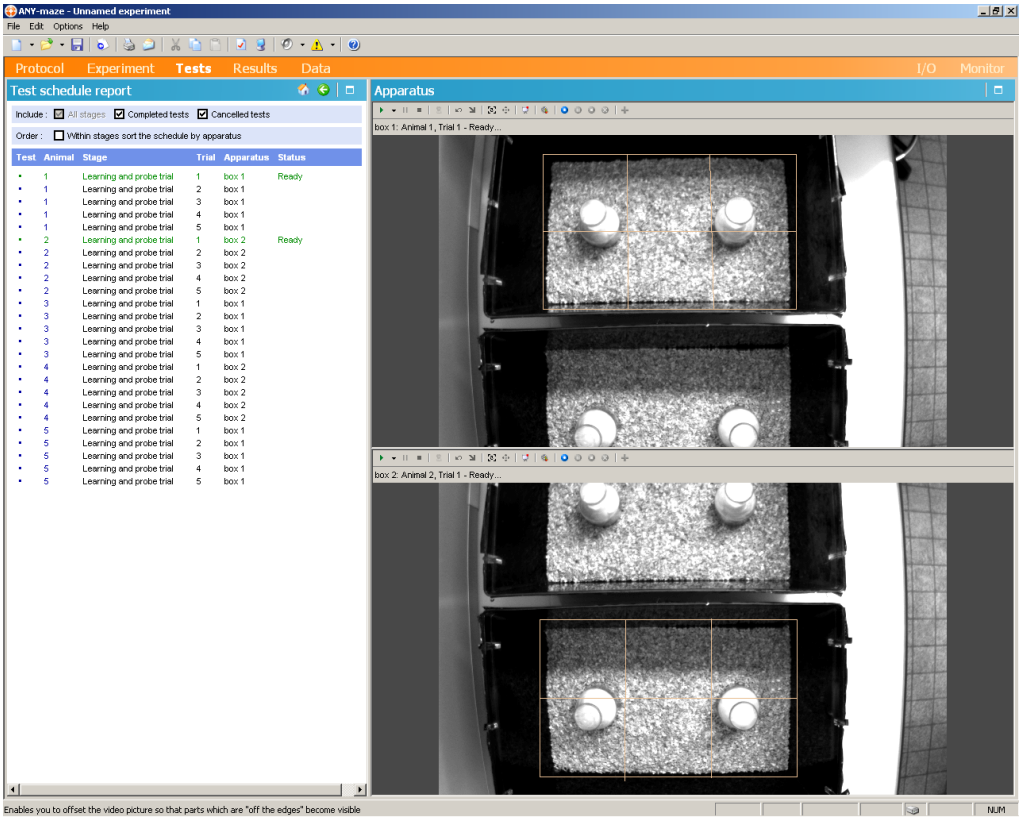


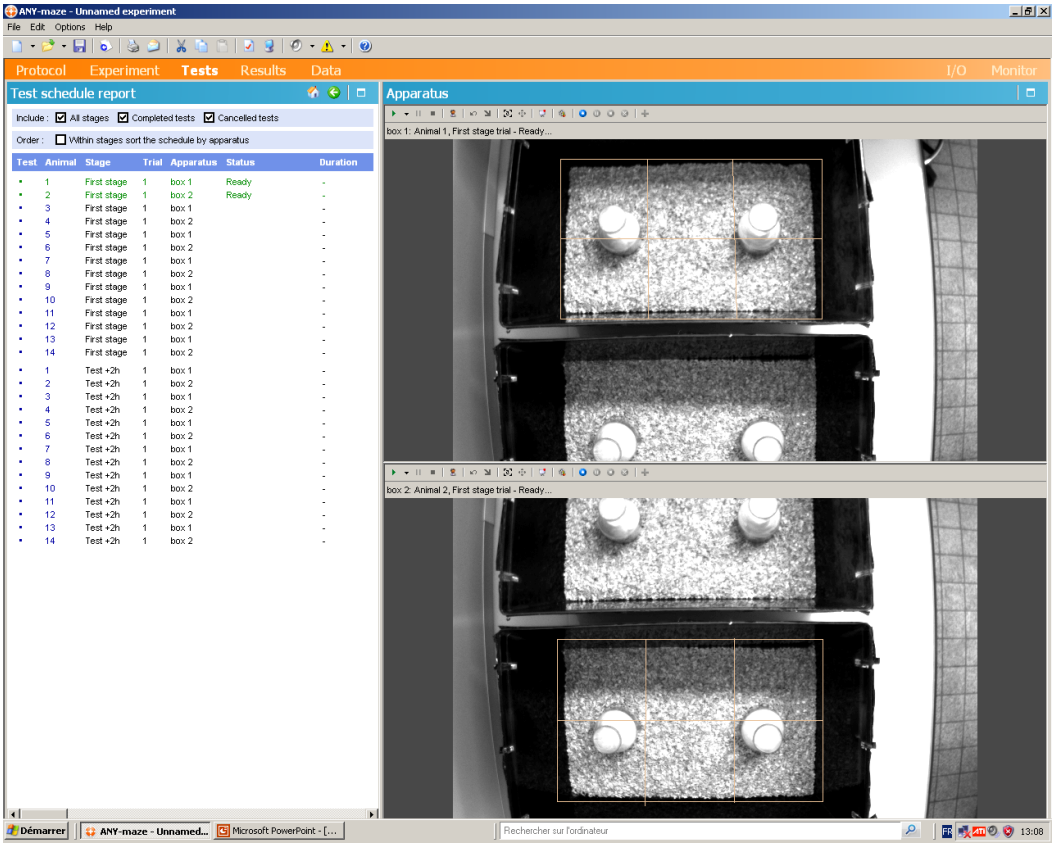


For the "4-sessions learning protocol", choose "perform all the trial for each animal before starting the next trial". For the "1-session learning protocol", choose "perform each trial for all the animals before starting the next trial".

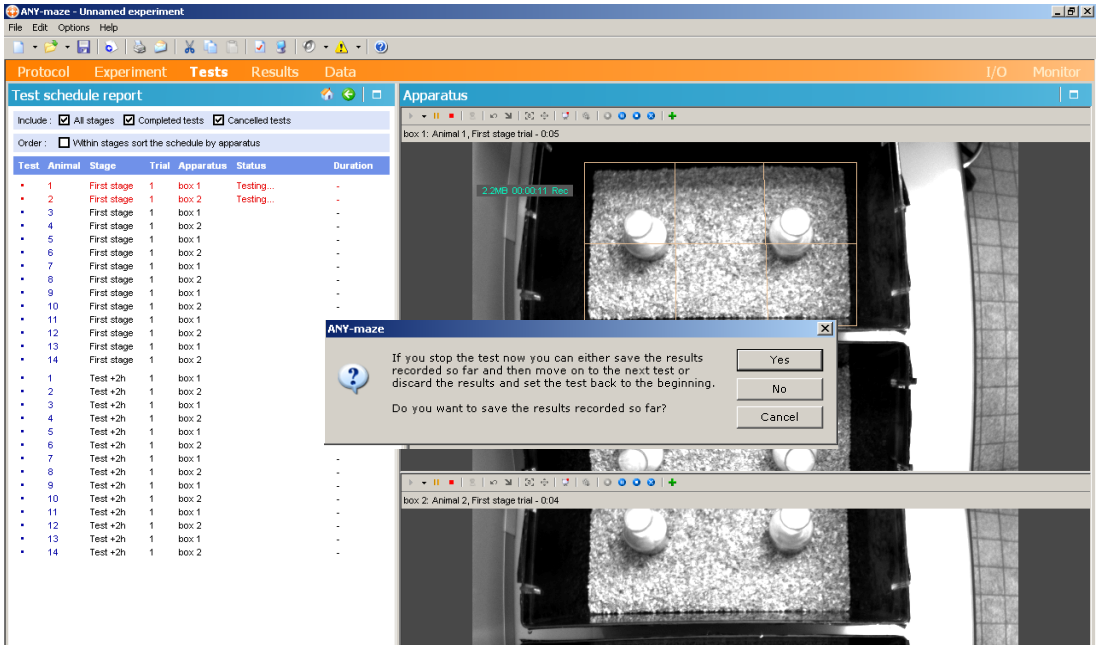
5. Select Experiment tab to name your experiment and to inform the number of animals. For the "1-session learning protocol" with a 2h-ITI, do not forget to calculate the number of animals you can test in two hours (~12-14 animals)

6. Select Tests tab when you are ready to begin the experiment. Select "Test schedule report" to follow each trial for each box.


ANY-maze - Unnamed experiment
File Edit Options Help
Protocol Experiment Tests Results Data I/O Monitor
Test schedule report
Include : All stages Completed tests Cancelled tests
Order : Within stages sort the schedule by apparatus
Test Animal Stage Trial Apparatus Status
Apparatus
box 1: Animal 1, Trial 1 - Ready...
box 2: Animal 2, Trial 1 - Ready...
Enables you to offset the video picture so that parts which are "off the edges" become visible
NUM


ANY-maze - Unnamed experiment
File Edit Options Help
Protocol Experiment Tests Results Data I/O Monitor
Test schedule report
Include : All stages Completed tests Cancelled tests
Order : Within stages sort the schedule by apparatus
Test Animal Stage Trial Apparatus Status Duration
Apparatus
box 1: Animal 1, First stage trial - Ready...
box 2: Animal 2, First stage trial - Ready...
Démarrer
ANY-maze - Unnamed...
Microsoft PowerPoint - [...
Rechercher sur l'ordinateur
13:08

Press on the green starting button to starting recoding (or click simultaneously on F1 and F2). An orange point detects the animal body. If you have to stop the experiment for any reason, press on the red button and according to your problem, save the results recorded so far or do not save. If you do not save, this same trial will begin again. If you do save, the software will be ready to start the next animal.



ANALYSIS

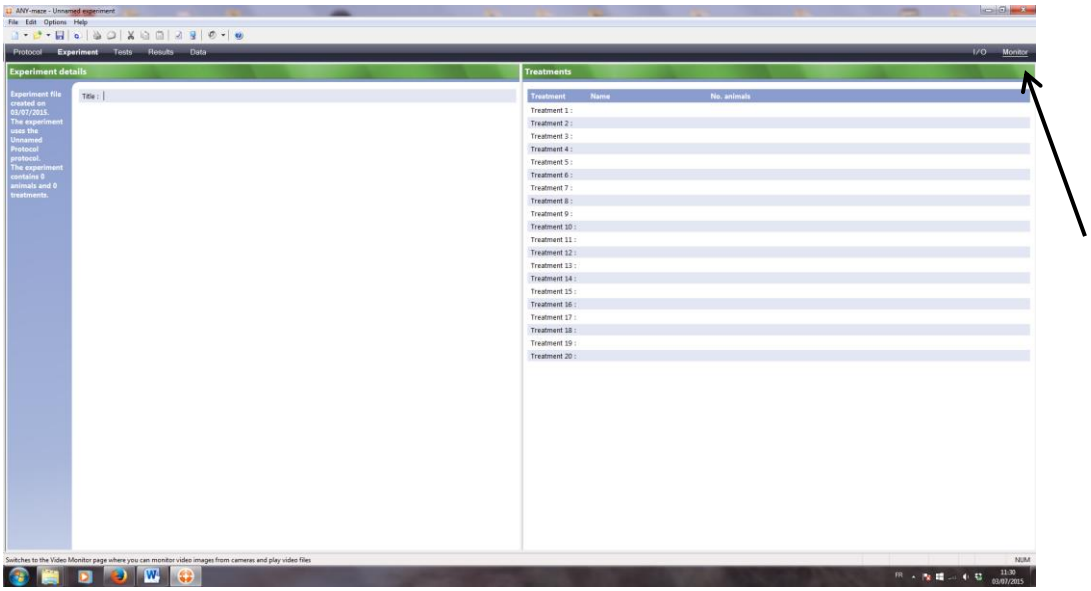
- The software will automatically create a videos file with the same file of your experiment file.

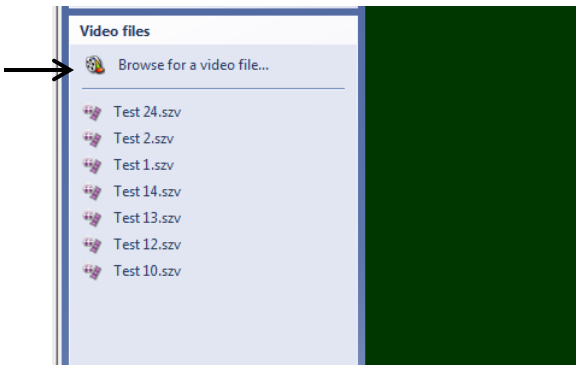
Each trial generates 2 videos (one video for Box1 and one video for Box2). When scoring videos, videos are numbered from 1 to n videos (without n° of animal, n° of trial or box). Before starting the test, write down carefully mice running order, in which box each mouse was tested, to avoid any confusion during analysis phase.

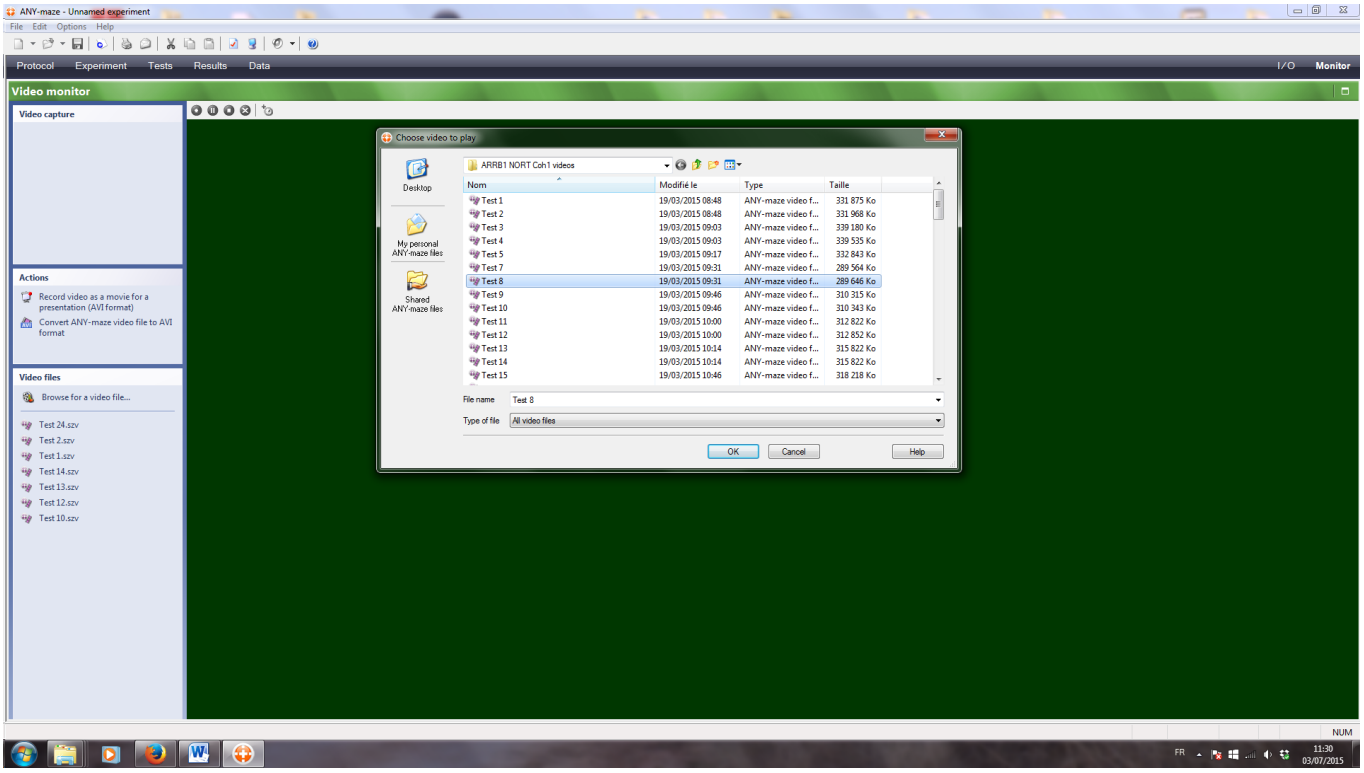
- Manual scoring can be done either simultaneously during the running test or after the experiment by watching the video file recording. Use STOPWATCH software to measure exploration of both objects.

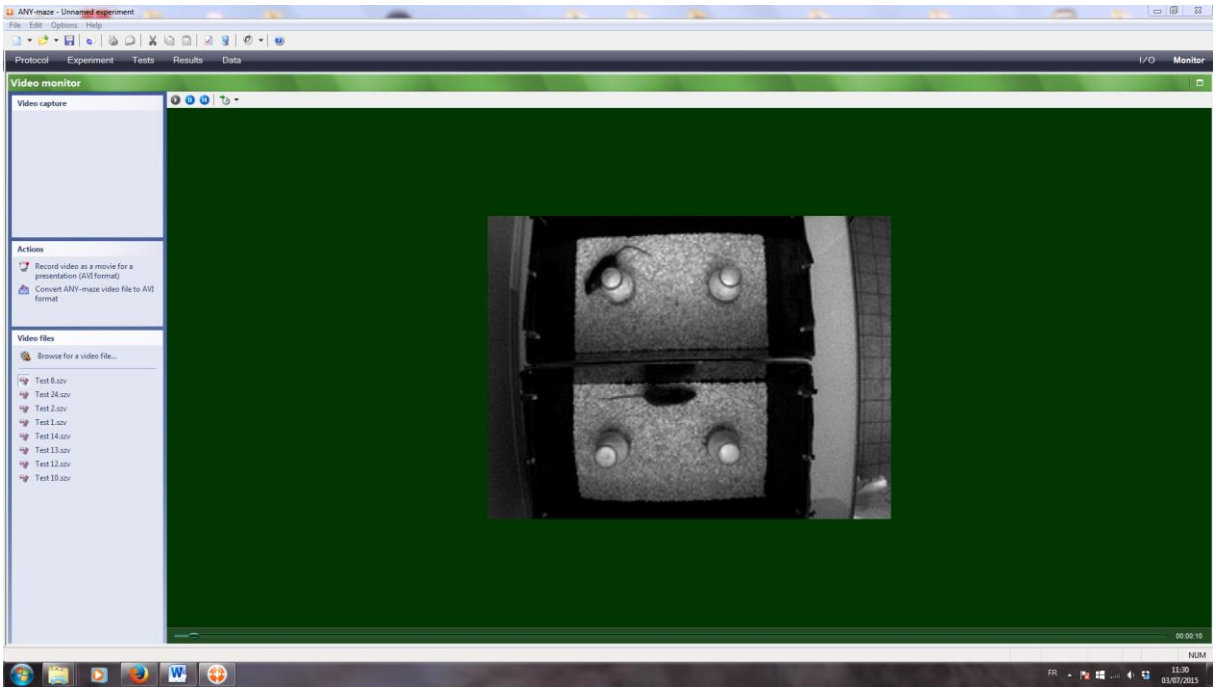
Latency to explore objects and frequency of exploration episodes can be used as additional parameters to interpret data. Select these options in STOPWATCH software.

- To visualize videos, open ANY-maze software, select Monitor on the left. Then, select “browse for a video file”, choose your video and score.




Video files
Browse for a video file...
Test 24.szv
Test 2.szv
Test 1.szv
Test 14.szv
Test 13.szv
Test 12.szv
Test 10.szv


ANY-maze - Unnamed experiment
Protocol
Experiment
Tests
Results
Data
I/O
Monitor
Video monitor
Video capture
Actions
Video files
Choose video to play
Desktop
Nom
Modifié le
Type
Taille
Test 8
File name
Type of file
OK
Cancel
Help



- Object exploration is defined as the orientation of the nose to the object at a distance ≤ 2 cm.
- Placing the forepaws on the objects was considered as exploratory behavior, but climbing on the objects was not.

Results for this test were expressed as:

a. exploration of each object (in seconds) during training and test sessions

b. exploration (in percent) of each object during the test session, calculated as time spent exploring familiar or novel object divided by total time spent exploring both objects

c. a discrimination index (DI) between objects during the test session, calculated as the difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects (DI = (N–F)/(N+F)).

LOCOMOTOR ANALYSIS

Locomotor activity was controlled during the entire experiment (parameter: ambulatory distance) using a videotracking procedure (ANY-maze Software, Bioseb, France).

Check with the following parameters that motor activity remains unchanged for all conditions:

- time in each zone (1 to 6)
- crossing in each zone (1 to 6)
- distance travelled in each zone (1 to 6)

Collect data from ANY-maze software and export them in an Excel file.

2. Fiche méthode du test de la piscine de Morris

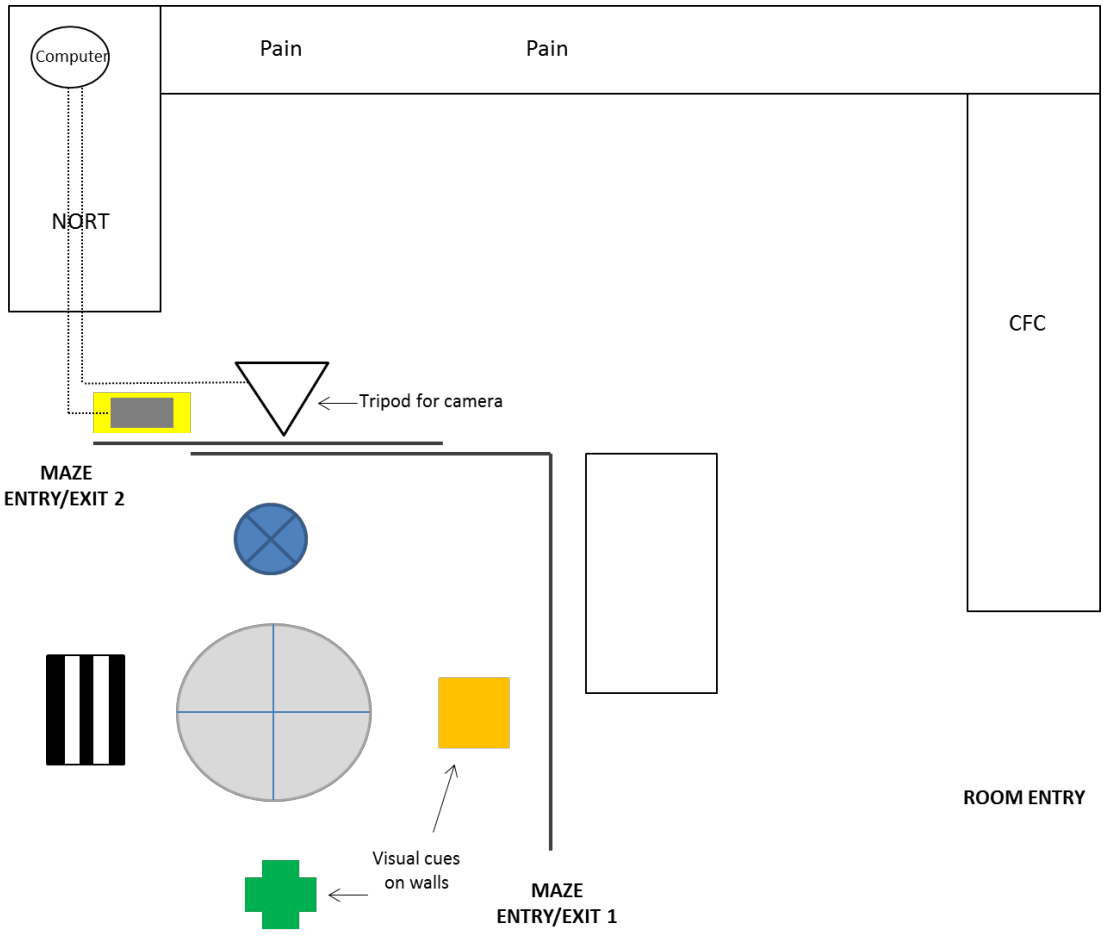
MATERIALS

Animals: C57Bl6/J male mice

Equipment:

- 1 maze : 125 cm in diameter (stainless steel tank); 50 cm height
- 1 platform : 10 cm diameter, height 30 cm
- Water : white milk powder (2/3 of packet per day), **22-23°C**
- 4 distal visual cues : different in shape, in color, surrounding the maze (one visual cue per cardinal point)
- 1 valve and pipes (to fill and remove the water and clean the tank)
- 1 thermometer (to check the water temperature)
- 1 bucket to fill the maze with hot water
- 1 remote control for ANY-maze software (if MWM is performed alone)
- ANY-maze software

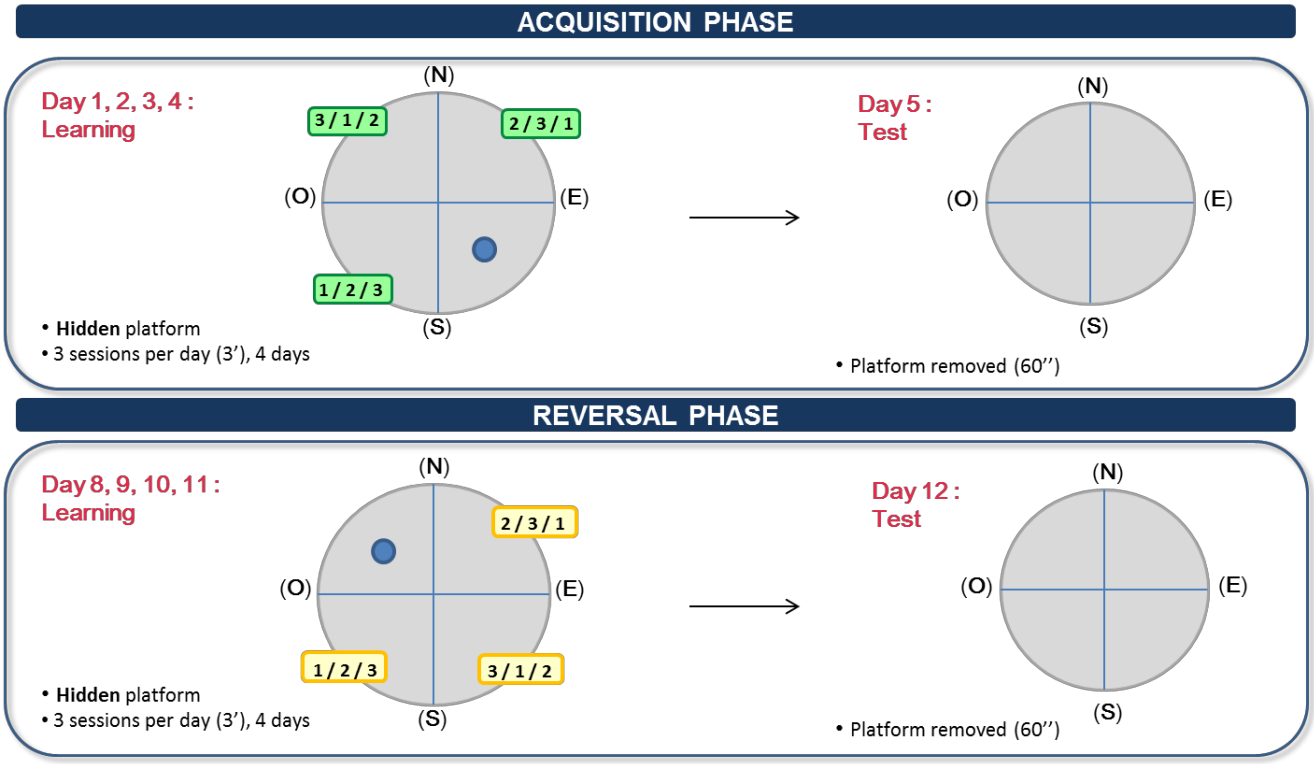
Room configuration:



AIM OF THE TEST:

Mice must learn to locate an unmarked submerged platform in a pool devoid of intramaze cues. Geometrical extra cues surround the maze to generate spatial learning. The maze is divided into four quadrants (North, South, West, and East). The escape platform is placed in the target quadrant (North for example). The MWM task is performed with three successive steps. The pre-training phase allows mice to accustom to the pool and the visible platform placed in clear water. The acquisition phase is divided into 4 training sessions and one probe trial. The reversal phase assesses cognitive flexibility performances. Animals must unlearn the initial rule (target quadrant) and adapt their behavior to a new rule (i.e locate the platform in the opposite quadrant).

PROTOCOL (adapted from Belzung and Sahay teams)**: rajouter les refs**



1. **Habituation = pre-training session = VISIBLE PLATFORM** (1 session, 3 trials, Day 0)

- Fill the maze with water and adjust temperature at 22-23°C (with hot water)
- Keep the water clear (do not add milk powder)
- Place the platform into the target quadrant of your choice
- The platform must be visible for the mice; the surface of the water must be 1 cm below the surface of the platform. If animals have difficulties to climb on the platform, add water.
- Place the mouse in the middle of the pool during a 60 sec-trial.
- Repeat this step 2 times after a 60 sec-ITI

Make sure that the platform is **stable** in the pool before starting the test. The platform must be in the center of the target quadrant. Be sure that the platform is not floating and does not move.

Keep in mind that hot water is available in the animal facility early in the morning. It is better to adjust water temperature in the morning (even if water is too hot).

2. **Acquisition phase = learning phase = HIDDEN PLATFORM** (Day 1–4)

- For this step, the platform must be hidden. The surface of the water must be 1-2 cm above the surface of the platform. The water is rendered opaque by addition of white milk powder (1/3 of a 750g-packet, Cora).
- Place the animal in the start position in the maze, facing the tank. Be careful not to drop the mouse but to release it gently into the water. The tracking system will start the moment the mouse is moving into the water.
- When the mouse reaches and climbs in the platform, stop the timer and the video and write down the latency to reach the platform on the parameter paper.
- There are 3 training trials per day (60 sec each) with a 60 sec inter trial interval (ITI), during 4 days.
- During ITI, animals have to stay on the platform.
- Starting position must be different at every trial and every day to generate spatial learning.

Mice that exceed 60 sec to find the platform will be gently guided to the platform by the experimenter. In this case, note 60 sec as escape latency for this animal. If the mouse jumps into the water before the end of the 60-sec ITI, replace it on the platform until it stays 60 sec.

Examples for starting positions:

- Day 1: SE - NW – SW
- Day 2: SW – SE – NW
- Day 3: NW – SW – SE
- Day 4: SE - SW – NW

3. Probe trial = retention phase (Day 5)

- Remove the platform from the opaque water.
- Place the animal in the opposite quadrant, facing the tank wall.
- Probe trial lasts 60 sec during which animals are free to explore the maze.

4. Reversal phase (Day 8–11)

If the aim of the study includes assessing cognitive flexibility, spatial reversal learning and retention starts 2 days after the acquisition probe trial in Day 5.

- Change the location of the escape platform from the target quadrant used during acquisition to the opposite target quadrant (which becomes the new target quadrant).
- Repeat the exact same procedure as acquisition phase : 4 training days, 3 trials per days, 60 sec per trial and ITI

5. Probe trial = retention phase for cognitive flexibility (Day 12)

Repeat the same procedure as Probe trial in Day 5.

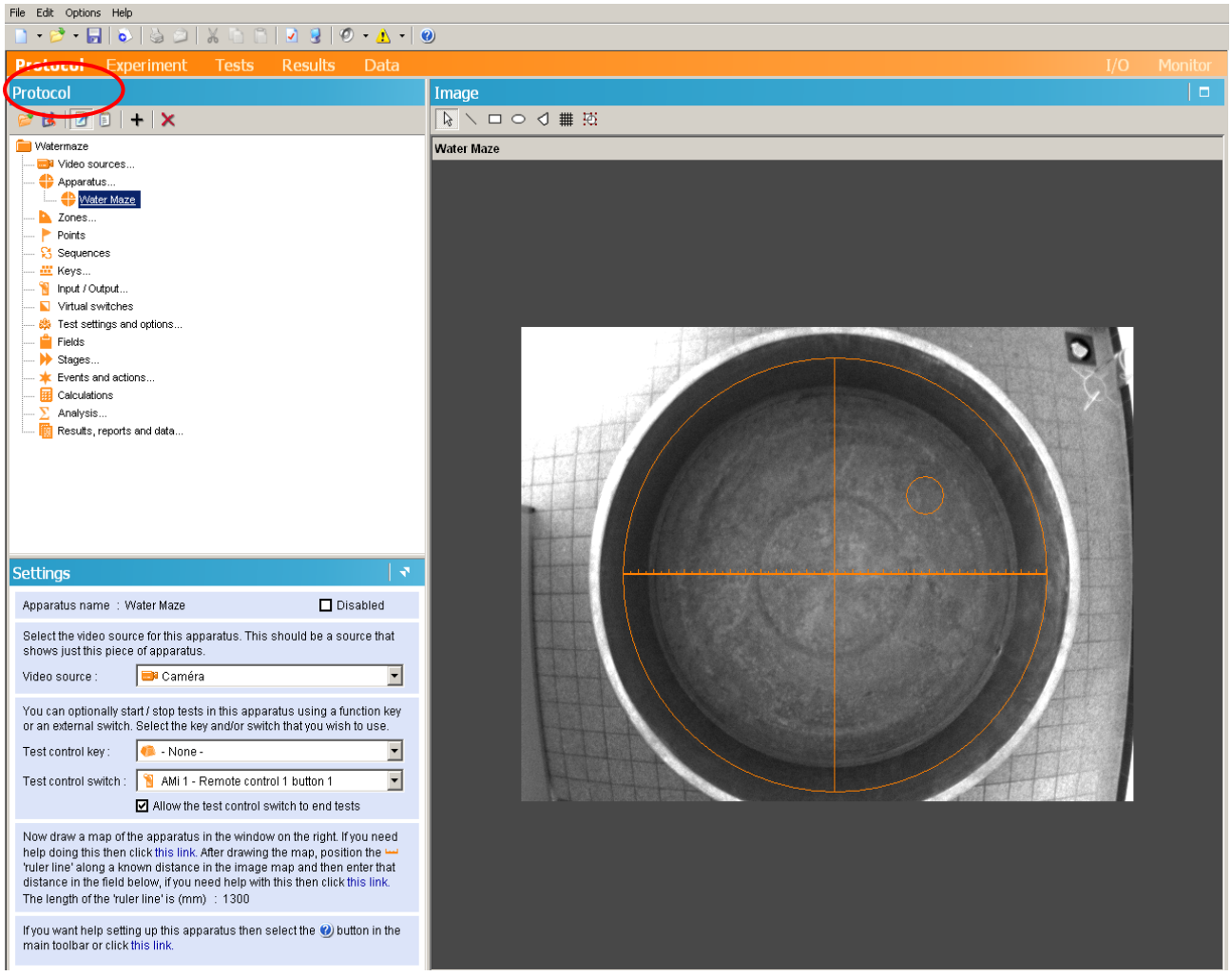
How to perform Morris Water maze with ANY-maze software?

1. Open ANY-maze icon on the desktop

2. Create a new experiment :
   - File
   - New experiment (immediately File, Save experiment as (in the folder of your choice))

3. Load a protocol (depending on the phase of the test)

4. Select **Protocol** tab (top left) to control all the settings

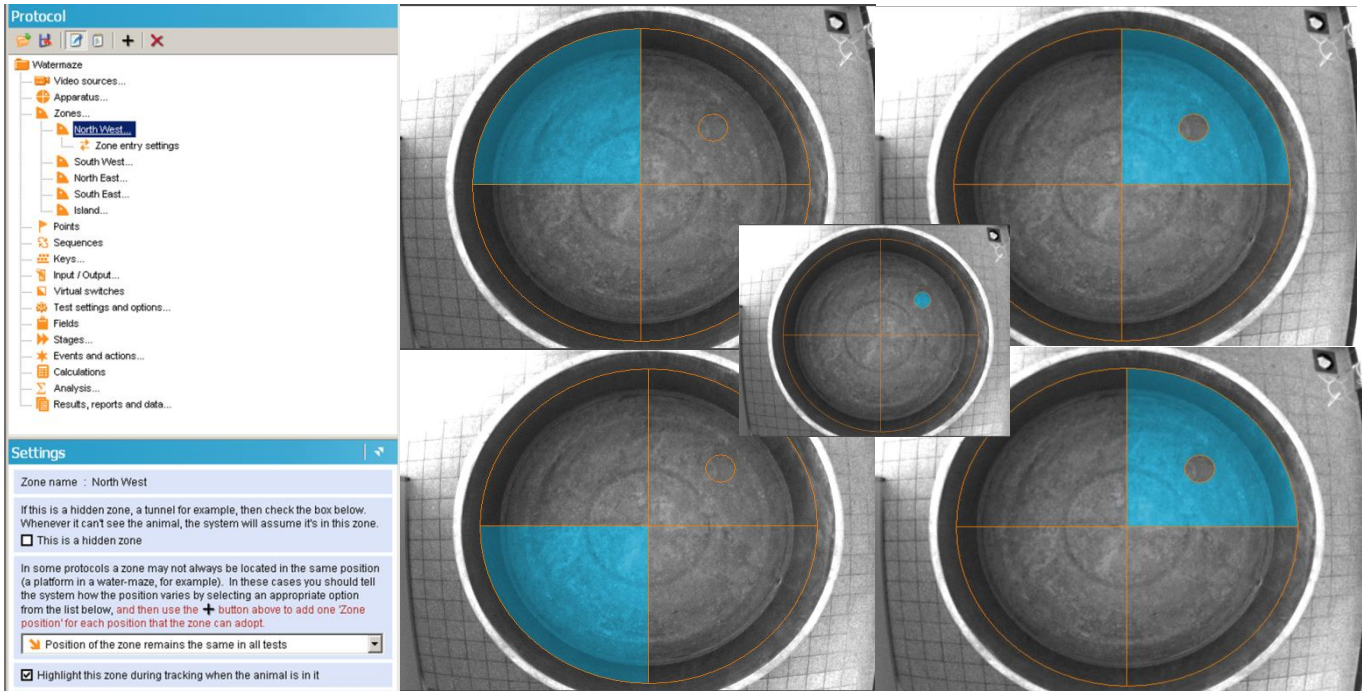


4.1 Apparatus -> Water maze:

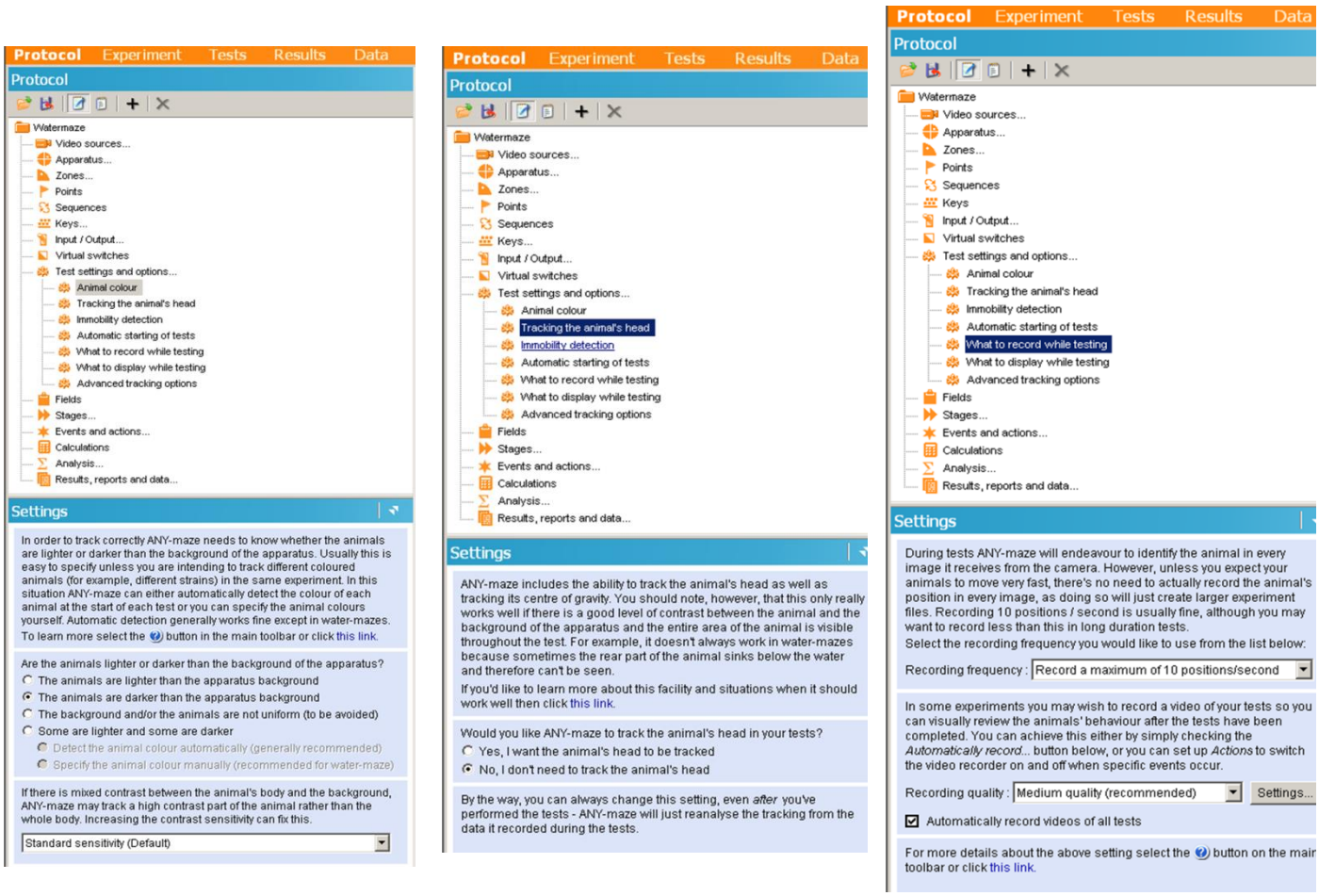
- You can visualize all the zones for the test via the camera screen.
- Choose in Test control switch: AM1- remote control button 1. The remote button is necessary when the test is performed by a single person to start recording videos.
- Check that the length of the ruler line is correct: 1250 mm.

4.2 Zones:

- Check that all the zones are defined properly.



4.3 Test settings and options



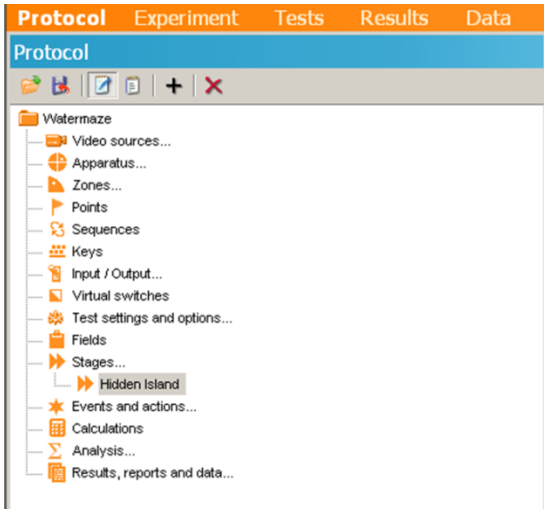
- Check the box:

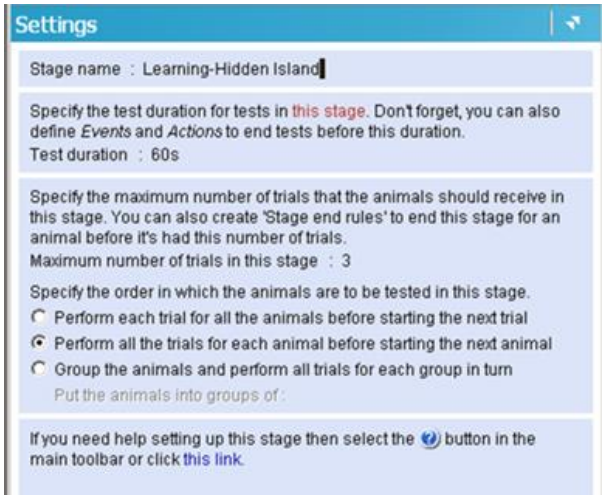
- The animals are darker than the apparatus background **(animal colour).**
- No, I don't want the animal's head to be tracked **(tracking the animal's head).**
- Automatically record videos of all tests **(what to record while testing)** -> a file will automatically be created in your experiment file.
- Suppress water maze reflections **(advanced tracking options).**

4.4 Stages

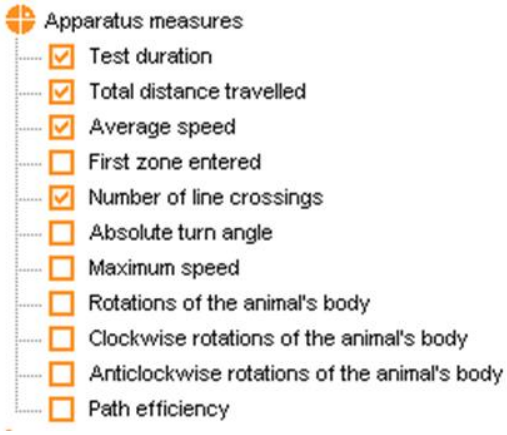
- Pre-training: Visible platform (60 sec; 3 trials).
- Learning: Hidden platform (60 sec; 3 trials).
- Retention: No platform (60 sec; 1 trial).
- Reversal learning: Hidden platform (60 sec; 3 trials).
- Reversal retention: No platform (60 sec; 1 trial).

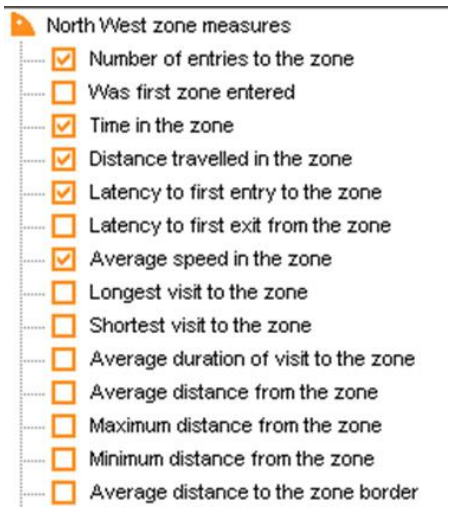
Choose: **Perform all the trials for each animal before starting the next animals**





4.5 Results, reports and data





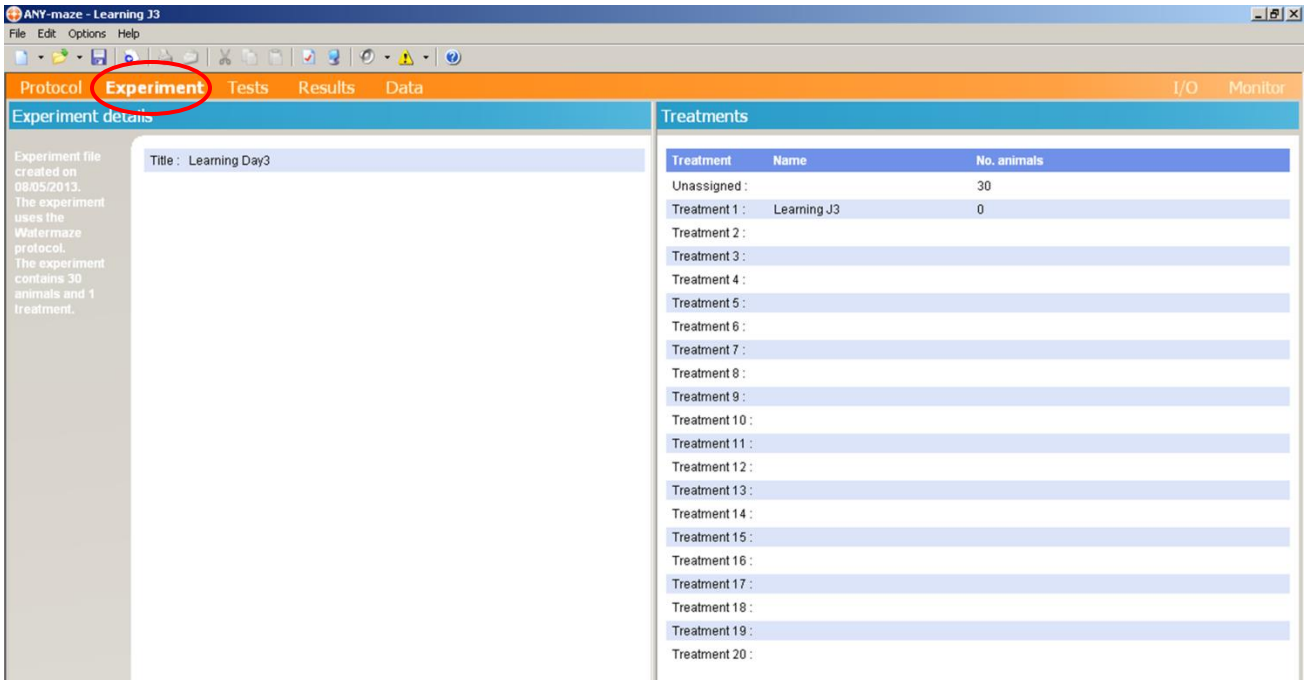
Check that all the following parameters are selected in each phase of the test:

✓ Apparatus measures :
- Test duration
- Total distance travelled
- Average speed
- Number of crossings

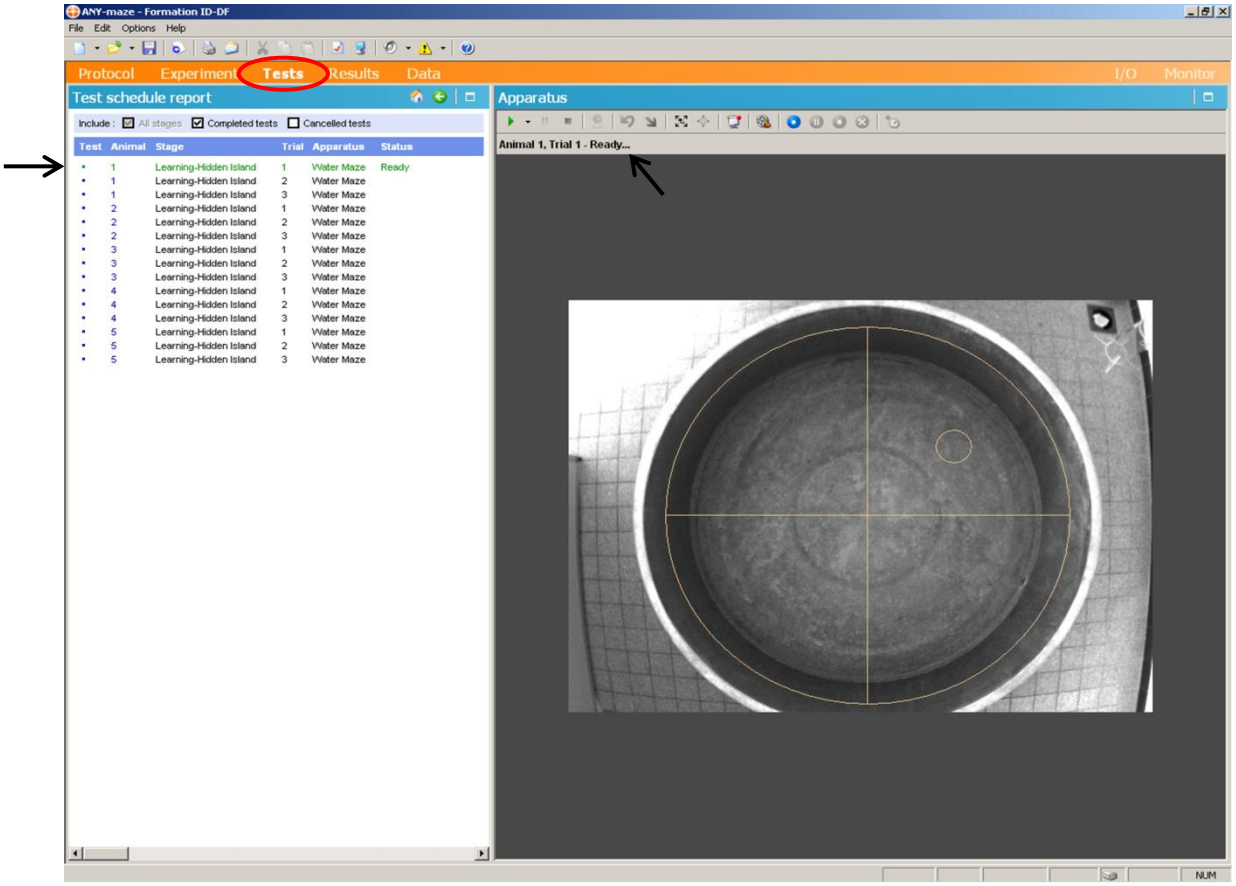
✓ Quadrants (N, S, E, W) :
- Number of entries to the zone
- Time in the zone
- Distance travelled in the zone
- Latency to first entry to the zone
- Average speed in the zone

✓ Platform/Island:
- Latency to first entry to the zone
- Distance travelled until first entry in the zone

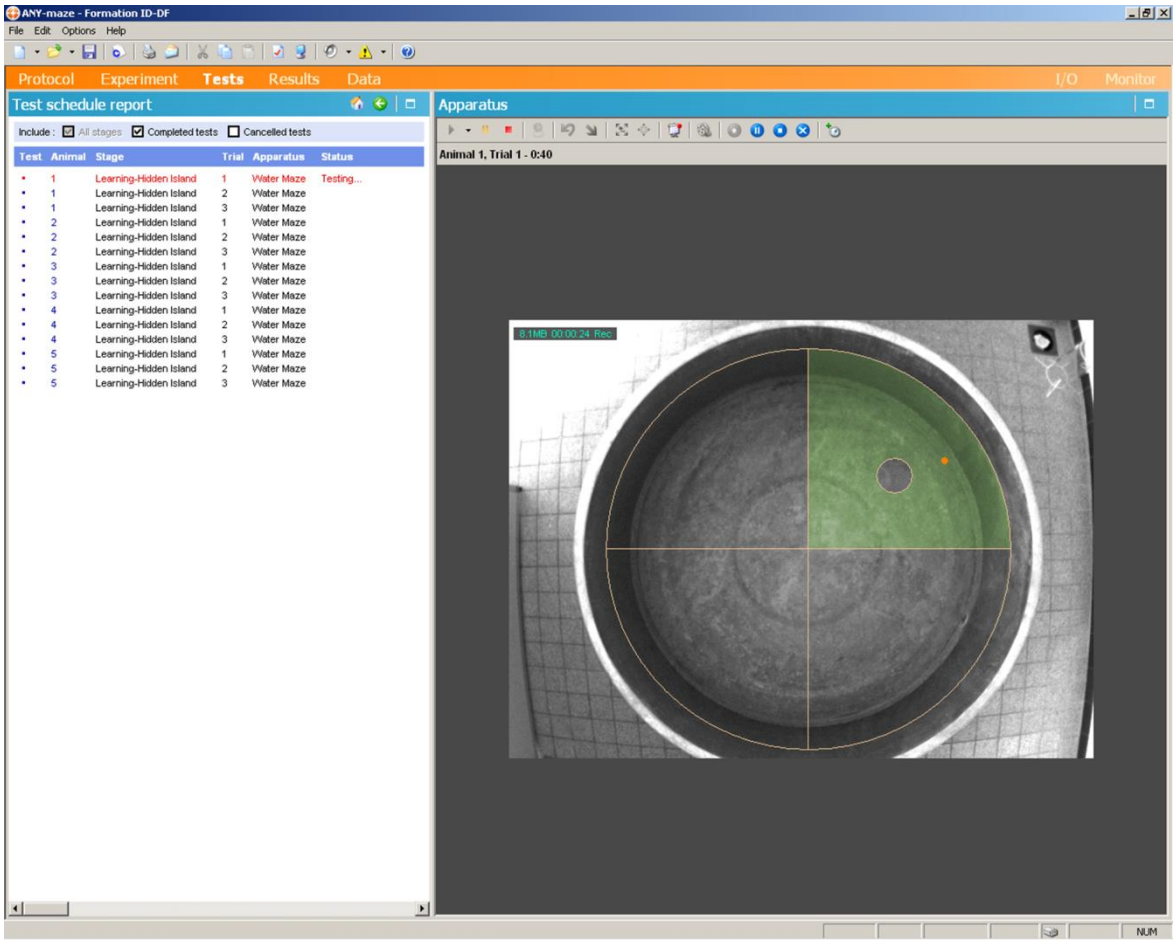
5. Go to **Experiment** tab to enter the phase and day (ex: learning Day 3) and the number of animals



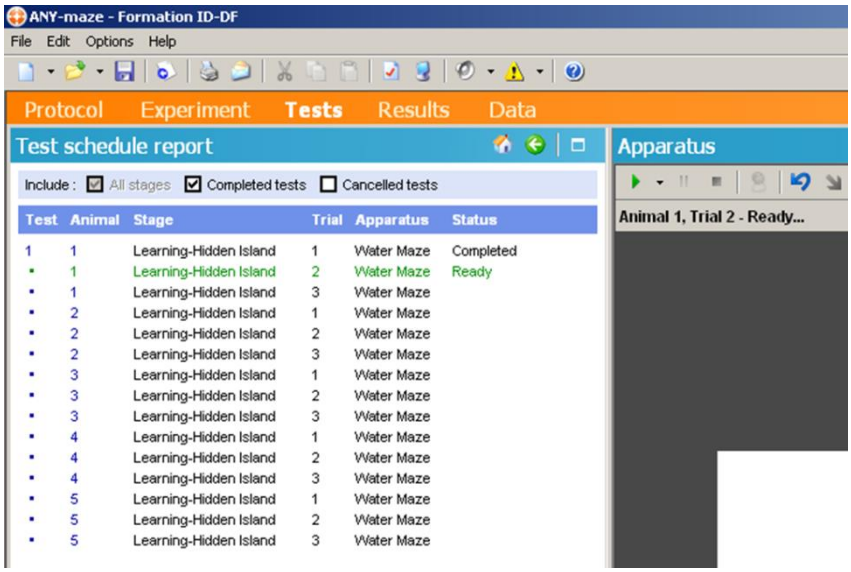
6. Go to **Tests** tab when you are ready to start the test (select test schedule report to visualize the order of trials)

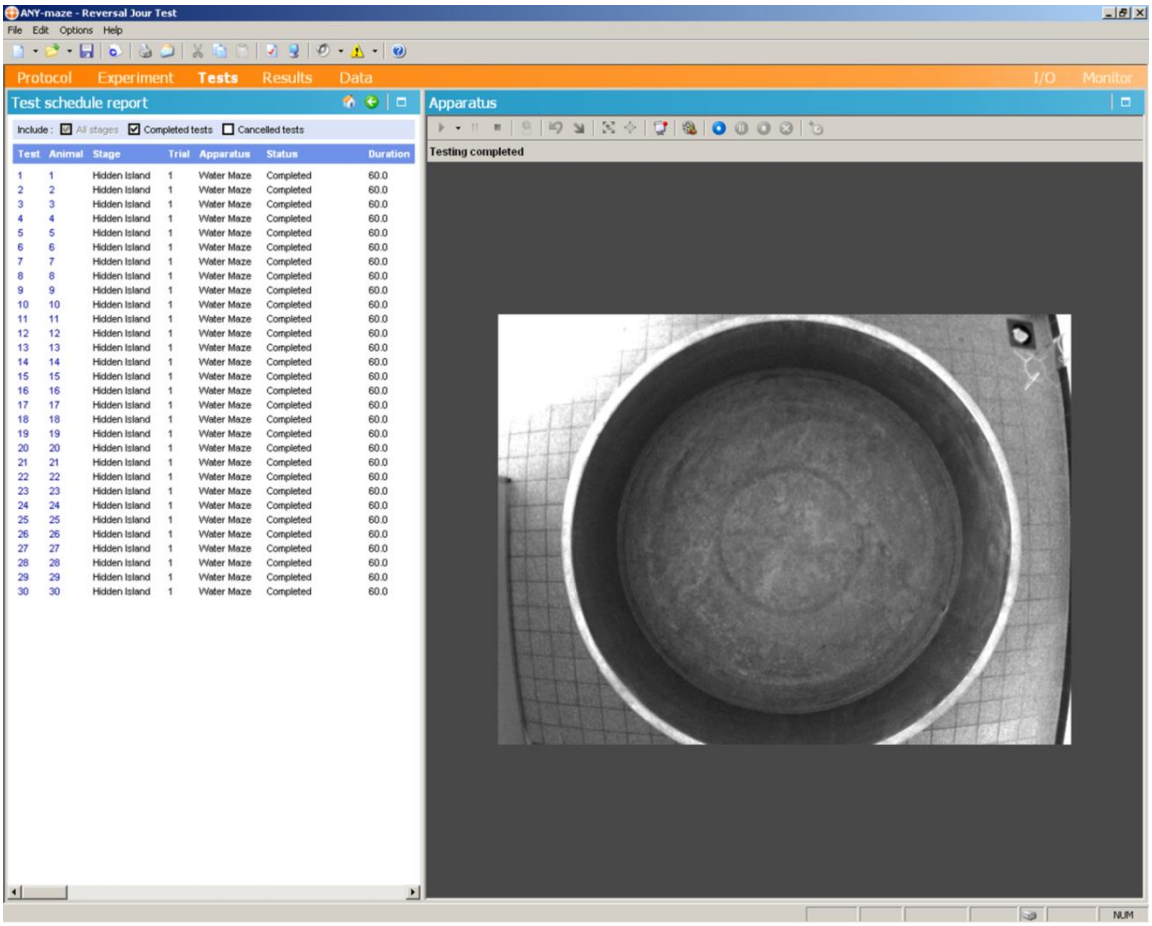


At this point, the software is ready to start the trials. Click on the start button (green triangle) or on the top of the remote control (**very close to the acquisition box +++, weak range**)



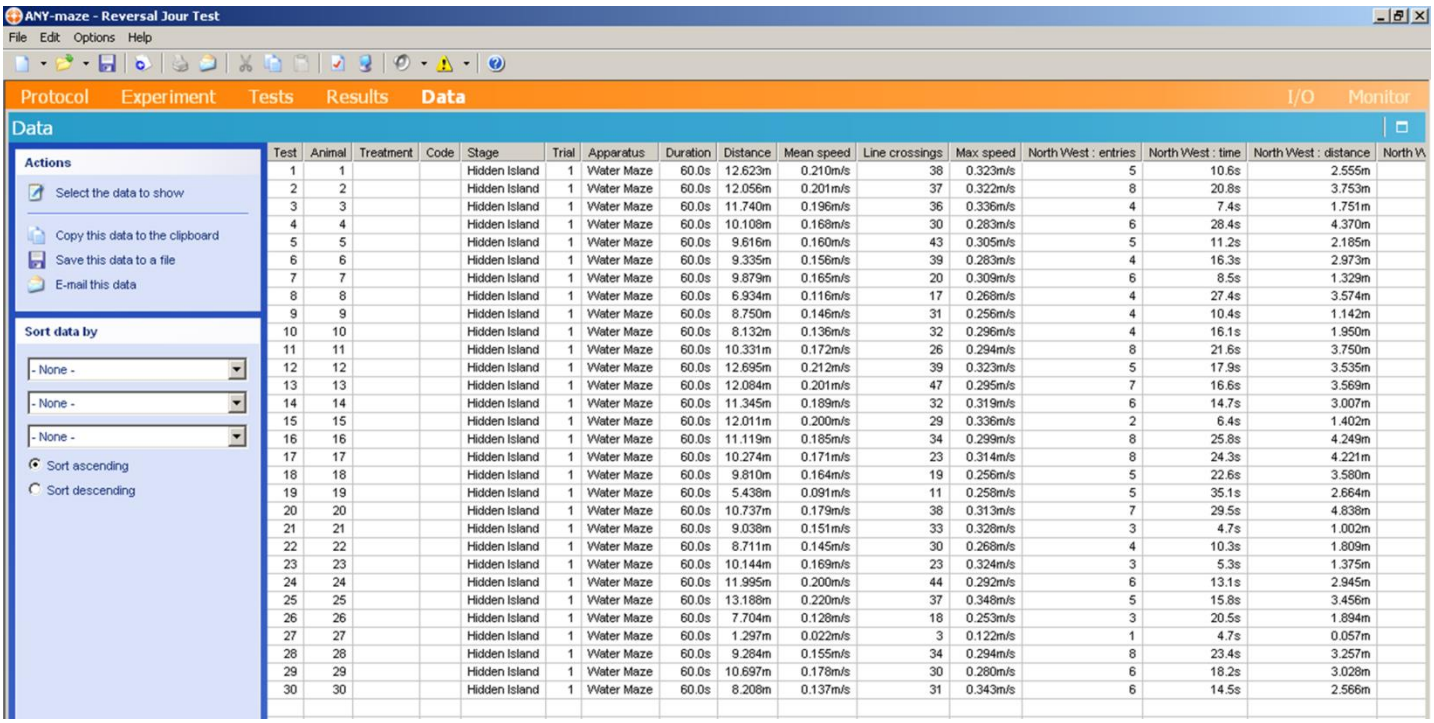
- During the trial, the software tracks the animal's body. Zones are lightened when the animal is detected.
- The trial will stop if the animal does not reach the platform in 60 sec.
- Stop the trial by clicking on the stop button (red square) when the animal climbs in the platform.
- When the trial is finished, the next trial will be ready to start.
- When all the trials are finished, "testing completed" appears.
- Do not forget to save all the results.


ANY-maze - Formation ID-DF
File Edit Options Help
Protocol Experiment Tests Results Data
Test schedule report
Include : All stages Completed tests Cancelled tests
Test Animal Stage Trial Apparatus Status
1 1 Learning-Hidden Island 1 Water Maze Completed
1 Learning-Hidden Island 2 Water Maze Ready
1 Learning-Hidden Island 3 Water Maze
2 Learning-Hidden Island 1 Water Maze
2 Learning-Hidden Island 2 Water Maze
2 Learning-Hidden Island 3 Water Maze
3 Learning-Hidden Island 1 Water Maze
3 Learning-Hidden Island 2 Water Maze
3 Learning-Hidden Island 3 Water Maze
4 Learning-Hidden Island 1 Water Maze
4 Learning-Hidden Island 2 Water Maze
4 Learning-Hidden Island 3 Water Maze
5 Learning-Hidden Island 1 Water Maze
5 Learning-Hidden Island 2 Water Maze
5 Learning-Hidden Island 3 Water Maze
Apparatus
Animal 1, Trial 2 - Ready...


ANY-maze - Reversal Jour Test
File Edit Options Help
Protocol Experiment Tests Results Data I/O Monitor
Test schedule report
Include : All stages Completed tests Cancelled tests
Test Animal Stage Trial Apparatus Status Duration
Apparatus
Testing completed
NUM

7. Go to the **Data** page

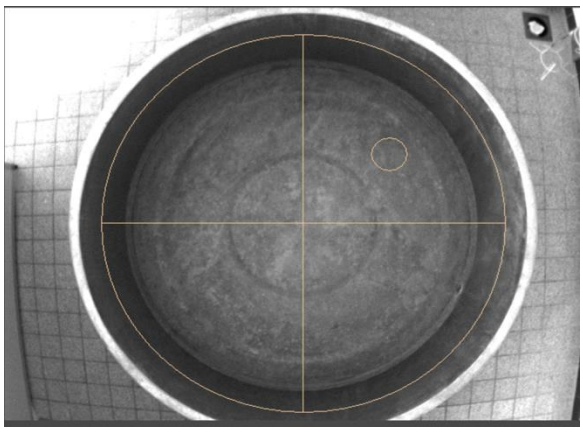
All the parameters asked in Protocol page are listed in this table.
Export the table in Excel to analyse the results or use Results sessions.

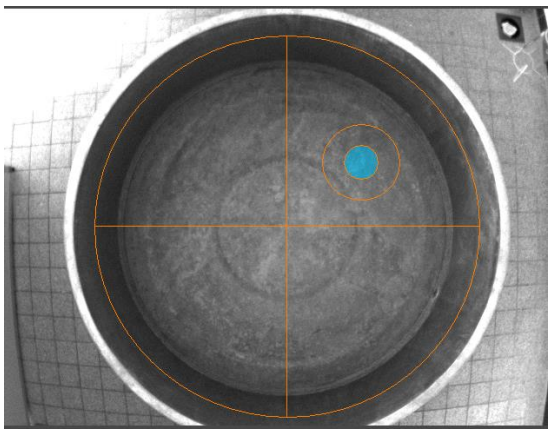


Test	Animal	Treatment	Code	Stage	Trial	Apparatus	Duration	Distance	Mean speed	Line crossings	Max speed	North West : entries	North West : time	North West : distance	North W
1	1			Hidden Island	1	Water Maze	60.0s	12.623m	0.210m/s	38	0.323m/s	5	10.6s	2.555m	
2	2			Hidden Island	1	Water Maze	60.0s	12.056m	0.201m/s	37	0.322m/s	8	20.8s	3.753m	
3	3			Hidden Island	1	Water Maze	60.0s	11.740m	0.196m/s	36	0.336m/s	4	7.4s	1.751m	
4	4			Hidden Island	1	Water Maze	60.0s	10.108m	0.168m/s	30	0.283m/s	6	28.4s	4.370m	
5	5			Hidden Island	1	Water Maze	60.0s	9.616m	0.160m/s	43	0.305m/s	5	11.2s	2.185m	
6	6			Hidden Island	1	Water Maze	60.0s	9.335m	0.156m/s	39	0.283m/s	4	16.3s	2.973m	
7	7			Hidden Island	1	Water Maze	60.0s	9.879m	0.165m/s	20	0.309m/s	6	8.5s	1.329m	
8	8			Hidden Island	1	Water Maze	60.0s	6.934m	0.116m/s	17	0.268m/s	4	27.4s	3.574m	
9	9			Hidden Island	1	Water Maze	60.0s	8.750m	0.146m/s	31	0.256m/s	4	10.4s	1.142m	
10	10			Hidden Island	1	Water Maze	60.0s	8.132m	0.136m/s	32	0.296m/s	4	16.1s	1.950m	
11	11			Hidden Island	1	Water Maze	60.0s	10.331m	0.172m/s	26	0.294m/s	8	21.6s	3.750m	
12	12			Hidden Island	1	Water Maze	60.0s	12.695m	0.212m/s	39	0.323m/s	5	17.9s	3.535m	
13	13			Hidden Island	1	Water Maze	60.0s	12.084m	0.201m/s	47	0.295m/s	7	16.6s	3.569m	
14	14			Hidden Island	1	Water Maze	60.0s	11.345m	0.189m/s	32	0.319m/s	6	14.7s	3.007m	
15	15			Hidden Island	1	Water Maze	60.0s	12.011m	0.200m/s	29	0.336m/s	2	6.4s	1.402m	
16	16			Hidden Island	1	Water Maze	60.0s	11.119m	0.185m/s	34	0.299m/s	8	25.8s	4.249m	
17	17			Hidden Island	1	Water Maze	60.0s	10.274m	0.171m/s	23	0.314m/s	8	24.3s	4.221m	
18	18			Hidden Island	1	Water Maze	60.0s	9.810m	0.164m/s	19	0.256m/s	5	22.6s	3.580m	
19	19			Hidden Island	1	Water Maze	60.0s	5.438m	0.091m/s	11	0.258m/s	5	35.1s	2.664m	
20	20			Hidden Island	1	Water Maze	60.0s	10.737m	0.179m/s	38	0.313m/s	7	29.5s	4.838m	
21	21			Hidden Island	1	Water Maze	60.0s	9.038m	0.151m/s	33	0.328m/s	3	4.7s	1.002m	
22	22			Hidden Island	1	Water Maze	60.0s	8.711m	0.145m/s	30	0.268m/s	4	10.3s	1.809m	
23	23			Hidden Island	1	Water Maze	60.0s	10.144m	0.169m/s	23	0.324m/s	3	5.3s	1.375m	
24	24			Hidden Island	1	Water Maze	60.0s	11.995m	0.200m/s	44	0.292m/s	6	13.1s	2.945m	
25	25			Hidden Island	1	Water Maze	60.0s	13.188m	0.220m/s	37	0.348m/s	5	15.8s	3.456m	
26	26			Hidden Island	1	Water Maze	60.0s	7.704m	0.128m/s	18	0.253m/s	3	20.5s	1.894m	
27	27			Hidden Island	1	Water Maze	60.0s	1.297m	0.022m/s	3	0.122m/s	1	4.7s	0.057m	
28	28			Hidden Island	1	Water Maze	60.0s	9.284m	0.155m/s	34	0.294m/s	8	23.4s	3.257m	
29	29			Hidden Island	1	Water Maze	60.0s	10.697m	0.178m/s	30	0.280m/s	6	18.2s	3.028m	
30	30			Hidden Island	1	Water Maze	60.0s	8.208m	0.137m/s	31	0.343m/s	6	14.5s	2.566m	

8. Different phases of the MWM

- Pre-training/learning sessions (Day 0 to 4) and Retention Probe test (Day5)



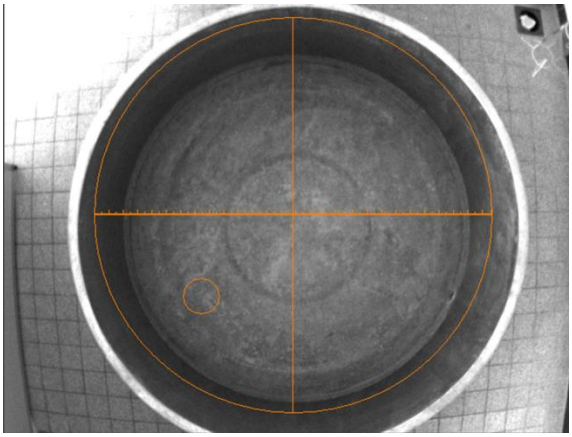


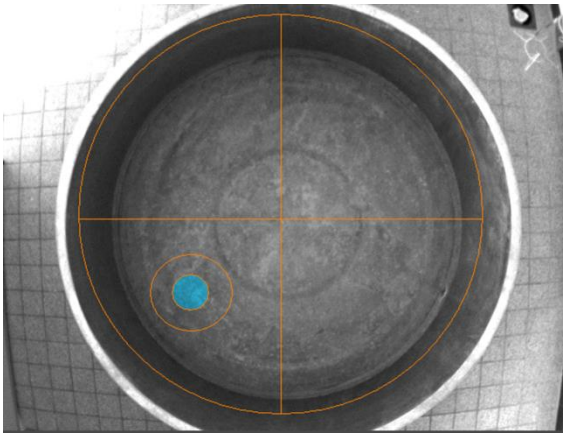
For Day 5 = Retention probe trial:

A new zone "Around Island" was created for retention.

Load "MWM Retention Probe trial" protocol.

- Reversal learning and reversal retention:





Load "MWM Reversal learning sessions" to evaluate reversal learning.

Load "MWM Reversal Retention Probe trial" to assess Reversal retention.

→ Use this protocol if you want to study mental flexibility.

Further methodological details in:

Vorhees CV, ***Williams MT***. *Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocol, 2006*

Patil SS, Sunyer B, Höger H, Lubec G. *Evaluation of spatial memory of C57B6/6J and CD1 mice in the Barnes maze, the Multiple T-maze and the Morris water maze.Behavioral Brain Researc, 2009*

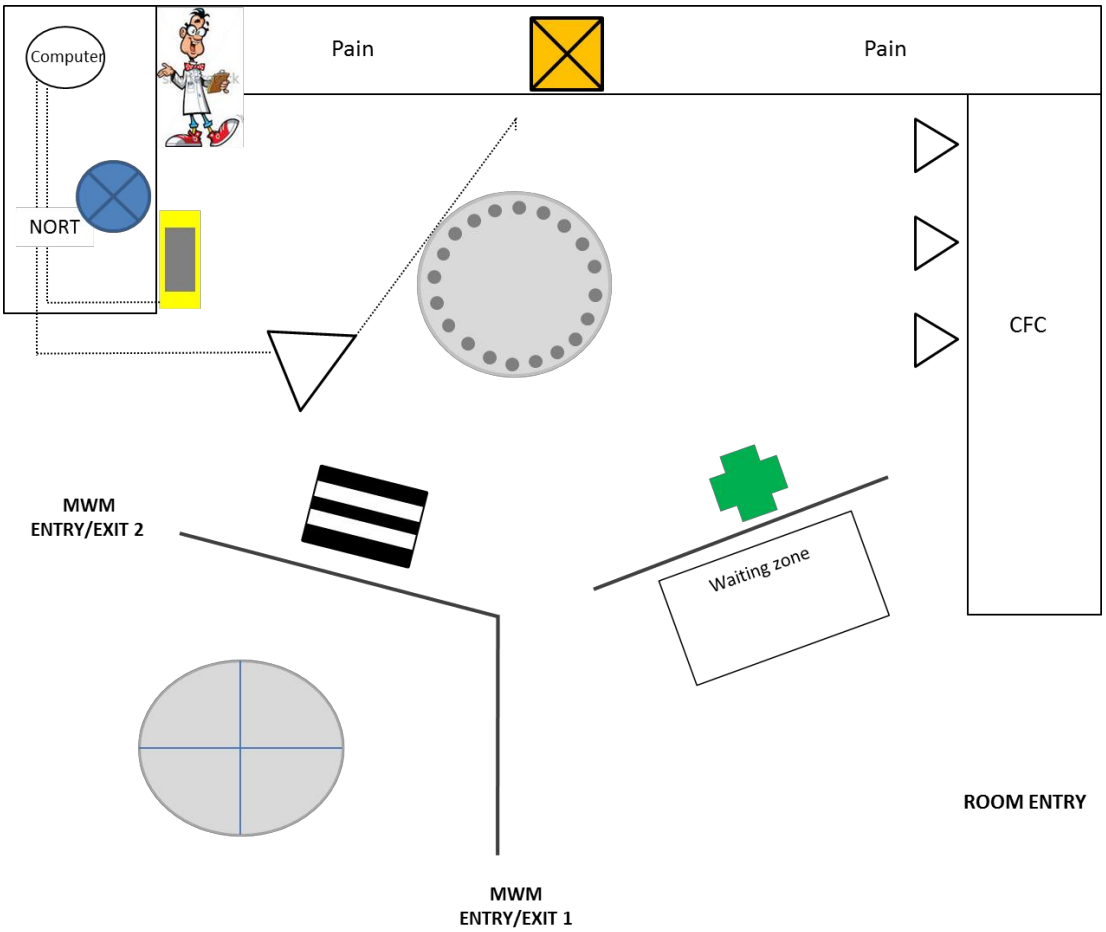
3. Fiche méthode du test du labyrinthe de Barnes

UMRS 1178 – Dépression, Plasticité, Résistance aux Antidépresseurs	
COGNITION PROTOCOLS	Date of creation : 01/14/2015 Page 1/10
How to perform the Barnes maze?	Author : FLAVIE DARCET

MATERIALS:

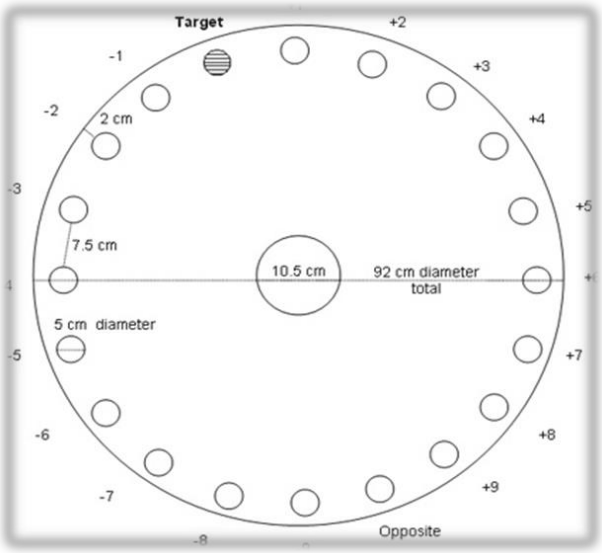
- A gray circular platform (Ø: 92 cm; height: 100 cm) with 20 holes (Ø: 5 cm) along the perimeter
- 20 “false” holes
- 1 target box (20 x 9 x 9 cm)
- 1 black starting cylinder (Ø 8 cm; height: 12.5 cm)
- 4 distal visual cues : different in shape, in color, surrounding the maze (one visual cue per cardinal point if possible, approximatively at the same distance from the center of the platform)
- 70% ethanol solution + soft paper
- Halogen light (near the pool, light pointed on the wall)
- 1 remote control for ANY-maze software to start recording
- ANY-maze software

ROOM CONFIGURATION:



In the Barnes maze, several learning and memory parameters are manually scored during different trials. The experimenter must stay around the computer to control the software and must see entirely the platform. The experimenter becomes a visual cue for animals so be careful to wear the same types of clothes during the whole experiment. Be careful not to change any object position during the whole experiment. In such an open environment, animals could take any changed or added object as a new visual cue.

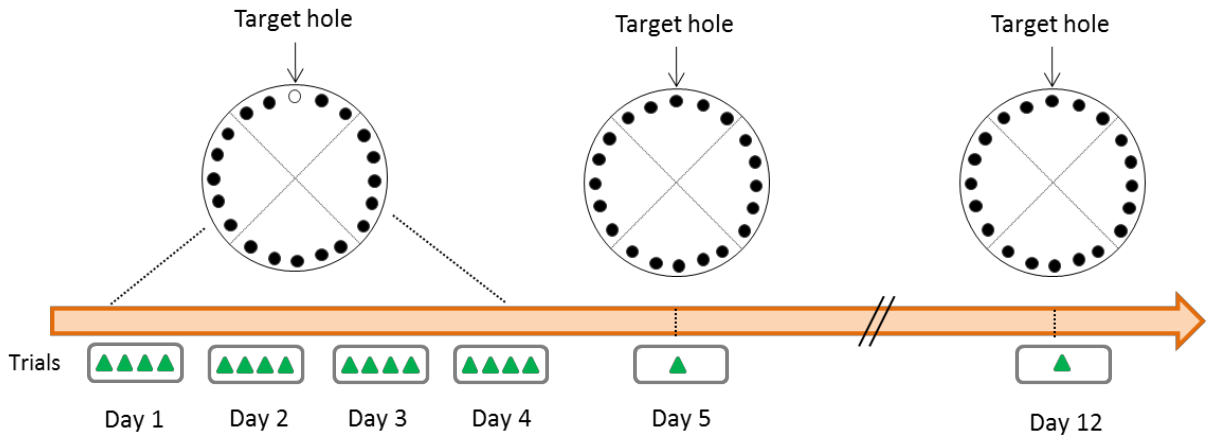
APPARATUS:



AIM OF THE TEST:

The aim of the Barnes maze is to assess reference spatial learning and memory performances. Various versions of the protocol exist depending on what type of spatial learning and memory the experimenter tries to evaluate (short-term memory, long-term memory or cognitive flexibility). The circular platform is 1-meter heigh. In this open environment, mice naturally seek a dark enclosed surrounding place, provided by the black goal box. There is no reinforcement in the goal box. From the surface of the maze, the open escape hole looks identical to the 19-closed holes so that the mice can locate the target box only with the spatial extra cues surrounding the maze.

PROTOCOL (adapted from Sunyer et al., 2009):



The Barnes maze is divided in:

- 4 training sessions (Day 1 to Day 4) which contain 4 trials of 3 minutes separated by 15-20 minutes intertrial interval (during which animals are returned in their home cage)

- 2 probe tests (on Day 5 or Day 12, depending on the type of memory you want to assess)

Day -2

Prepare the room according to the figure before the experiment (place visual cues, dividing walls, platform and camera).

Day -1 (habituation trial)

To reduce anxiety behavior, mice are habituated to the platform and to the target box the day before the beginning of the experiment. During habituation and training sessions, 19 holes are closed. Only one hole is the target box.

- A 30-minute room habituation before the start of the experiment is required for the mice.
- Recording is not necessary for this step.
- Place the mouse in the black starting cylinder. Wait for 10 sec and remove the cylinder.
- Let the mouse explore the apparatus during 30 sec.
- If the mouse enters into the goal box, cover the hole so that the mouse cannot escape during 30 supplemental seconds.
- If the mouse does not enter into the goal box within 30 sec, gently guide it towards the target hole, encourage it to enter and restrain it into the target box during 30 supplemental seconds.
- Replace the mouse in its home cage.

Day 1 to Day 4 (training sessions)

- Mice are transported into the testing room 30 minutes before the start of the experiment
- Place the mouse in the black starting cylinder
- After 10 sec, remove the starting cylinder and start recording immediately (use the remote button or click play directly on the software)
- Let animals explore during 3 minutes
- Manually score the following learning parameters for each trial:

    - **Primary latency** : time required to the mice to make the first contact with the target hole (nose poke)
    - **Primary errors :** number of errors (nose poke or forepaws) before the first contact with the target hole
    - **Total latency** : time for mice to enter the whole body in the target box (4 forepaws out the platform)
    - **Total errors** : total number of errors before entering in the target hole (4 forepaws out the platform)

- Ambulatory parameters are recorded by the software (see details above in "How to perform Barnes maze with ANY-maze software").
- Once animals have entered into the target box, let them stay in during 60 supplemental seconds.
- If they failed to find or enter into the target box, gently guide animals towards the target box entry. For those mice, 180 seconds are recorded for escape latency.
- Return the animals in their home cage during the 15-20 minutes intertrial interval.
- Clean the apparatus, the target box and false boxes between each trial and each animal with a 70% ethanol solution to avoid any olfactory cues.
- Repeat the same protocol for trial 2, 3 and 4.
- Repeat the same procedure for Day 2, Day 3 and Day 4.

A hole was considered visited when mice tilted their head over it (nose poke) or introduced theirs paws in the hole.

You can count errors and measure latencies either facing the maze (directly looking at the mouse on the platform) either looking on the computer screen (back to the maze, indirectly looking at the mouse). Both options are possible, but once you decided, keep it that way for the rest of the experiment.

Always control on the screen that the mouse is correctly tracked. If you rapidly realize that the tracking is not working, you can stop the recording (do not save the trial) and start again **without stopping the timer for latency to identify and enter into the target hole**. Tracking measures are mostly dedicated to distance and speed parameters used to verify that there is no differences in motor activity or motivational behavior between groups.

If you feel that one of your manual scoring is uncertain, you will be able to score again with videos of each trial.

Day 5 and Day 12 (retention)

On Day 5, reference memory was evaluated by a probe/retention trial (90 s) during which the target box was removed and the target hole was closed. Mice were allowed to explore the maze and to visit the target and the adjacent holes.

- Mice are transported into the testing room 30 minutes before the start of the experiment.
- Remove the target box and replace it with a false hole.
- Place the mouse in the starting cylinder, wait for 10 seconds and remove the cylinder.
- Let the mouse explore the apparatus and all the holes during 90 sec.
- Manually score retention parameters:
    - Primary latency
    - Primary errors
    - Total errors
    - Errors distribution (which holes were visited and how many times)
- Return the mouse in the home cage.

Others ambulatory and memory parameters are automatically recorded by the software (time spent in quadrants, distance and speed in quadrants).

On Day 12, mice were once again submitted to a probe trial (recall) in the same conditions as Day 5 to evaluate long-term retention. No training occurred between Days 5 and 12.

 Make sure that no objects have moved between Day 5 and Day 12. Inform other experimenters who could use the room, not to move objects or to replace them correctly.

 Before starting the experiment, identify with attention how many animals you can test per experiment. Count around 1h15 to test 4 animals. Testing 30 animals will take you the day (from 7 a.m to 17 p.m)

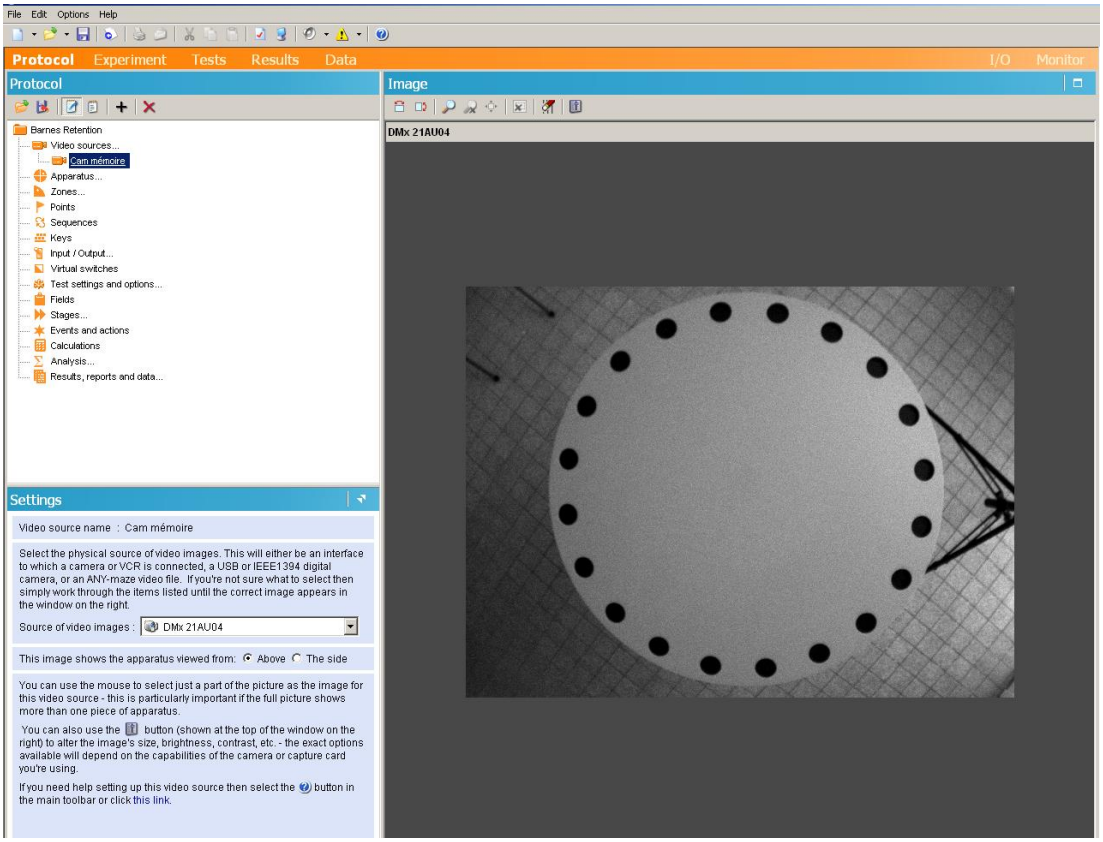
How to perform Barnes maze with ANY-maze software?

5. Open ANY-Maze software, create and name a new experiment (File/New experiment) Save your experiment now and at the end of the experiment (File/Save as)

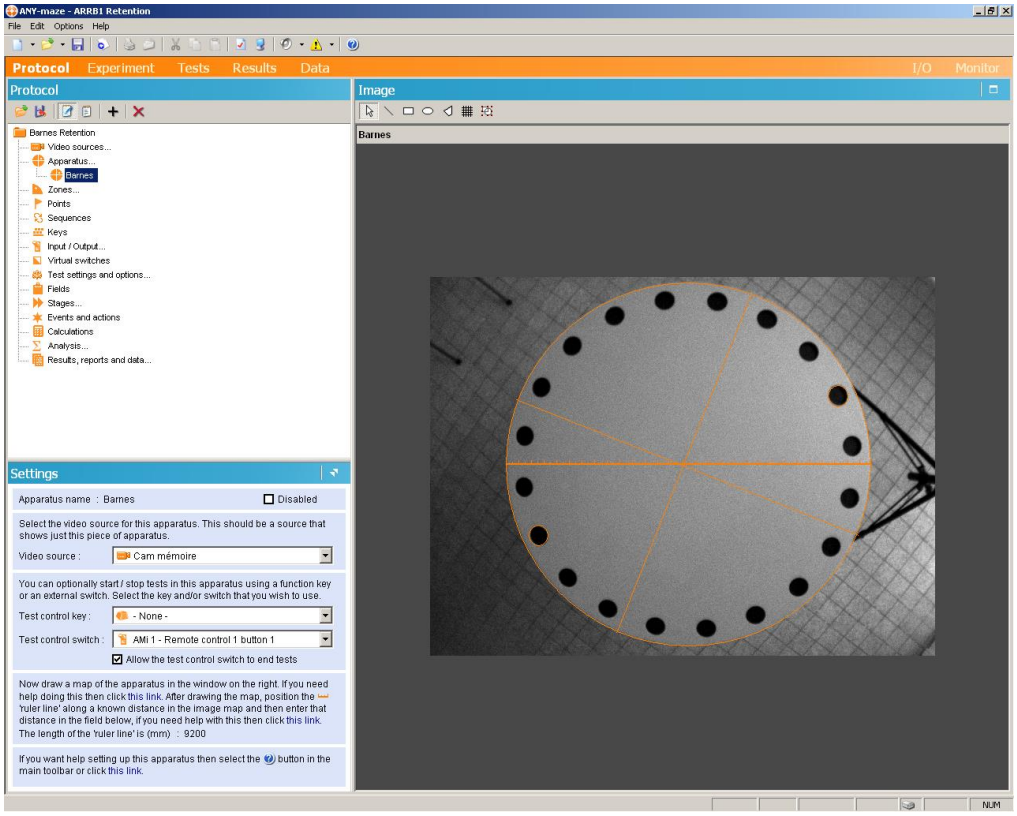
6. Download "BM Learning" protocol

7. Check the following parameters

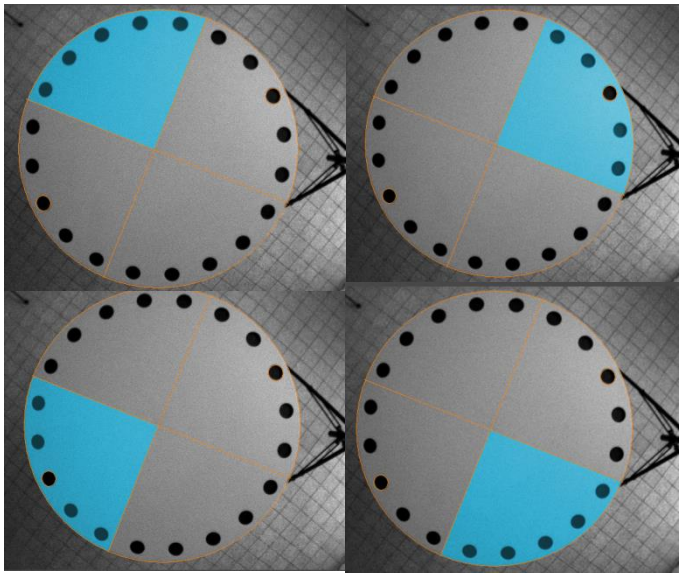
    a. Camera (DMx 21AU04)

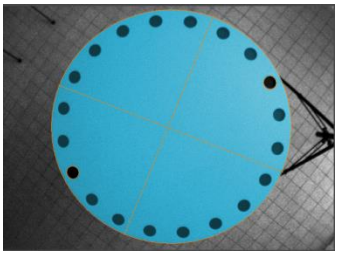


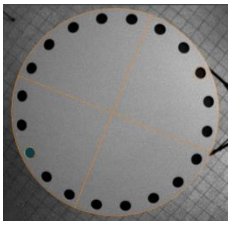
    b. Apparatus:
        i. Draw or adjust equipment delimitation and quadrants
        ii. If you want to use the remote button, select in test control switch : AMi Remote control button
        iii. Check the position of the "ruler line" and its length : 9200 mm

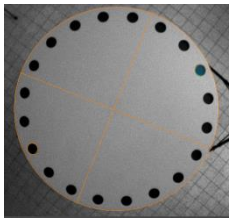


c. Zones : check successively all the zones









d. For the acquisition phase, check learning stages:

   i. Stage name: learning

   ii. Test duration: 180s

   iii. Max number of trials in this stage: 4 (or 3 for 129Sv background)

   iv. Select "Perform each trial for all animals before starting the next trial"

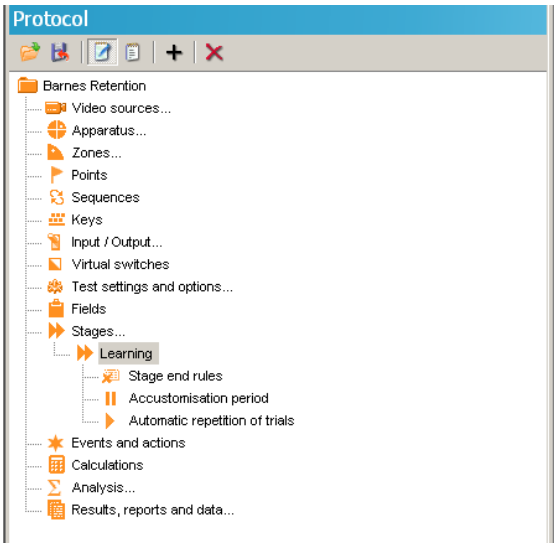
e. For the retention phase, check parameters:

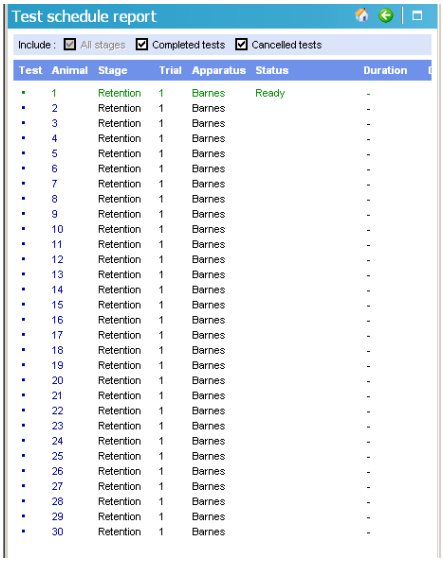
   i. Stage name: retention

   ii. Test duration: 90s

   iii. Max number of trials in this stage: 1

   iv. Select "Perform all the trials for each animals before starting the next animals"





f. In Results, reports and data, check that at least the following parameters are crossed for each zone :

- Time in the zone
- Distance in the zone
- Mean speed in the zone
- Number of entries in the zone

DATA ANALYSIS

Click on the "data" page

- All the parameters you requested in Results, reports and data section or Protocol page are listed in the table
- Export the table in an Excel file to analyze the results with the statistical software of your choice

4. Fiche méthode du test de conditionnement par la peur

UMRS 1178 – Dépression, Plasticité, Résistance aux Antidépresseurs	
COGNITION PROTOCOLS	Date of creation : 02/23/2015 Page 1/18
How to perform the One trial Contextual Fear Conditioning?	Author : FLAVIE DARCET

MATERIALS

- 4 conditioning soundproof boxes (67x53x55 cm)
- 4 conditioning chambers (25x25x25 cm)
- 4 stainless steel grids
- 4 pans underneath grid floors
- PACKWIN Software
- 1 STARTLE AND FEAR INTERFACE LE 118-8
- 4 LOAD CELL COUPLER LE 111
- 4 SHOCKER LE 100-26
- Ethanol 70% solution
- Paper
- One video camera per conditioning box

PROTOCOL:

The protocol was adapted from Wiltgen et al. (2006) and Drew et al. (2010). The one-trial fear conditioning procedure takes place over two consecutive days.

On Day 1, mice were placed in the conditioning chamber and received a terminating shock (2s, 0.4 mA) and were removed 15 s following the shock.

On the following day (+24 h), mice were returned to the conditioning chamber in the exactly same context for 240 s for a test of context-elicited freezing.

A video camera was positioned in front of the chambers to allow the subjects' behavior to be observed and recorded by an experimenter.

***Wiltgen BJ**, Sanders MJ, Anagnostaras SG, Sage JR, Fanselow MS. Context fear learning in the absence of the hippocampus. J Neurosci. 2006*

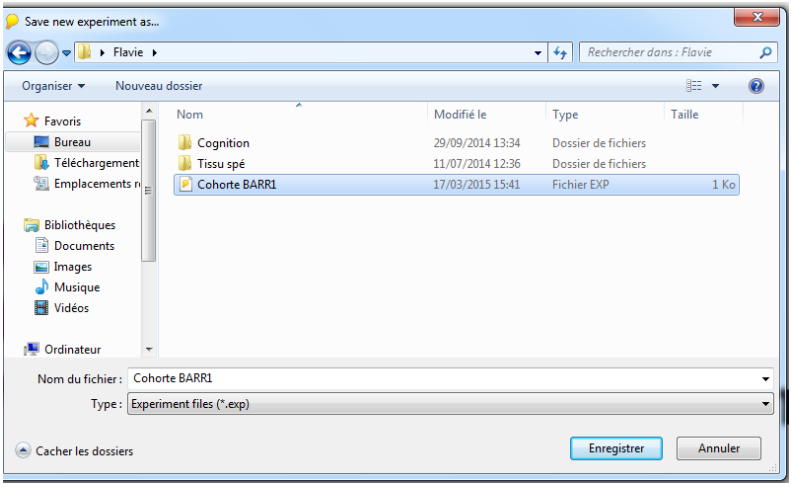
***Drew MR**, Denny CA, Hen R. Arrest of adult hippocampal neurogenesis in mice impairs single- but not multiple-trial contextual fear conditioning. Behav Neurosci. 2010*

PREPARATION OF CONDITIONING CHAMBERS

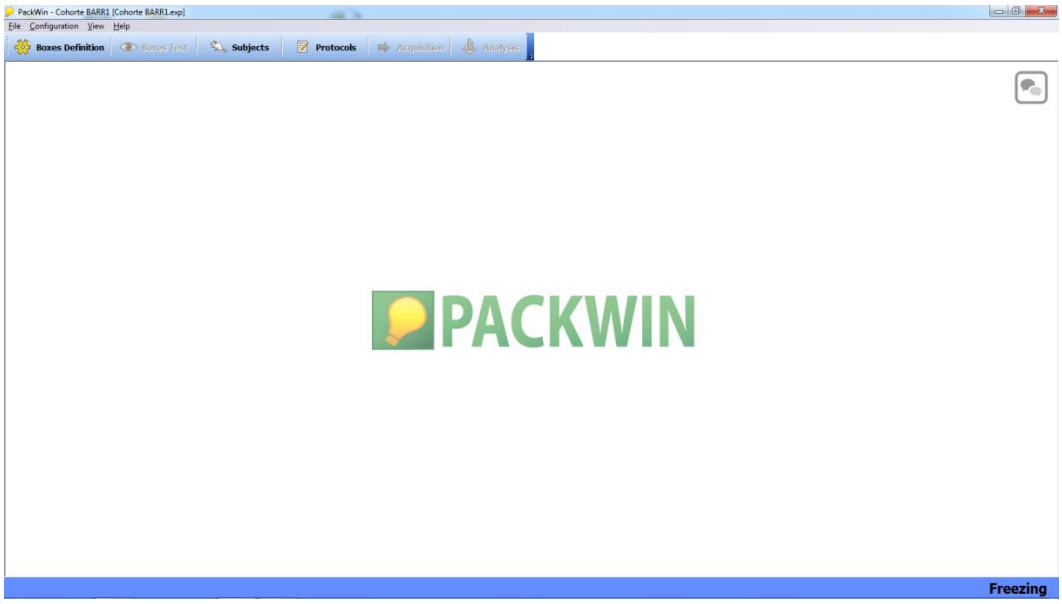
1. Turn on the computer
2. Turn on conditioning boxes and lights (switch behind or between boxes). For Box 1, the light is independent, on the left.
3. First, switch on STARTLE AND FEAR INTERFACE LE 118-8
4. Then, switch on SHOCKER LE 100-26 and LOAD CELL COUPLER LE 111 for Box 1 to 4.
5. Check the following parameters for each Box:
    a. Button on Use (1)
    b. By pushing the button from AC to DC (2), adjust the red hand on O mA playing with the spanner adjuster "Zero (3) **(for this step, the grates have to be connected)**
    c. Button on Shock (4), check that calibration is 0.4 mA by pushing on Calibration
    d. Check inside the box that sensors behind conditioning boxes are well connected

START AN EXPERIMENT

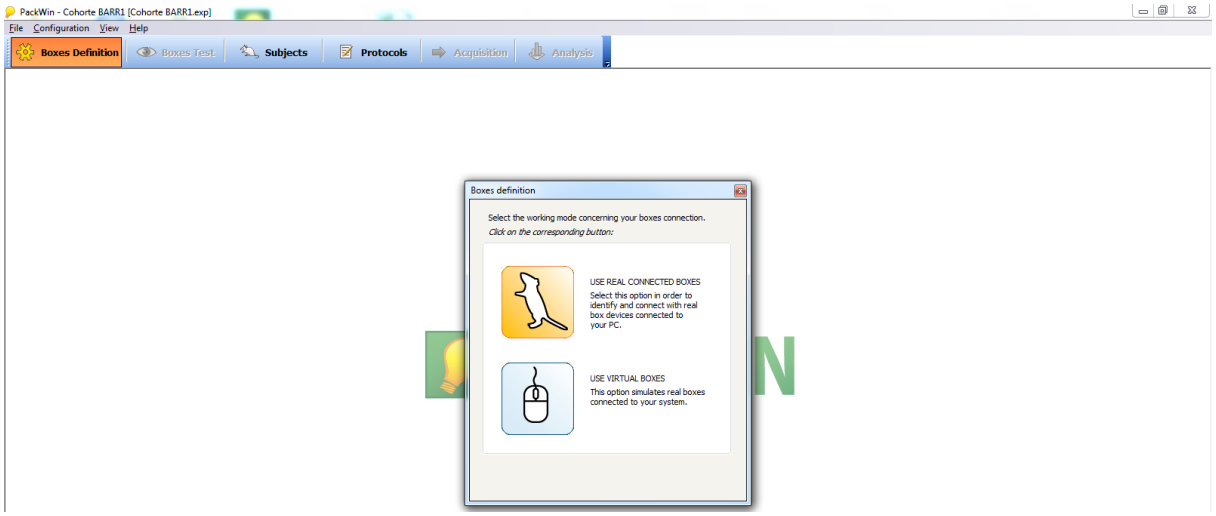
6. A 30-minute room habituation before the start of the experiment is required for the mice.
7. Open PACKWIN 2.0 software (Panlab) on the desktop
8. Select New experiment or Continue if your protocol is already set
9. Name your experiment → Save or Open your experiment

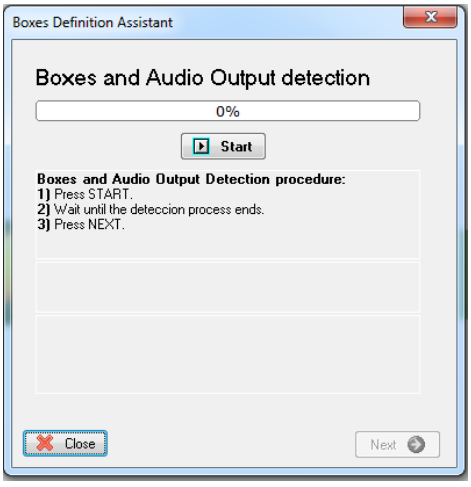


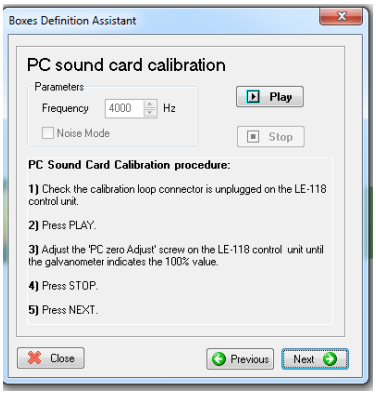
10. The following window opens :

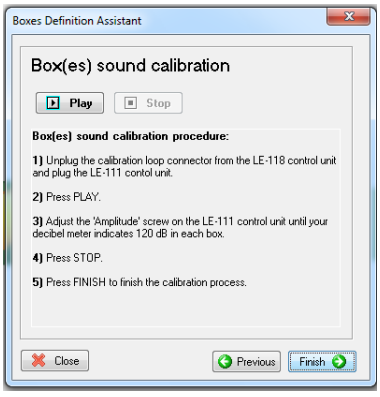


11. Select "Boxes Definition" → "USE REAL CONNECTED BOXES" → Start → Next → Play → Next → Finish

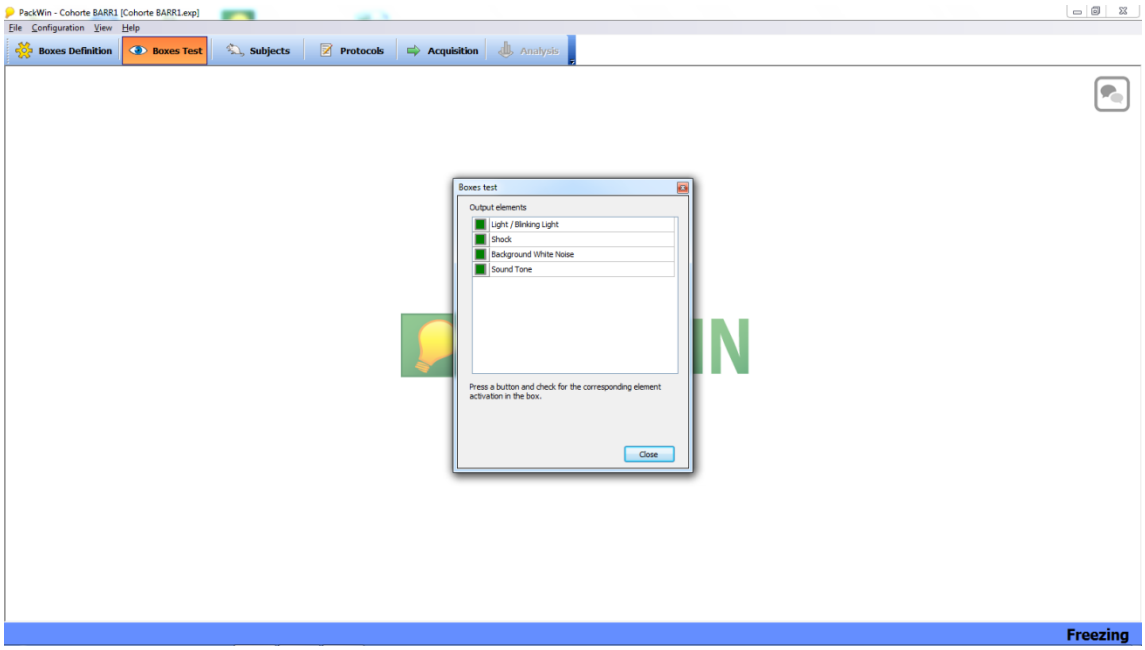


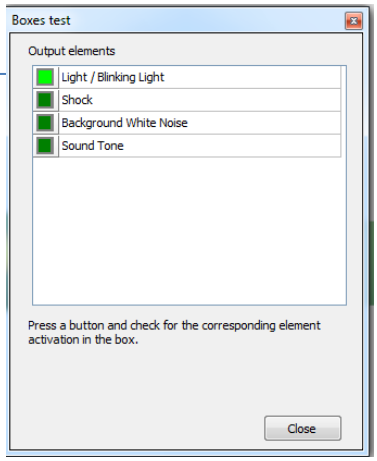


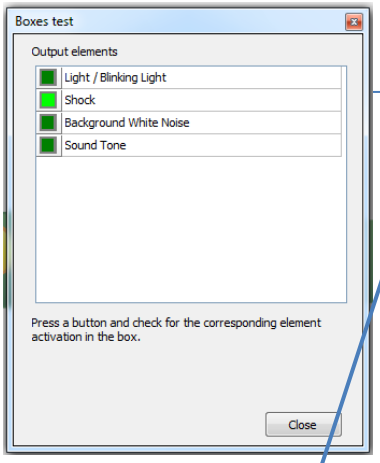




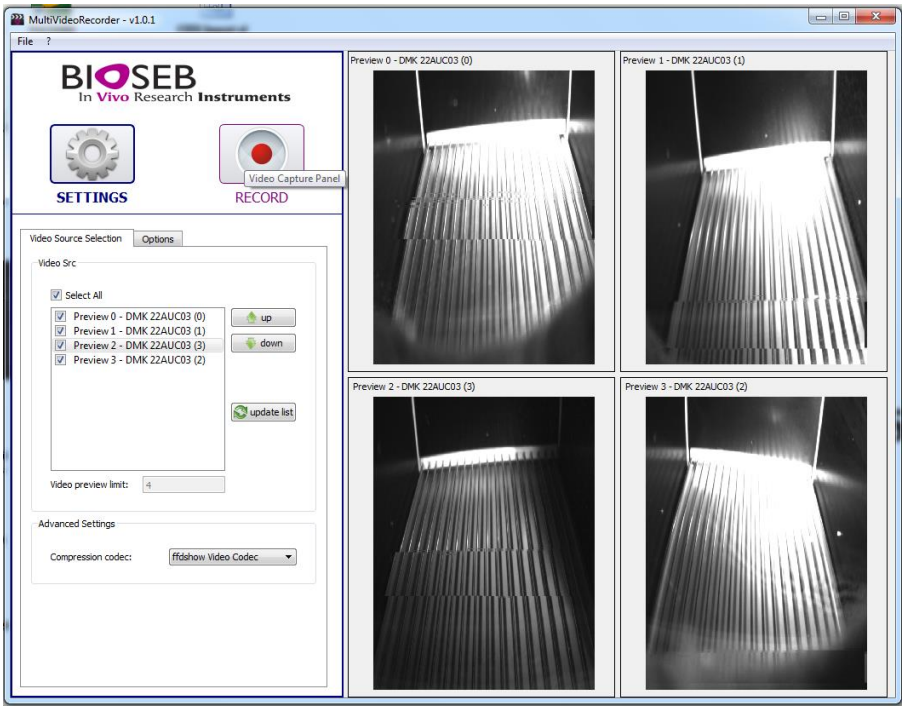
12. Select "Boxes test" → Light → Shock ; Check that the light/shock is on when you select light/shock on the screen







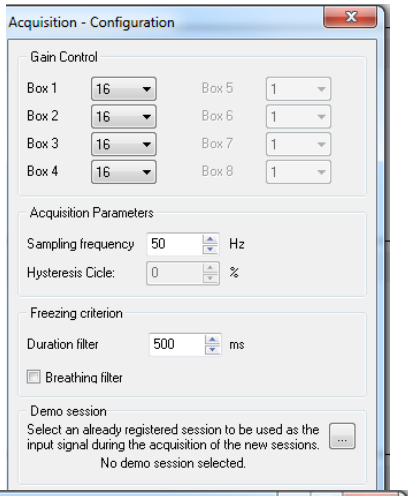
13. Simultaneously, prepare cameras to record videos.
    a. Open Multi Video Recorder on the desktop → Continue
    b. Settings → Pull up Camera 4 and check that all video cameras are working
    c. Settings → Option → Browse → Choose your video file location
    d. Record → "Start all"



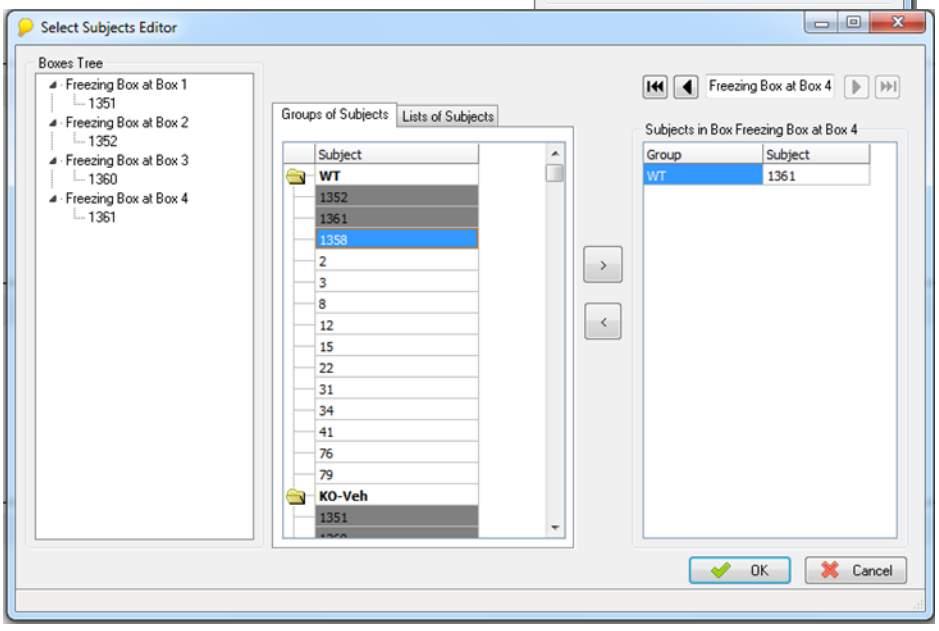
14. Select "Acquisition"

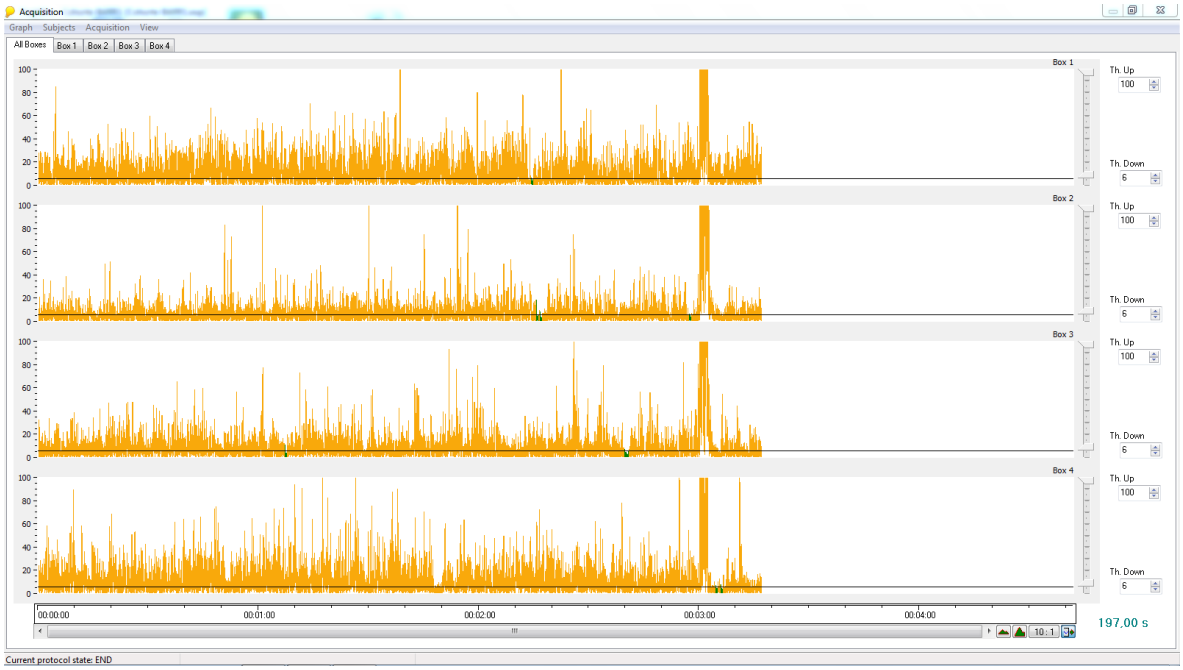
a. Select your protocol (Day 1 or Day 2)
b. Select the number of chambers used (Box 1 to Box 4)
c. Acquisition → Configuration → Gain control 16 for each box **(1)**
d. Subjects → Select subjects → Assign to each box one animal **(2)**
e. Acquisition → Start a run
f. For each box, put the animal in conditioning chambers, close the Plexiglas gate, push the grids into the connected holes, close the chamber and place the camera in the window.
g. Threshold high and low activity for C57Bl 6J (threshold: 6)
h. Click on OK when all the animals and cameras are ready. Start video recorder simultaneously. Movement variations are represented in yellow an freezing episodes are represented in green.

(1)



(2)



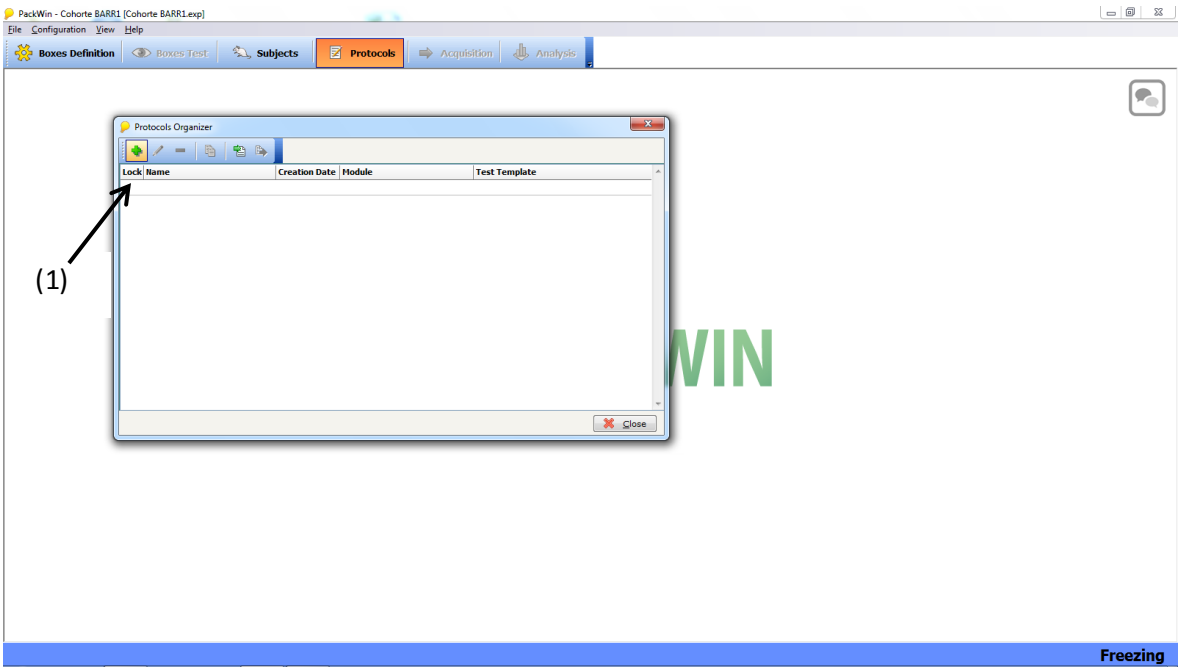


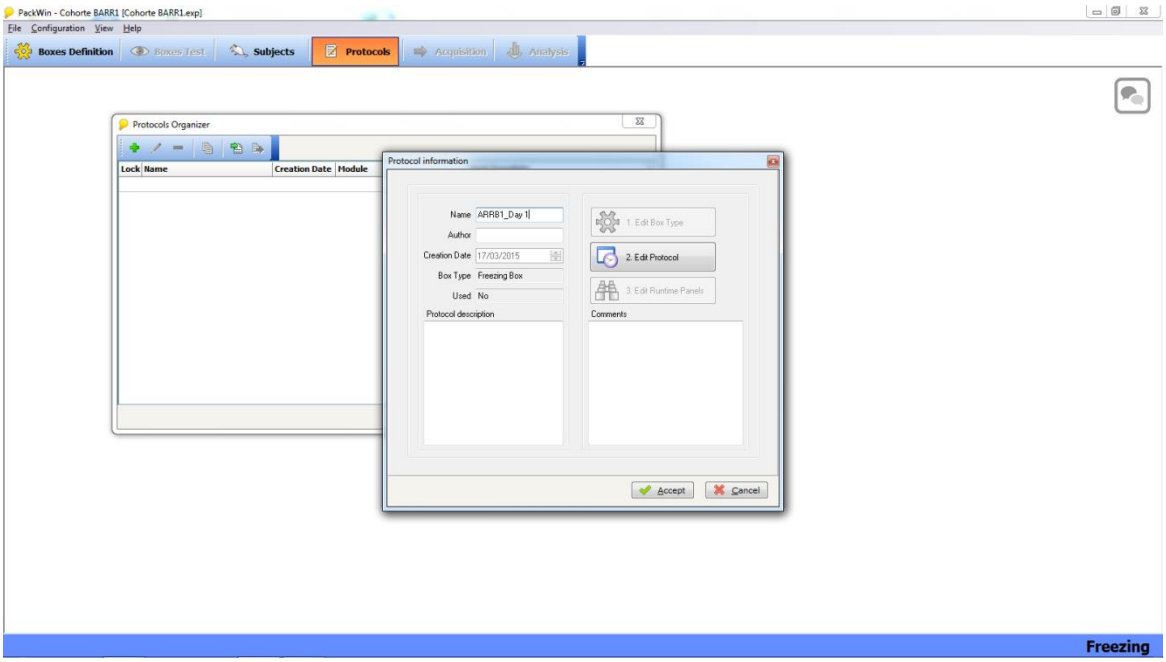
i. At the end of the run, save all and close the window.
j. Remove animals and clean grids and floors
k. Start again from all the procedure to start a new run.

Protocols and the list of subjects are previously prepared to save time during experiments days. See next parts for details.

CREATE A PROTOCOL

15. Select “Protocols” and click on the green cross (1) to add a protocol → “Edit protocol” → Accept





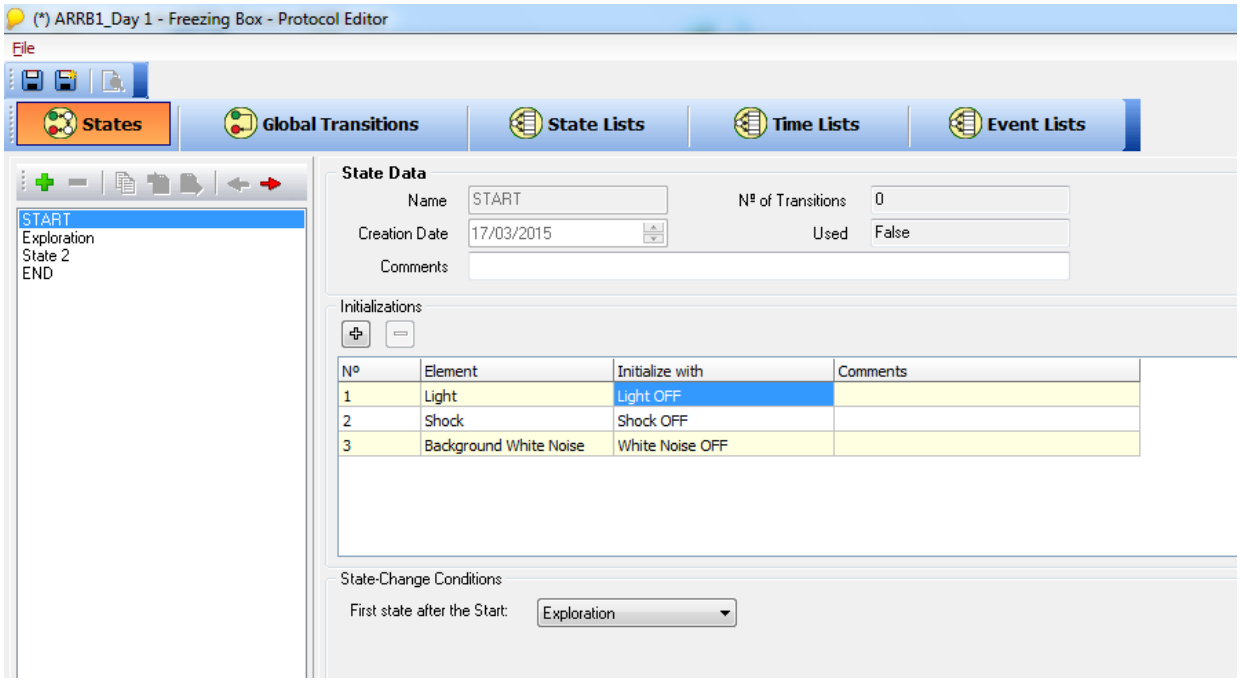
16. Create different phases of the test :

a. For Day 1 : 5 states

Day 1	Start	Exploration 1	Shock	Exploration 2	End
Light	OFF	ON	ON	ON	OFF
Shock	OFF	OFF	ON	OFF	OFF
Destiny	Exploration 1	Shock	Exploration2	End	-
Time	-	0:03:00,000	0:00:02,000	0:00:15,000	-

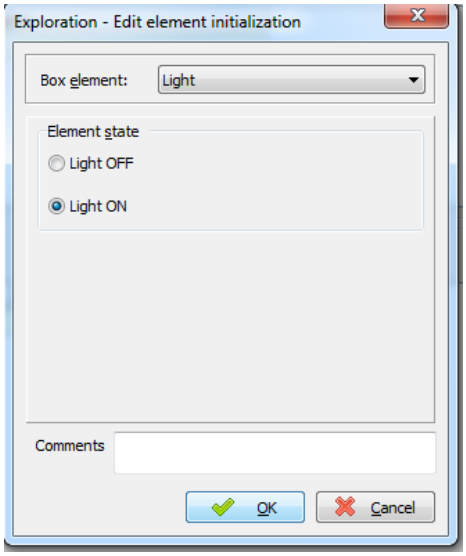
b. For Day 2 : 3 states

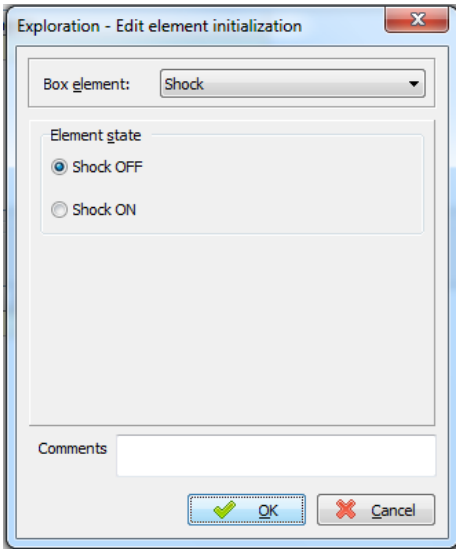
Day 2	Start	Exploration	End
Light	OFF	ON	OFF
Shock	OFF	OFF	OFF
Destiny	Exploration	End	-
Time	-	0:04:00,000	-

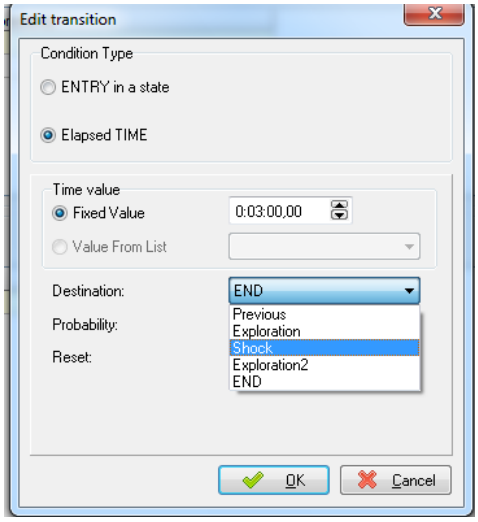


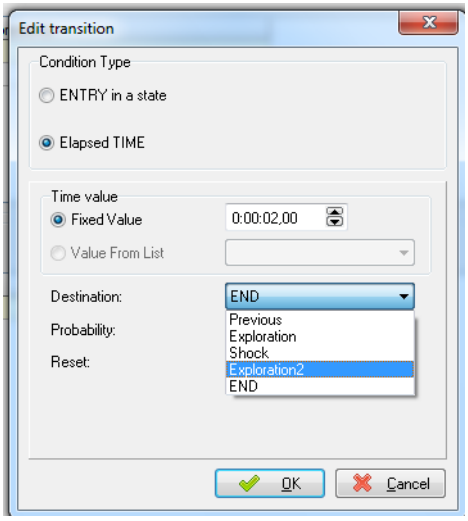
-

- Click on the green cross to add a new state
- Click on Light or Shock to select OFF or ON
- Adjust duration and destiny according to the state

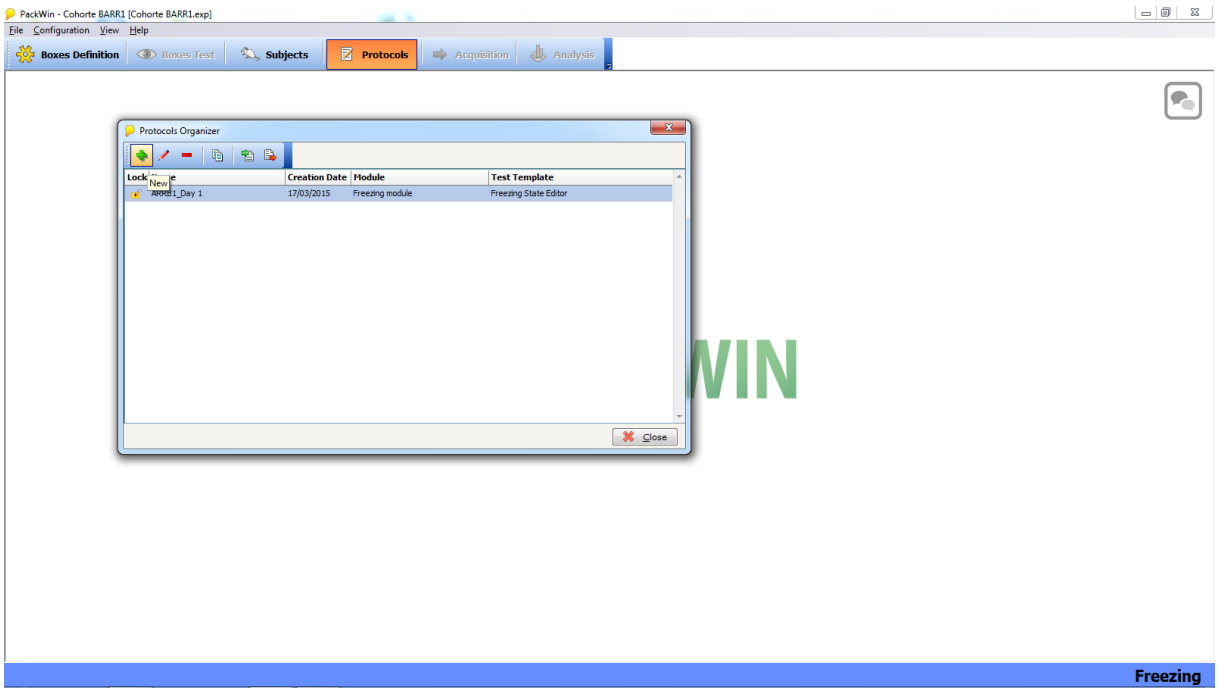


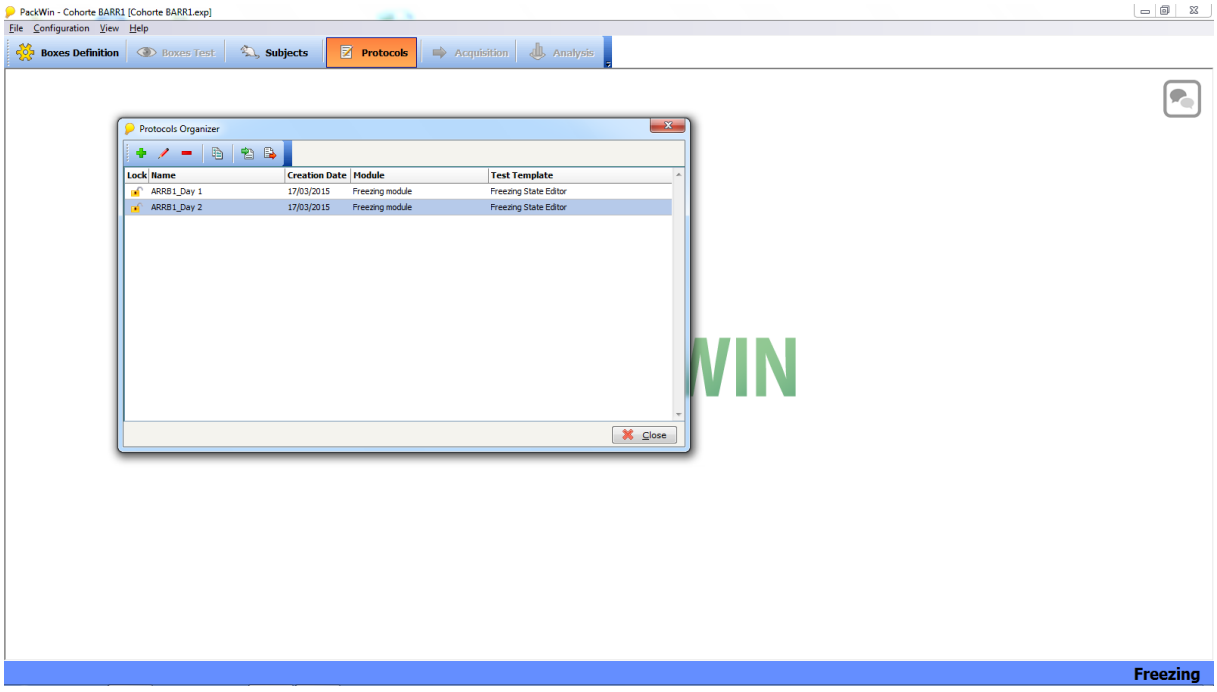






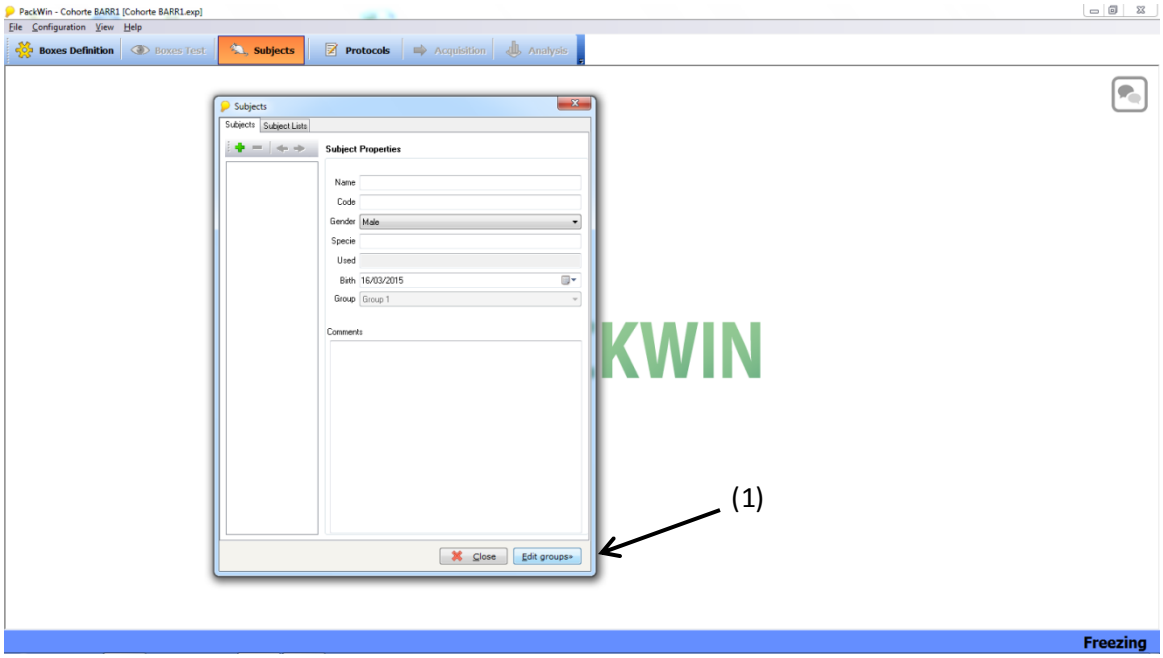
17. Once each state is completed, save the protocol Day 1 and create protocol for Day 2 using the same method and following the Day 2 table for the settings.

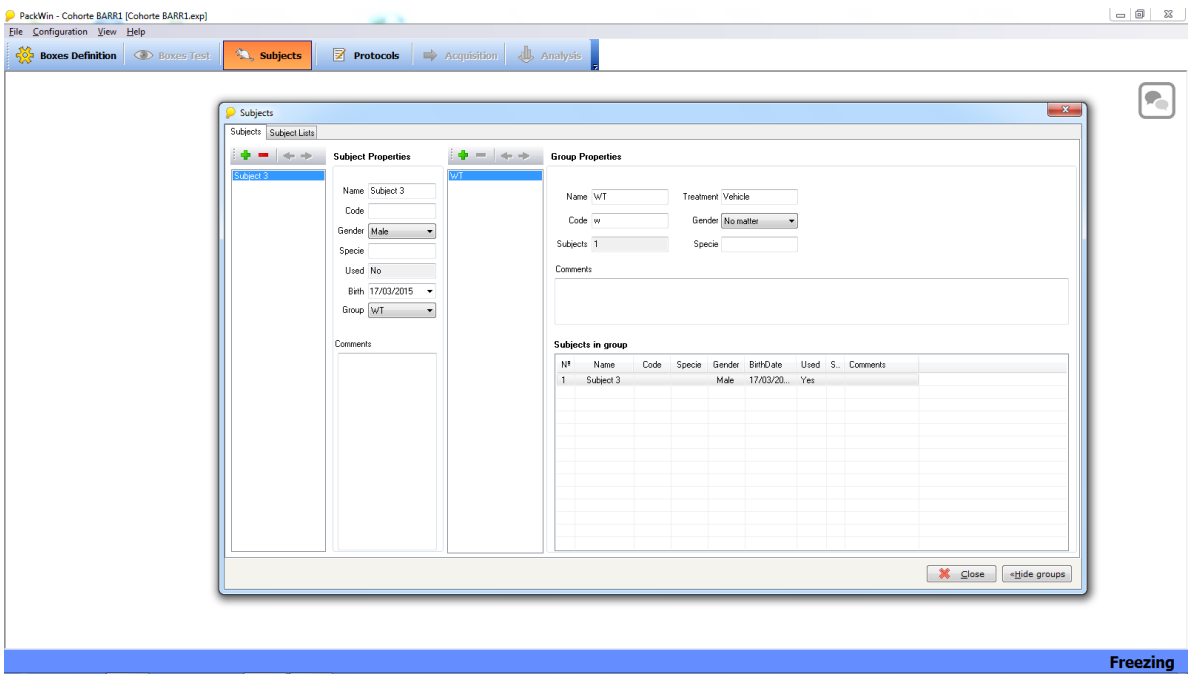




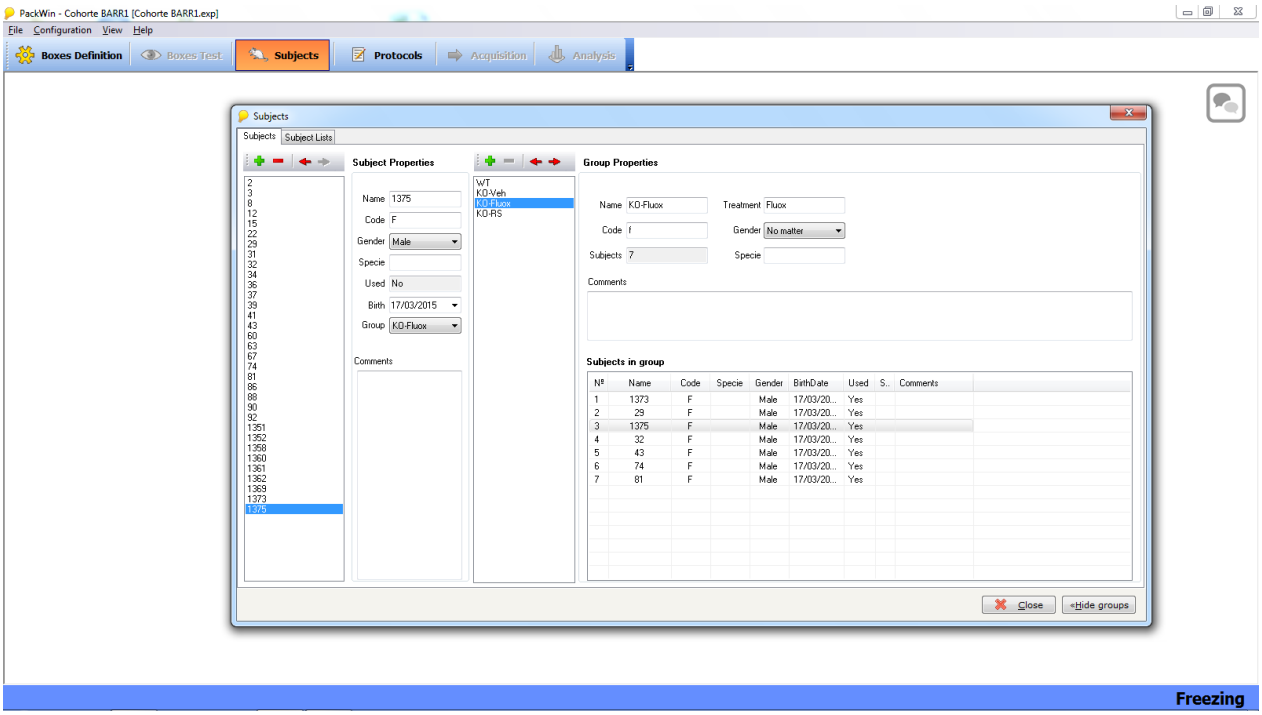
CREATE A SUBJECT LIST

18. Select "Subjects" → "Edit groups" (1)



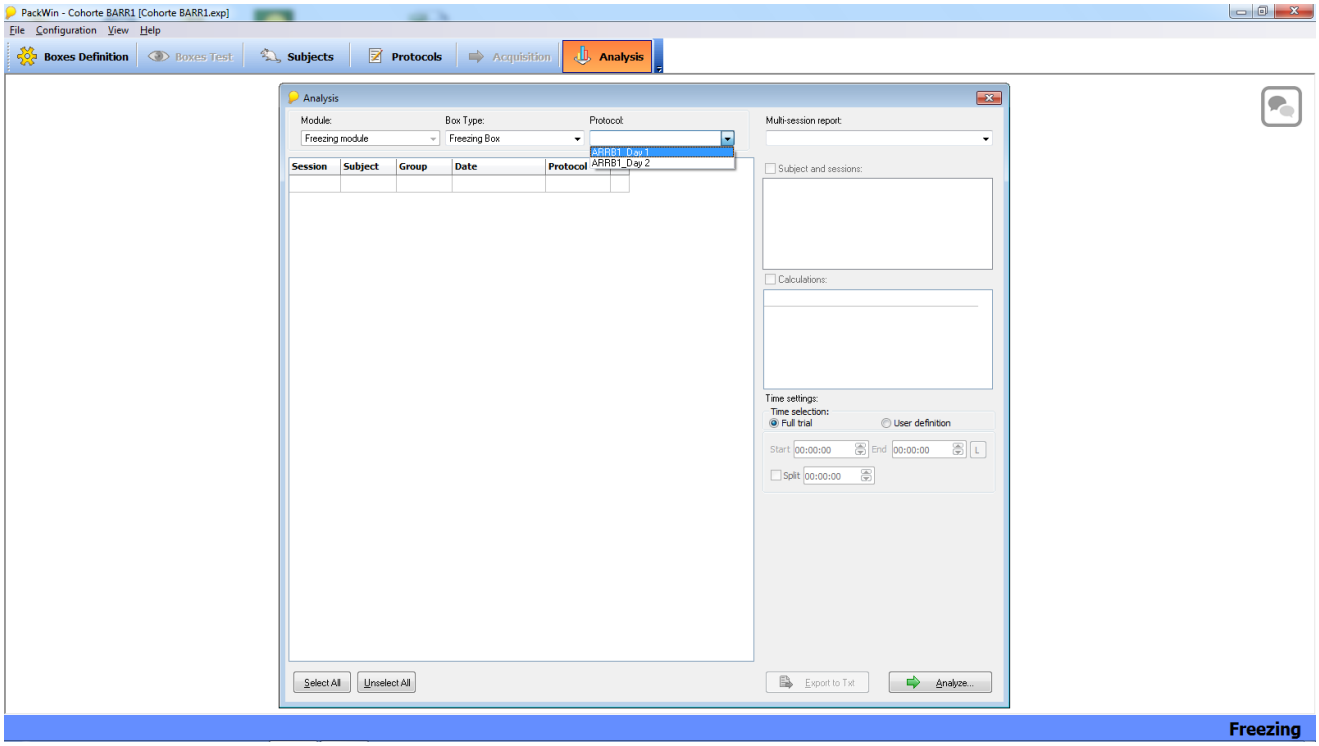


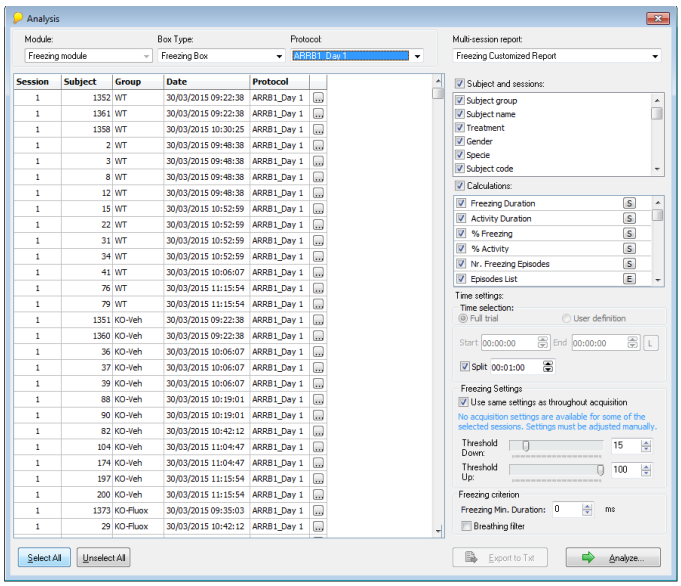
Add all the subjects and select properties of subjects to facilitate the analysis.



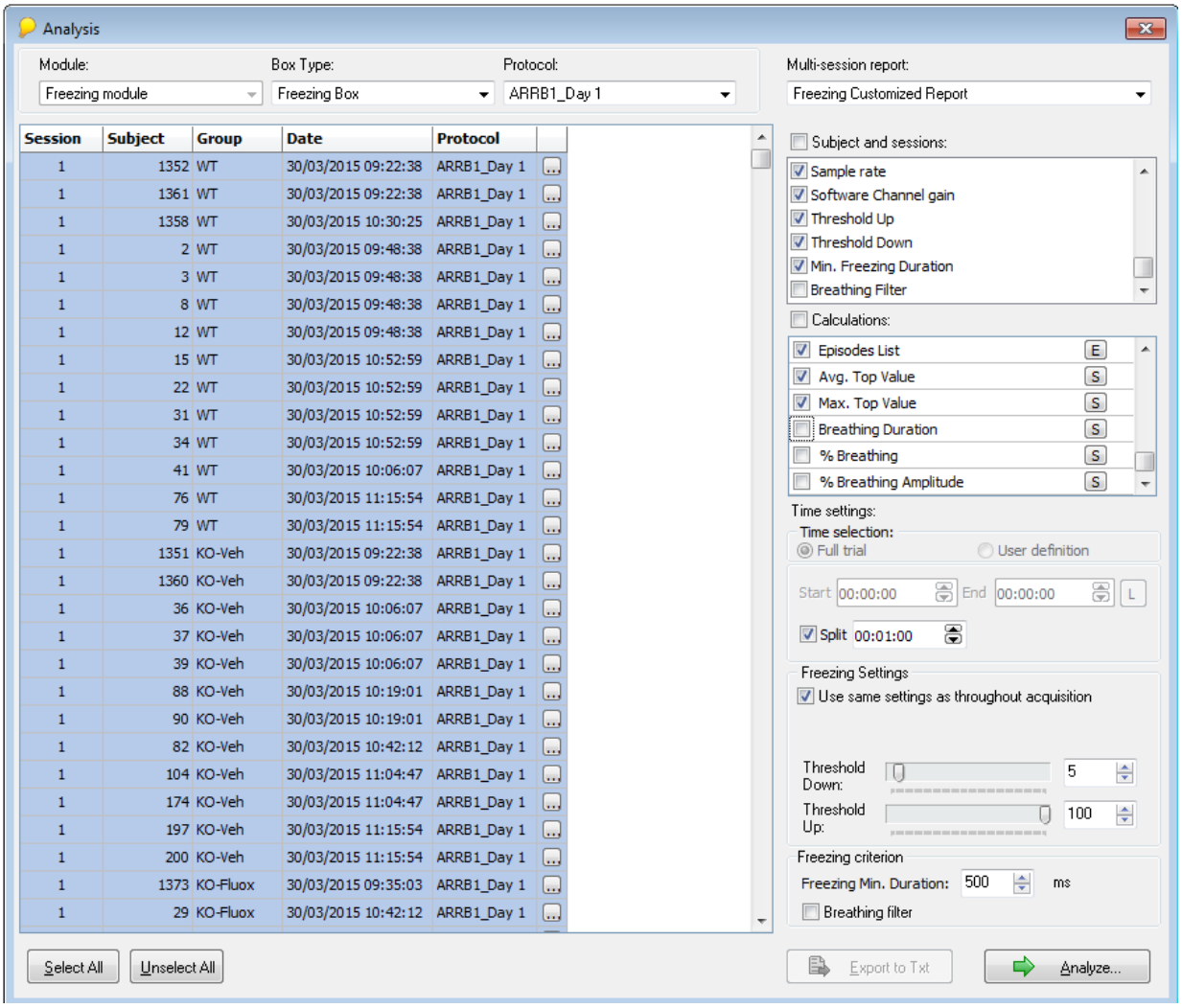
ANALYSE DATA OF EXERIMENTS

19. Open your file
20. Select "Analysis" → Choose your protocol (Day 1 or Day 2) → the list of every animal tested appears





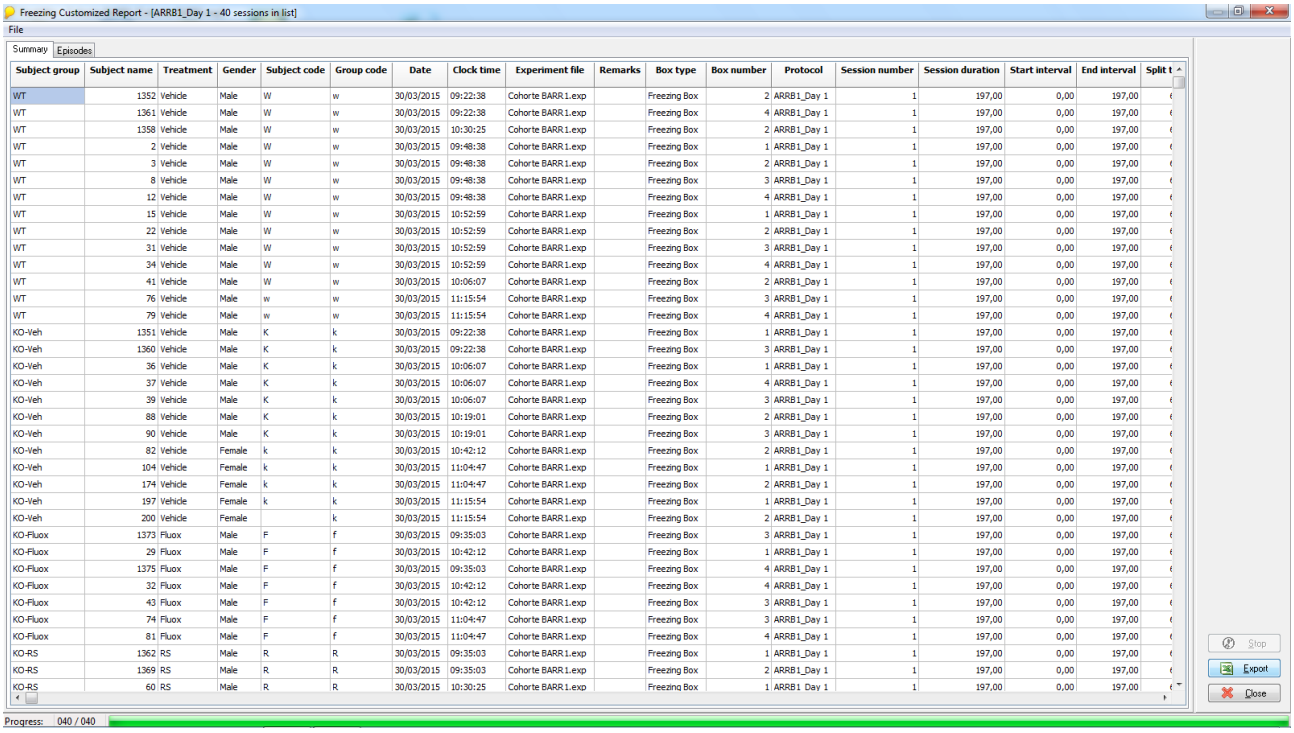
21. Select all → Select or uncheck parameters that you are interested in → Adjust Threshold Down (5 or 6 according to genetic background) → Analyze

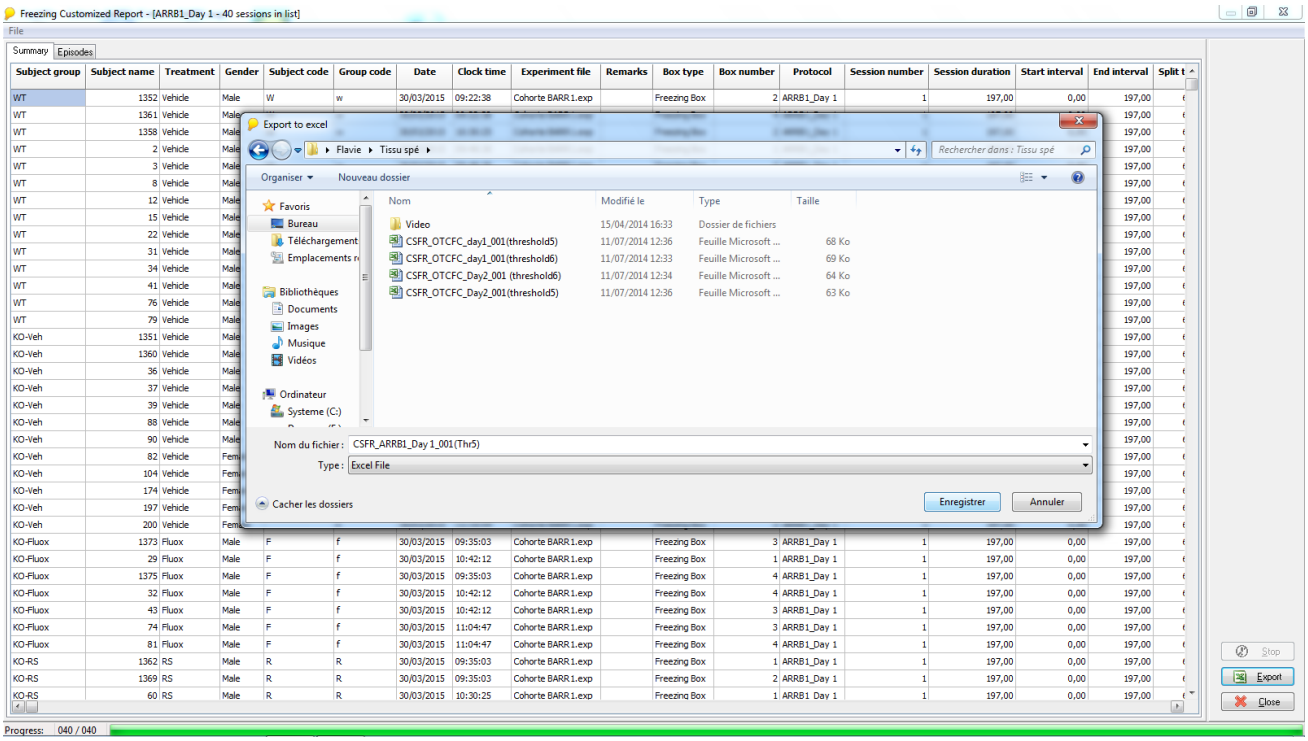


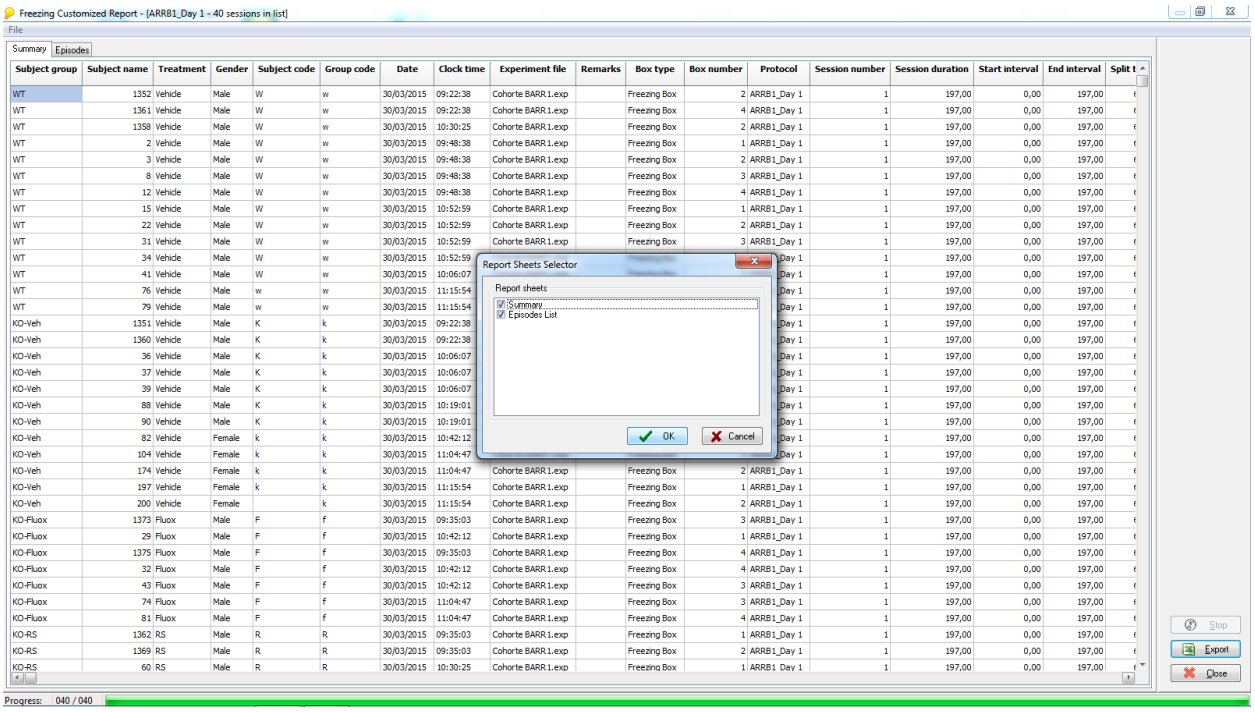
22. When the analysis is finished, data appear in a new window. Select "Export" → Choose your file location and name you data file→ OK

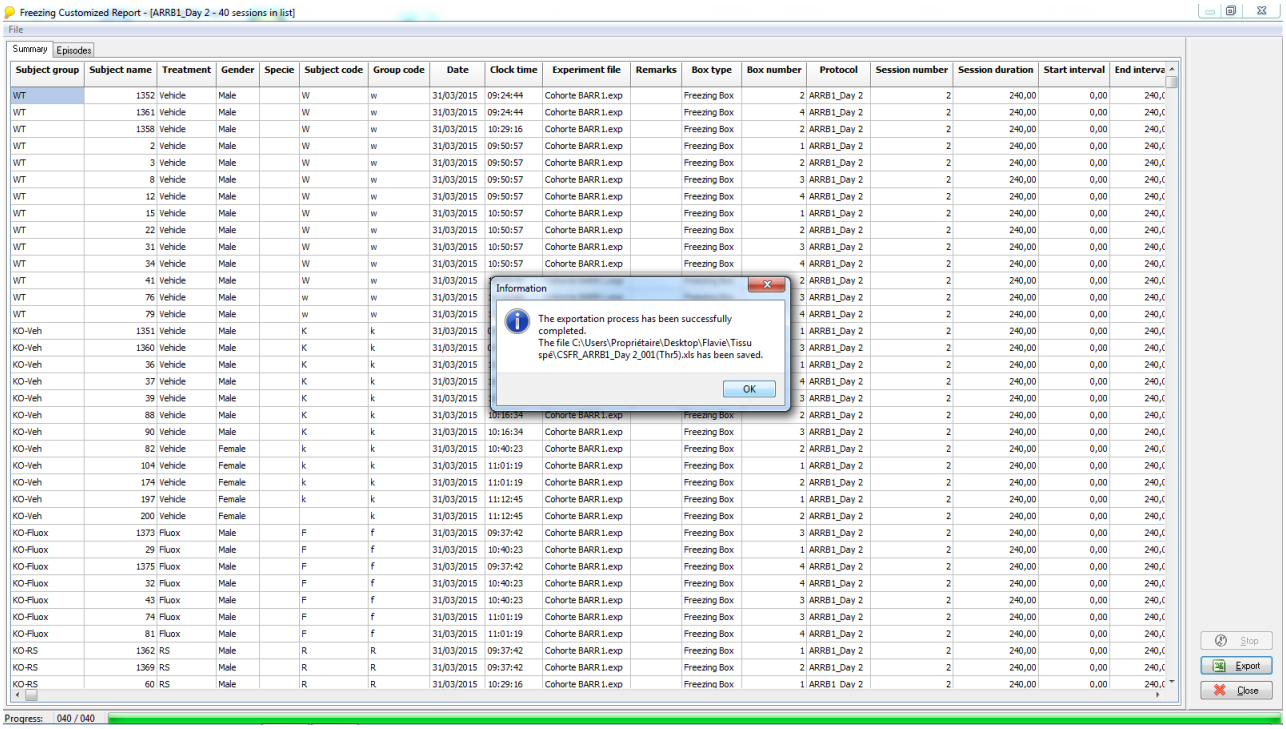
23. Check Summary and Episodes → OK → OK

24. Reproduce the same method to analyze Day 2.


Freezing Customized Report - [ARRB1_Day 1 - 40 sessions in list]
File
Summary
Episodes
Stop
Export
Close
Progress: 040 / 040


Freezing Customized Report - [ARRB1_Day 1 - 40 sessions in list]
File
Summary
Episodes
Export to excel
Flavie ▸ Tissu spé ▸
Rechercher dans : Tissu spé
Organiser
Nouveau dossier
Favoris
Bureau
Téléchargement
Emplacements r
Bibliothèques
Documents
Images
Musique
Vidéos
Ordinateur
Systeme (C:)
Nom
Modifié le
Type
Taille
Video
15/04/2014 16:33
Dossier de fichiers
CSFR_OTCFC_day1_001(threshold5)
11/07/2014 12:36
Feuille Microsoft ...
68 Ko
CSFR_OTCFC_day1_001(threshold6)
11/07/2014 12:33
69 Ko
CSFR_OTCFC_Day2_001 (threshold6)
11/07/2014 12:34
64 Ko
CSFR_OTCFC_Day2_001(threshold5)
11/07/2014 12:36
63 Ko
Nom du fichier : CSFR_ARRB1_Day 1_001(Th5)
Type : Excel File
Cacher les dossiers
Enregistrer
Annuler
Stop
Export
Close
Progress: 040 / 040


Freezing Customized Report - [ARRB1_Day 1 - 40 sessions in list]
File
Summary
Episodes
Report Sheets Selector
Report sheets
Summary
Episodes List
OK
Cancel
Stop
Export
Close
Progress: 040 / 040


Freezing Customized Report - [ARRB1_Day 2 - 40 sessions in list]
File
Summary
Episodes
Information
The exportation process has been successfully completed.
The file C:\Users\Propriétaire\Desktop\Flavie\Tissu spé\CSFR_ARRB1_Day 2_001(Thr5).xls has been saved.
OK
Stop
Export
Close
Progress: 040 / 040

2.2 Etudes des performances liées à l'émotion

2.2.1 Tests prédictifs d'un phénotype ou d'une activité de type anxiolytique

2.2.1.1 Le test du champ ouvert ou test de l'Open Field

L'open field (OF, ou test de champ ouvert) est l'un des plus utilisés en psychopharmacologie. Il est réalisé en plaçant l'animal dans une cage de 40x40 cm ouverte pendant 30 minutes. Les mouvements de l'animal sont mesurés par un système de suivi par des capteurs infrarouge (Activity Monitor, MED associates, Georgia, VT, USA). L'Open Field est utilisé afin de prédire une activité de type anxiolytique (Figure 20). Pour cela, une aire virtuelle représentant un carré de 12,3 cm de côté est définie. En général, les animaux présentent un haut degré d'évitement de l'aire centrale par rapport à la périphérie. L'administration unique d'un anxiolytique, comme une benzodiazépine ou chronique d'un antidépresseur augmentent le nombre d'entrées et le temps passé dans cette aire centrale (Prut and Belzung, 2003). Au final, les variables mesurées dans l'OF sont l'activité ambulatoire totale, le nombre d'entrées dans l'aire centrale, le temps passé dans le centre de l'arène. Ce test peut être utilisé pour une mesure simple de l'activité locomotrice.

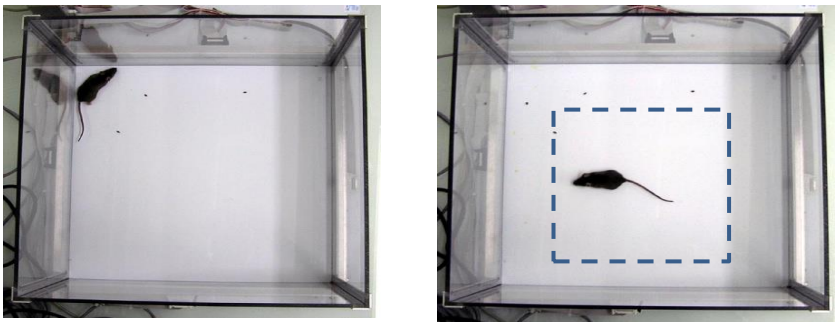


Figure 20: Test de Champ Ouvert chez la Souris. Une administration unique d'anxiolytique ou chronique d'antidépresseur augmentent le nombre d'entrées et le temps passé dans l'aire centrale de l'arène chez la Souris (photo gauche) tandis que classiquement les souris contrôles se déplacent à la périphérie de la cage (photo droite).

2.2.1.2 Le labyrinthe en croix surélevé

Ce paradigme, d'abord développé chez le Rat (Pellow and File, 1986) puis chez la Souris, est également un test de mesure d'un comportement de type anxieux (Figure 21). Le labyrinthe est composé de 2 bras ouverts et 2 bras fermés reliés par une plateforme centrale, à 50 cm au-dessus du

sol et éclairé par une lampe de faible puissance (20 watts). Les souris sont placées individuellement sur la plateforme centrale, face à un bras ouvert et peuvent explorer librement l'ensemble du labyrinthe pendant 5 minutes. Une caméra placée au-dessus du dispositif et reliée à un logiciel de suivi (EPM3C, Bioseb, France) permet l'enregistrement des déplacements. Afin de discriminer un effet anxiolytique d'un effet sédatif, le nombre total d'entrées dans les 4 bras est mesuré. Un phénotype anxieux se caractérise par une diminution du temps passé dans les bras ouverts par rapport au temps passé dans les bras fermés.

Figure 21: Test du labyrinthe en croix surélevé. Un traitement aigu par des anxiolytiques ou chronique d'antidépresseur augmentent le nombre d'entrées et le temps passé dans les bras ouverts (photo gauche) et diminue le temps passé dans les bras fermés (photo droite)

2.2.1.3 Le test des 4-plaques

Le test des 4 plaques (Aron et al., 1971) est un modèle de test d'anxiété lié à la peur, dans lequel l'exploration du nouvel environnement est inhibée par la délivrance d'un choc électrique doux à la patte. L'appareil consiste en une cage de dimensions 25 cm × 18 cm × 16 cm dont le sol est équipé de 4 plaques rectangulaires métalliques identiques (11 cm × 8 cm) séparées les unes des autres par un espace de 4 millimètres (Bioseb, France). Les plaques sont connectées à un générateur de choc électrique (0.6 mA; 0.5 s) au niveau des pattes de l'animal (Figure 22). La cage est fermée par le haut grâce à un couvercle transparent en Plexiglas qui empêche les animaux de s'enfuir et permet de d'observer facilement leurs déplacements et ainsi de déterminer précisément quand administrer le choc. Après une période d'habituation de 15 sec, l'animal reçoit un choc électrique au niveau des pattes lorsque celui-ci se déplace d'une plaque à une autre (le courant ne passe que lorsque l'animal a les deux pattes avant sur une plaque et les deux pattes arrières sur une autre plaque). Le phénotype anxieux est mesuré par le nombre de punitions administrées à l'animal dans une période de 60 secondes.

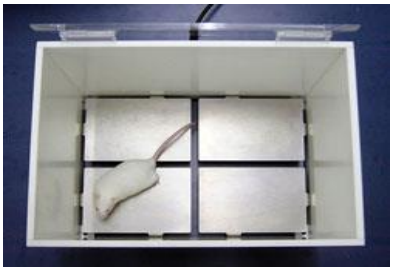
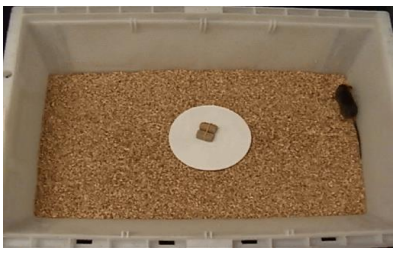


Figure 22 : Test des 4 plaques. Un traitement par un composé anxiolytique augmente le nombre de ces passages, donc le nombre de punitions.

2.2.2 Test prédictif d'un phénotype ou d'une activité anxiolytique et antidépressive : le test d'alimentation supprimée par la nouveauté ou « novelty suppressed feeding »

Ce test induit une situation de motivations conflictuelles chez l'animal, entre celle dirigée vers la nourriture et la peur de s'aventurer au centre de l'enceinte fortement éclairée. Ce test a montré son aptitude à mettre en évidence des changements dans le comportement des rongeurs comme le Rat et la Souris, après un traitement anxiolytiques (traitement aigu) et antidépresseurs (traitement chronique) (Santarelli et al., 2003). L'animal à jeun depuis 24 heures est placé dans une cage rectangulaire de 50x40x20 cm. Au centre de cette cage est disposé un cercle blanc éclairé dans lequel sont déposés 2 granulés de nourriture. L'animal est alors placé dans un coin du dispositif la tête face à la paroi, puis un chronomètre est immédiatement lancé. La latence pour mordre manifestement le granulé (croquer dans le granulé en utilisant ses pattes avant) est enregistrée (Figure 23). Les animaux sont testés individuellement pendant une période de 10 minutes (David et al., 2009; Santarelli et al., 2003). A la suite de ce test, l'animal est replacé dans sa cage et on mesure sa consommation de nourriture pour vérifier que les variations du temps de latence entre des animaux traités et des animaux non traités sont dues à l'activité anxiolytique/antidépresseur des molécules étudiées et non à un appétit moindre.



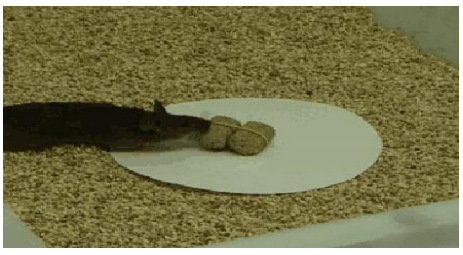


Figure 23 : Test du Novelty Suppressed Feeding. Un traitement aigu par des anxiolytiques ou chronique par antidépresseur diminuent le temps de latence des animaux pour se nourrir. Le dispositif est constitué d'une enceinte dont le sol est recouvert de sciure, et dans le centre duquel est placé de la nourriture sur un disque blanc cartonné (photo gauche). La latence de la prise alimentaire est mesurée (photo droite).

2.2.3 Tests prédictifs d'un phénotype ou d'une activité de type antidépressive

2.2.3.1 Index d'état dépressif : quantification de l'état du pelage

Cette mesure est un indicateur de la dégradation de l'activité de toilettage chez la souris et par corrélation de son état « dépressif » (Figure 24) (Ducottet et al., 2003; Griebel et al., 2002). Le score du pelage est évalué dès le début de l'expérience et répété une fois par semaine, le même jour, jusqu'à la fin du protocole. Ce score est défini après l'évaluation par l'expérimentateur sur 5 zones du corps de l'animal : la tête, le cou, le dos, le ventre, les pattes. Un score de 0 est attribué pour un pelage en bon état, 0.5 si le pelage est peu dégradé, et 1 pour un pelage dégradé. Le score global est ensuite calculé.

Figure 24 : **Dégradation de l'état du pelage.** En conditions standards (photo gauche), l'animal présente un pelage lisse et brillant. Après 4 semaines d'exposition à la corticostérone dans l'eau de boisson, le pelage de l'animal se dégrade en paquets disparates (photo droite).

2.2.3.2 Le splash test

Ce test est assimilé au caractère dépressif puisqu'il met en œuvre la capacité de toilettage chez le rongeur. Les animaux d'une même cage sont placés 30-60 min dans une nouvelle cage (cage de pré-test). Au moment du test, on vaporise une fois et de façon modérée une solution de sucrose à 10% sur le cou de l'animal. Cette solution est non seulement collante, ce qui doit initier un toilettage chez le rongeur, mais également appétante. L'animal est directement introduit dans sa cage initiale sans nourriture ni eau pour la durée du test. Un chronomètre est lancé simultanément. Plusieurs paramètres sont mesurés : la latence à initier un toilettage (qui doit durer plus de deux secondes et comprend une action des pattes avant sur la face, ou un léchage du pelage ventral ou dorsal), le temps total ainsi que la fréquence des toilettages (sur une période de 5 minutes).

2.2.3.3 Le test de préférence à la saccharine

Ce test repose sur la préférence naturelle des souris pour une boisson sucrée (à la saccharine par exemple) par rapport à de l'eau. Il permet ainsi de mesurer le niveau d'anhédonie des animaux, considérée comme un des principaux symptômes de la dépression. D'une façon pratique, le test est réalisé directement dans la salle d'élevage. Les souris sont individualisées la veille du début de l'expérience et sont habituées à boire de l'eau provenant de 2 tubes plastiques de 15 mL (troués au culot) pendant 3 jours consécutifs. Le 4ème jour, les souris ont un libre accès à un tube plastique contenant de l'eau et un autre tube plastique contenant une solution de saccharine à 0,1% pendant une période de 48h. La quantité consommée d'eau et de saccharine est mesurée toutes les 12h à 8h et à 20h chaque jour. De plus, les tubes plastiques sont interchangés toutes les 12h pour éviter le développement d'une préférence de place (droite/gauche). La préférence à la saccharine (en %) est calculée comme suit : Préférence = [consommation de saccharine (mL)/consommation totale (mL) (eau + saccharine)]*100.

2.2.3.4 Le test de suspension caudale

Le TST est un modèle animal (Steru et al., 1985), dérivé du FST où la suspension de la souris ou du rat par la queue induit une immobilité. Il mesure la durée de l'immobilité (pendant 6 minutes) ainsi que la puissance des mouvements de l'animal (Porsolt et al., 1987; Steru et al., 1985) permettant de distinguer différentes classes de psychotropes. Le TST résoudrait plusieurs problèmes rencontrés avec le FST : l'immobilité est mesurée objectivement (automatique) et aucune hypothermie n'est induite après immersion dans l'eau froide (Thierry et al., 1986). Les études montrent que le TST peut être prédictif de l'activité de nombreux antidépresseurs. Un scotch doux est placé au bout de la partie caudale de l'animal, ce qui permet de le suspendre par la queue à un crochet. Le crochet est relié à un capteur qui enregistre les variations de mouvements (Bio-TST2, Bioseb, Vitrolles, France, Figure 25). L'enregistrement des données est effectué sur une période de 6 minutes.

Figure 25: Dispositif d'enregistrement du test de suspension caudale. Une souris traitée par un antidépresseur voit son temps d'immobilité diminué.

3 Manipulations *ex vivo*

3.1 Culture de cellules souches neurales à partir de gyrus dentelé d'hippocampe adulte de Souris

De façon à vérifier la délétion spécifique de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé de l'hippocampe, des cultures de cellules souches provenant du gyrus dentelé de souris ARRB1$^{-/-}$ ont été réalisées en collaboration avec le laboratoire de Sandrine Humbert de l'Institut des Neurosciences de Grenoble. La culture des cellules souches neurales à partir du gyrus dentelé de l'hippocampe est effectuée comme décrit dans Babu et al., 2011 (Babu et al., 2011). La culture de cellules souches de la zone sous ventriculaire des mêmes souris est réalisée en suivant le même protocole. Brièvement, des souris adultes (de 2 à 4 mois) mâles ARRB1$^{+/+}$ et ARRB1$^{-/-}$ sont tuées par dislocation cervicale puis les tissus sont rapidement prélevés dans une solution de PBS stérile. Après digestion enzymatique, les cellules viables sont séparées des débris cellulaires et de la myéline par centrifugation sur gradient de Percoll. Les cellules sont mises en culture dans du DMEM/F12/Glutamax complémenté avec 1% de Pénicilline/Streptomycine, 1% de B27, 20 ng/ml d'EGF et 20 ng/ml de FGF-2 dans des boîtes de pétri préalablement traitées à la poly-D-lysine (20 µg/ml) et à la laminine (20 µg/ml) pour promouvoir l'adhérence des cellules souches et leur croissance en monocouche. Les cultures se développent dans l'incubateur pendant 7 jours (zone sous-ventriculaire) et 14 jours (gyrus dentelé) puis les protéines sont récoltées dans du RIPA classique supplémenté en inhibiteurs de protéases et phosphatases en vue d'un dosage de la protéine β-arrestine 1 par Western Blot. Les cultures ont été réalisées par Fabienne Agasse et Caroline Benstaali de l'Institut des Neurosciences de Grenoble.

3.2 Mesure de l'expression protéique par Western Blot

3.2.1 Dosage des protéines

La quantité de protéines présentes dans les différents échantillons est préalablement déterminée afin de déposer la même quantité de protéines dans chaque puit. La quantification des protéines a été effectuée en utilisant une technique combinant la réaction de Biuret (Gornall et al., 1949) avec une détection colorimétrique hautement sensible et sélective des ions cuivre, par un réactif contenant l'acide bicinchoninique (Thermo Scientific Pierce BCA Protein Assay Kit, Pierce Biotechnology).

3.2.2 Technique du Western Blot

L'électrophorèse est réalisée dans un gel de polyacrylamide additionné de 10% de laurylsulfate de sodium (SDS-PAGE) pendant 1h à 160 V (Amersham ECL Gel Running®). Les protéines sont ensuite transférées sur une membrane de polyvinylidène difluoride (PVDF) (Amersham, Biosciences, Les Ulis, France) dans du tampon de transfert pendant 2h à 100V à 4°C. Après une série de rinçages et le blocage des sites non spécifiques, les membranes sont incubées avec des anticorps primaires pour détecter les quantités totales des protéines d'intérêts et leurs formes phosphorylées quand nécessaire (Tableau 13).

Tableau 13 : Récapitulatif des anticorps utilisés en Western Blot

Anticorps primaires	Blocage	Dilution	Référence	Fournisseur	Poids (KDa)
β-arrestine 1	Lait	1 :500	D24H9	Becton Dickinson	50
ERK	BSA	1 :500	T202/Y204	Cell Signaling	42-44
Phospho-ERK	BSA	1 :500	T202/Y204	Cell Signaling	42-44
AKT	BSA	1 :1000	C67E7	Cell Signaling	60
Phospho-AKT	BSA	1 :1000	T308	Cell Signaling	60
β-actine (HRP)	BSA/Lait	1 :10000	SC47778	Santa Cruz Biotechnology	37

Les anticorps primaires sont détectés grâce aux anticorps secondaires appropriés couplés à la HRP (horseradish peroxydase), et le signal ECL (SuperSignal West Pico Chemiluminescent Substrate, Pierce, Belgique) est quantifié grâce au système ChemiDoc XRS (Bio-rad, Marnes-La-Coquette, France). Pour s'assurer qu'une quantité égale de protéines a été déposée dans chaque puit, les taux de β-actine ont été mesurés comme contrôle pour chaque échantillon. Les bandes sont ensuite quantifiées en utilisant le logiciel Image Lab. Lors de cette expérience, la même membrane a été utilisée pour révéler les différentes protéines. Le traitement de la membrane par une solution de Stripping pendant 30 minutes permet de « défixer » les complexes anticorps primaire-secondaire de la membrane et de réaliser un nouveau marquage.

3.3 Cartographie de l'expression d'ARN messager par technique d'hybridation *in situ*

Afin de vérifier l'expression et la localisation de l'ARNm de la protéine β-arrestine 1 dans le cerveau des souris ARRB1$^{-/-}$, la technique d'hybridation *in situ* a été réalisée [RNAscope® 2.0 HD, ACDBio]. Cette méthode est une technique de détection indirecte qui vise les acides nucléiques et repose sur la propriété qu'ont 2 séquences nucléiques complémentaires de s'apparier de façon

spécifique. Des sections coronales d'hippocampes issues de souris ARRB1$^{+/+}$ et ARRB1$^{-/-}$ de 20 µm d'épaisseur ont été coupées au cryostat, fixées au paraformaldéhyde (PFA) puis déshydratées dans des bains successifs d'éthanol à température ambiante. Les sections sont d'abord prétraitées par une solution de Pretreat pendant 10 minutes, rincées, puis hybridées par la sonde appropriée, un oligodeoxyribonucléotide complémentaires des bases de l'ARNm de la protéine β-arrestine 1 chez la souris. Après avoir été incubées avec la sonde pendant 2h à 40°C puis lavées avec un tampon de lavage, les sections sont hybridées avec différents réactifs stabilisants à 40°C pendant 15 à 30 minutes selon le réactif. Pour amplifier le signal, les sections sont mises en contact avec des substrats du DAB pendant 10 minutes à température ambiante. Après 2 lavages à l'eau, les sections sont colorées à l'hématoxyline 50% puis à l'eau Ammoniaquée 0,02% pendant 2 minutes. Pour finir, les coupes sont déshydratées dans un bain d'éthanol à 70%, 2 bains d'éthanol à 100% et un bain de xylène. L'ARNm de la protéine β-arrestine 1 est visualisée grâce à un microscope à champ clair et les sections scannées par un scanner de lames (ZEISS Axio Scan.Z1) en collaboration avec l'équipe de Fabrice CHRETIEN (Unité d'Histopathologie humaine et modèles animaux, Institut Pasteur, Paris). L'intensité d'expression de β-arrestine 1 est déterminée par comptage en utilisant les logiciels Guide ZEN 2012 – Slidescan et FIJI. La délétion devant être spécifique à la zone sous granulaire du gyrus dentelé de l'hippocampe, la zone de comptage se limite au premier tiers interne du gyrus dentelé. Une fois le seuil de détection correctement réglé, le rapport du nombre de pixels noirs sur le nombre de pixels blancs correspond au pourcentage d'expression de la β-arrestine 1 dans la zone étudiée (Figure 26).

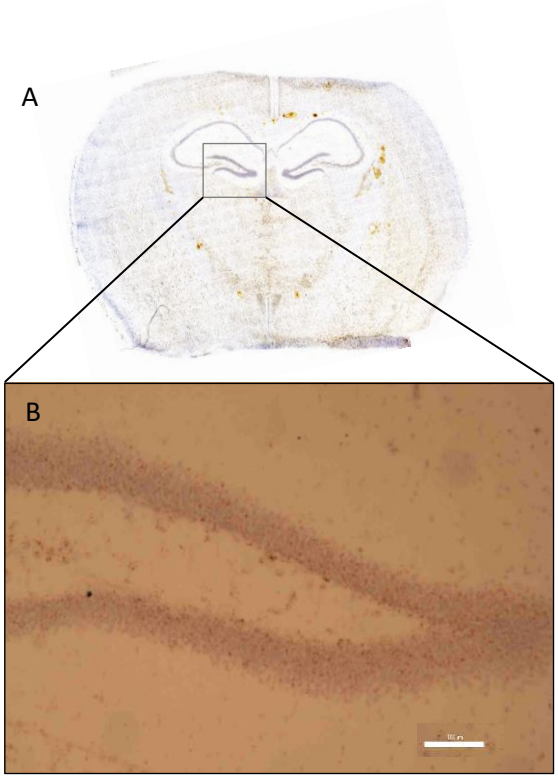


Figure 26: Exemple de marquage β-arrestine 1 par hybridation *in situ* sur une coupe coronale complète au niveau du gyrus dentelé après traitement par scanner de lames (A) ou par microscopie optique (B).

3.4 Etude de la neurogenèse hippocampique adulte

3.4.1 Préparation des tissus

3.4.1.1 Perfusions intra-cardiaque

La souris est anesthésiée à l'aide d'un mélange kétamine/xylazine (120 mg/kg et 10 mg/kg, respectivement). On contrôle l'anesthésie en effectuant une pression sur la patte arrière de l'animal. L'absence de réaction permet d'entreprendre la chirurgie. L'animal est fixé sur une planche en liège, sur le dos. Une incision au niveau médian de l'abdomen permet d'introduire les ciseaux de chirurgie et de remonter jusqu'au diaphragme par les flancs de l'animal. Le diaphragme sectionné laisse entrevoir le cœur. La peau qui recouvrait l'abdomen est rabattue en arrière puis fixée, permettant un accès dégagé jusqu'au cœur. Il est primordial d'opérer rapidement l'animal pour conserver la propriété pulsatile du cœur. Cette capacité va permettre de rincer et fixer les tissus cérébraux, ce qui est nécessaire pour obtenir des marquages sans pollution par des cellules sanguines résiduelles. Un cathéter relié à une pompe péristaltique (pompe péristaltique, W40578, Fisher Scientific SAS, Illkirch, France) est inséré dans le ventricule gauche du cœur. Cette pompe a pour fonction de perfuser en premier lieu une solution de NaCl 0.9% conservée dans la glace. Une fois le cathéter en place, une incision de l'oreillette droite va permettre au cœur et à la pompe d'évacuer le sang du corps de l'animal. Après 5 minutes, on peut constater une décoloration de certaines zones du corps de l'animal tels que : le foie, les pattes avant, la langue, les oreilles etc. Ces indicateurs permettent de poursuivre le processus de perfusion en changeant le NaCl 0.9% par une solution de paraformaldéhyde 4% dans 0,2M de solution de Sørensen pendant 10 minutes. Cette solution va permettre de fixer les tissus et de stopper toute activité cellulaire. Le cerveau est ensuite prélevé et conservé pendant la première journée dans une solution de paraformaldéhyde 4% à 4°C. Elle sera ensuite substituée par une solution de tampon phosphate (PBS) + sucrose 30% + azide 0.1% à 4° ce qui permettra de protéger et conserver les tissus jusqu'à leur prochaine utilisation.

3.4.1.2 Préparation des cerveaux

Les cerveaux sont placés à l'envers dans des blocs en plastique individuels préalablement remplis avec de l'OCT (Tissue-Tek, Optimal Cutting Temperature). Ainsi préparés, les blocs sont plongés dans une boite en polystyrène contenant de la carboglace (-60 °C). La propriété de l'OCT à se solidifier à basse température va contenir le cerveau dans un bloc. Ils sont ensuite démoulés puis conservés au congélateur à -80°C. Le bloc d'OCT est fixé sur une platine amovible encore une fois à

l'aide d'OCT, à l'intérieur d'un dispositif réfrigéré à -20°C (Leica CM 3000). Cet appareil va permettre de faire des sections du bloc, ici définies à 35 µm d'épaisseur. On équilibre ensuite le cerveau par rapport à la lame du couteau du cryostat de façon à obtenir une morphologie symétrique des structures cérébrales. Après l'identification du début de l'hippocampe à l'aide de l'atlas pour Souris (Bregma -1.20 to -3.80 mm; Franklin et Paxinos, 2007), chaque section de l'hippocampe est collectée. Le stockage des coupes de cerveaux se fait à l'aide d'une boîte de 24 puits. Chaque ligne correspond à un cerveau. Les puits sont remplis par une solution de PBS + sodium azide (0.1%) qui préservera les tissus d'une éventuelle dégradation par les bactéries ou champignons. L'hippocampe se subdivisera par 13 séries de 6 coupes. Dans chaque puit, nous retrouverons 1/6 de l'hippocampe et chaque section est espacée de la suivante par 210 µm. Cette méthode permet d'économiser le nombre d'animaux en utilisant différents marqueurs d'immunohistochimie pour le même cerveau.

3.4.2 Etude de la prolifération des cellules progénitrices

L'étude de la prolifération cellulaire est réalisée grâce à une technique d'immunohistochimie utilisant le marquage au Ki67. La protéine Ki67 est une protéine nucléaire exprimée par la cellule lorsqu'elle est entrée dans le cycle cellulaire. Son expression augmente progressivement au cours des phases de synthèse, G2 puis M du cycle (Cattoretti et al., 1992).

Après avoir été rincées 3 fois 15 minutes dans une solution de tampon phosphate contenant du triton PBST (PBS+Triton 0,3%), les coupes de cerveaux sont plongées pendant deux heures dans une solution de 10% de sérum d'âne afin de bloquer les sites non spécifiques. Les coupes sont ensuite immédiatement incubées dans l'anticorps primaire anti-Ki67 fabriqué chez le lapin (Eurobio, France) le temps d'une nuit à 4°C sous agitation. Le lendemain, après 3 rinçages de 15 minutes dans un tampon phosphate (PBS), les tissus reposent deux heures dans l'anticorps secondaire d'âne anti-lapin couplé à un fluorochrome Cy2 (Jackson, dilution 1 :500) (Sahay et al., 2011). Le marquage est visualisé grâce à un fluorochrome couplé à l'anticorps secondaire. La fluorescence permet d'apprécier la proportion de cellules en cycle et donc la prolifération cellulaire.

3.4.3 Etude de la survie des cellules progénitrices

L'étude de la prolifération cellulaire dans l'hippocampe de la souris adulte nécessite un schéma spécial d'injection de 5-bromo-2-deoxyuridine (BrdU), 2 fois par jour pendant 3 jours 3 semaines avant le sacrifice de l'animal (100 mg/kg) (Cameron and McKay, 2001; Wang et al., 2015).

Le BrdU est un analogue synthétique de la thymine. Au moment de la réplication de l'ADN (pendant la phase S du cycle cellulaire) le BrdU va s'incorporer au nouveau brin formé et permet de marquer ainsi les cellules nouvellement formées. Les anticorps spécifiques anti-BrdU vont permettre de révéler ces jeunes cellules en suivant le protocole suivant. Les sections de cerveaux sont montées sur des lames (lames adhésives super Frost plus, Fischer Scientific SAS, Illkirch, France), puis placées pendant 5 minutes dans une solution d'acide citrique (pH 6.0) à 96°C. Après des rinçages successifs avec su PBS, les tissus sont traités avec de la trypsine (0.01%) dissoute dans du Tris/CaCl2 pendant 10 minutes.

Suite à une nouvelle série de rinçage, du 2N HCl est déposé sur les lames pendant 30 minutes. Le blocage des lames est effectué à l'aide d'une préparation de sérum de Chèvre (normal goat serum, NGS) 5% dans du PBS. Pendant la nuit, on dépose sur les lames une solution d'anticorps anti-souris BrdU (1 :100). Après une série de rinçage au PBS, les lames sont recouvertes d'une solution d'anticorps secondaire (1: 200 chèvre anti-souris biotinylé) pendant une heure, suivie d'une amplification par un complexe avidine/biotine. Le marquage est visualisé par le 3,3'-Diaminobenzidine (DAB). Les tissus sont plongés dans des bains successifs permettant le contremarquage par FAST-RED (Vector Nuclear Fast Red H-3403, ABCYS, Paris, France) pendant 2 min puis un rinçage à l'eau ultra pure (1min) ainsi qu'une déshydratation progressive (dans de l'éthanol 70% puis 100% pendant 5 et 10 minutes, respectivement).

Les lames sont recouvertes par une lamelle entre lesquelles on dépose un milieu de montage qui permet de fixer définitivement les tissus. La quantification des cellules marquées se fait comme précédemment décrite par David et al., 2009 à l'aide d'un microscope (Olympus BX51 microscope, Allemagne) au grossissement 40X (Figure 27).

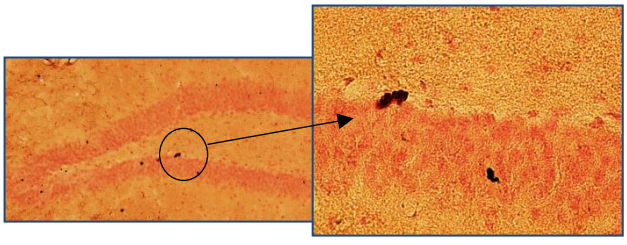


Figure 27: Photographies de coupes histologiques du gyrus dentelé de l'hippocampe adulte après marquage au BrdU à différents grossissements.

3.4.4 Etude de la maturation neuronale

L'étude de la maturation neuronale ne nécessite pas d'injecter un marqueur exogène. En effet, on utilise l'expression endogène d'un marqueur de neurone immature la Doublecortine (DCX) exprimé à la membrane des cellules neuronales, qui permet de révéler les neurones nouvellement formés dans la couche granulaire de l'hippocampe (Couillard-Despres et al., 2005).

La procédure suit celle décrite par Wang et collaborateurs, 2008 (Wang et al., 2008). Les sections de cerveaux sont rincées avec du tampon tri-phosphate (TBS), puis traitées dans une solution de H_2O_2 1% (qsp TBS) pendant 15 minutes. Les tissus sont incubés dans une préparation à 10% de sérum d'âne avec 0.3% de Triton X- 100 pendant 30 minutes. Les tissus sont maintenus toute la nuit avec l'anticorps primaire doublecortine (chèvre 1 :500; Santa Cruz Biotechnology, Santa Cruz, CA, USA). L'anticorps secondaire est biotinylé selon la construction âne anti-chèvre (1 :500 ; Jackson ImmunoResearch, West Grove, PA) dans du TBS pendant 2 heures à température ambiante.

Le marquage est révélé par un complexe avidine/biotine (Vector USA) et un kit DAB (SK-4100 DAB kit, Vector, USA). Une fois la révélation effectuée, les coupes de cerveaux sont montées sur lames et recouvertes par une lamelle. Les cellules marquées avec l'anticorps doublecortine (DCX+) sont catégorisées selon leur morphologie dendritique (Figure 28) : DCX+ sans arborisation tertiaire et celles avec une arborisation plus complexe. L'index de maturation est défini comme le ratio entre les cellules avec arborisation complexe sur le total des cellules DCX+ (Wang et al., 2008).

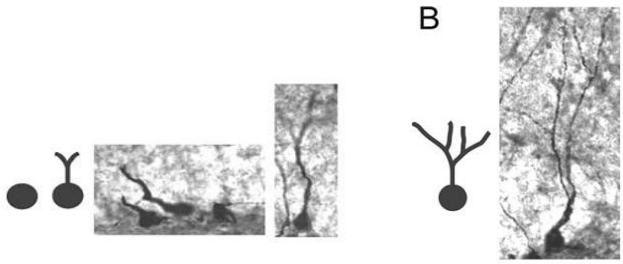


Figure 28: Caractérisation des neurones immatures marqués à la doublecortine en fonction de leur morphologie dendritique. a : cellules doublecortine positives sans dendrites tertiaires (neurone immatures) ; b : cellule doublecortine positive avec arborisation tertiaire (neurone mature). D'après Wang et al., 2008

Le Tableau 14 ci-dessous récapitule les anticorps utilisés pour les techniques d'immunohistochimie.

Tableau 14: Récapitulatif des anticorps utilisés en immunohistochimie.

Anticorps	Source	Dilution	Référence	Fournisseur
Anticorps primaires				
Ki-67	Monoclonal lapin	1 :100	VP-RM04	Eurobio
BrdU	Souris	1 :100	347850	Becton Dickinson
DCX	Polyclonal chèvre	1 :500	C-18 (H2812)	Santa Cruz Biotechnology
Anticorps secondaires				
Biotine	Chèvre anti-souris	1 :200	BA-9200	Vector Labs
	Ane anti-chèvre	1 :500	705-065-003	Jackson ImmunoResearch
Cy2	Ane anti-lapin	1 :500	715-175-150	Jackson ImmunoResearch

Résultats expérimentaux

Article 1:

Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression.

Darcet et al., Front Behav Neurosci. 2014 May 1;8:136. doi: 10.3389/fnbeh.2014.00136.

Article 2:

Rapid anxiolytic effects of a 5-HT4 receptor agonist are mediated by a neurogenesis-independent mechanism.

Mendez-David et al., Neuropsychopharmacology. 2014 May;39(6):1366-78. doi: 10.1038/npp.2013.332.

Article 3:

Chronic 5-HT_4 receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression.

Darcet et al., Neurosci Lett. 2016 Feb 2. pii: S0304-3940(16)30054-4.

Article 4:

Selective β-arrestin 1 deletion in stem cells of the dentate gyrus alters emotional state and dampens antidepressant-like effects in adult mice.

En préparation

Résultats complémentaires:

Selective β-arrestin 1 deletion in stem cells of the dentate gyrus alters cognitive phenotype and prevents pro-cognitive-like effects of chronic fluoxetine and 5-HT_4 receptor agonist treatments in adult mice

En préparation

ARTICLE 1: Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression

Flavie Darcet, Indira Mendez-David, Laurent Tritschler, Alain M Gardier, Jean-Philippe Guilloux, Denis J David

Publié dans Frontiers in Behavioral Neuroscience, doi :10.3389/fnbeh.2014.00136

Questions posées :

La modélisation d'un phénotype d'anxiété/dépression chez la souris par l'administration chronique de corticostérone (modèle CORT) induit-elle des déficits d'apprentissage et de mémoire comparables à ceux observés chez l'Homme présentant une dépression caractérisée ? Si oui, quels sont les types de mémoire affectés chez ces souris « anxio/dépressives » ?

Résumé de l'étude

Les troubles cognitifs sont souvent reportés comme des symptômes invalidants chez les patients souffrant de dépression caractérisée. L'incapacité à se concentrer fait d'ailleurs partie des critères de diagnostic de la dépression majeure définis par le DSM-5. Dans de nombreuses études précliniques, des altérations cognitives ont été observées dans différents modèles d'anxiété/dépression (voir **Revue Pharmaceuticals**). Cependant, peu d'études se sont intéressées aux effets d'un traitement chronique par la corticostérone sur les performances d'apprentissage et de mémoire. Dans cette étude, nous avons mené une caractérisation complète des fonctions cognitives (dont la mémoire de type épisodique, la mémoire spatiale et la mémoire associative) chez des animaux ayant été soumis pendant 4 semaines à un régime de corticostérone (modèle CORT) (David et al., 2009).

Afin d'éviter que les différents tests cognitifs n'influent les uns sur les autres, l'étude a été menée avec 4 cohortes différentes de souris C57BL/6Rj, chaque cohorte étant testée pour une tâche cognitive précise après 4 semaines de traitement à la corticostérone (35 µg/mL) dans l'eau de boisson. Le phénotype anxio/dépressif est vérifié grâce à un test de l'Open Field au début de la période de comportement et un Splash test après le test cognitif pour les cohortes n°1 et 2. La mémoire de type épisodique est étudiée grâce au test de reconnaissance d'objet, la mémoire spatiale est estimée à l'aide tests de navigations spatiales dans un environnement aquatique ou sec (la piscine de Morris et le Barnes maze, respectivement) et la mémoire à composante associative est mesurée grâce à un test de conditionnement par la peur.

Tout d'abord, nous avons observé une diminution de la capacité de discrimination des objets chez les animaux présentant un phénotype « anxio/dépressif » par rapport aux contrôles, suggérant un déficit de la mémoire de type épisodique. D'autre part, les tests spatiaux mettent en évidence une altération globale des capacités d'apprentissage et de mémoire chez les animaux traités par la corticostérone. La flexibilité mentale - un des aspects des fonctions exécutives de la mémoire - ainsi que la mémoire à long-terme semblent également affectées par le phénotype anxio/dépressif. Enfin, l'absence d'augmentation du comportement d'immobilité ou « freezing » observée dans le test de conditionnement par la peur chez les animaux anxio/dépressifs indique un trouble de la mémoire associative.

Ces résultats suggèrent que le phénotype anxio/dépressif induit par le traitement chronique de corticostérone est associé à un déficit cognitif global, affectant tous les aspects de la mémoire ayant été testés.

Contribution personnelle :

Au cours de ce travail :

- J'ai mis au point et validé 3 tests comportementaux de cognition chez la Souris : le test de reconnaissance d'objet, la piscine de Morris et le Barnes maze.
- J'ai réalisé le suivi des animaux incluant la préparation et l'administration du régime chronique de corticosterone.
- J'ai mené l'ensemble des tests comportementaux émotionnels et cognitifs (excepté le test de conditionnement par la peur) sur les 3 cohortes différentes.
- J'ai analysé les résultats et rédigé l'intégralité de l'article sous la supervision du Pr. Denis David et du Dr Jean-Philippe Guilloux

frontiers in
BEHAVIORAL NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 01 May 2014
doi: 10.3389/fnbeh.2014.00136

Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression

Flavie Darcet, Indira Mendez-David, Laurent Tritschler, Alain M. Gardier, Jean-Philippe Guilloux† and Denis J. David*†

EA3544, Neuropharmacologie des troubles anxio-dépressifs et neurogenèse, Faculté de Pharmacie, Université Paris-Sud, Châtenay-Malabry, France

Edited by:
Benjamin Adam Samuels, Columbia University/Research Foundation for Mental Hygiene, USA

Reviewed by:
Michael Drew, University of Texas at Austin, USA
Christine Ann Denny, Columbia University, USA

***Correspondence:**
Denis J. David, EA3544 "Pharmacologie des Troubles Anxio-Dépressifs et Neurogenèse," Faculté de Pharmacie, Université Paris-Sud, 5 rue J-B Clément, Tour D1, 2e etage, F-92296 Chatenay-Malabry, France
e-mail: denis.david@u-psud.fr

† *Last co-authorship.*

Cognitive disturbances are often reported as serious incapacitating symptoms by patients suffering from major depressive disorders (MDDs). Such deficits have been observed in various animal models based on environmental stress. Here, we performed a complete characterization of cognitive functions in a neuroendocrine mouse model of depression based on a chronic (4 weeks) corticosterone administration (CORT). Cognitive performances were assessed using behavioral tests measuring episodic (novel object recognition test, NORT), associative (one-trial contextual fear conditioning, CFC), and visuo-spatial (Morris water maze, MWM; Barnes maze, BM) learning/memory. Altered emotional phenotype after chronic corticosterone treatment was confirmed in mice using tests predictive of anxiety or depression-related behaviors. In the NORT, CORT-treated mice showed a decrease in time exploring the novel object during the test session and a lower discrimination index compared to control mice, characteristic of recognition memory impairment. Associative memory was also impaired, as observed with a decrease in freezing duration in CORT-treated mice in the CFC, thus pointing out the cognitive alterations in this model. In the MWM and in the BM, spatial learning performance but also short-term spatial memory were altered in CORT-treated mice. In the MWM, unlike control animals, CORT-treated animals failed to learn a new location during the reversal phase, suggesting a loss of cognitive flexibility. Finally, in the BM, the lack of preference for the target quadrant during the recall probe trial in animals receiving corticosterone regimen demonstrates that long-term retention was also affected in this paradigm. Taken together, our results highlight that CORT-induced anxio-depressive-like phenotype is associated with a cognitive deficit affecting all aspects of memory tested.

Keywords: depression, anxiety/depression model, corticosterone, recognition memory, spatial learning maze, associative memory, cognitive impairments, cognitive flexibility

INTRODUCTION

The prevalence of depression, a severe psychiatric disease, is constantly high worldwide to the extent that World Health Organization (WHO) estimates that Major Depressive Disorder (MDD) will be the second largest cause of disability in year 2020 (WHO, 2008). Major depression is characterized by a set of emotional and behavioral alterations, including persistent depressed mood and loss of interest or pleasure as core symptoms. Since cognitive symptoms are common among patients with MDD (Fava et al., 2006; Hammar and Ardal, 2009; Murrough et al., 2011; Lee et al., 2012; Millan et al., 2012), investigators have examined the nature of difficulties in cognitive functioning that are associated with depression such as attention (Landro et al., 2001; Ravnkilde et al., 2002; Porter et al., 2003; Lampe et al., 2004), processing speed (Ravnkilde et al., 2002; Hammar et al., 2003; Lampe et al., 2004), executive function (Naismith et al., 2003; Lampe et al., 2004) and learning and memory (Landro et al., 2001; Fossati et al., 2002; Ravnkilde et al., 2002; Porter et al., 2003; Vythilingam et al., 2004). A recent literature review e assessed abnormalities in neural circuits and cognition early in the course of MDD (Trivedi and Greer, 2014). Interestingly, cognitive deficits in memory and decision-making are detected early in the course of MDD and may be associated with structural abnormalities in the hippocampus or cortex (Trivedi and Greer, 2014). New antidepressant drug strategies that also target cognitive symptoms are needed to improve long-term outcomes, particularly functional recovery.

In order for basic research to provide potential advances in the field, it is critical to use animal models that present behavioral, neurochemical and brain morphological phenotype reminiscent of some symptoms of depression including cognitive impairments. A variety of studies have assessed cognitive disorders in anxiety or depression models in rodents (Patki et al., 2013; Richter et al., 2013). However, some of these only focused their work on a single aspect of learning and memory. Cognitive dysfunctions in Chronic Mild Stress-exposed rats (CMS) have been shown in several behavioral paradigms. For example, CMS induces spatial learning and memory impairments in the Morris water maze (MWM) test in mice (Song et al., 2006), recognition memory deficits in both rats (Orsetti et al., 2007) and mice (Elizalde et al., 2008) and suppression

of fear extinction (Garcia et al., 2008). Similarly, maternal separation and social defeat models were employed to highlight spatial reference memory deficits (Couto et al., 2012; Patki et al., 2013).

Here, we performed a thorough characterization of cognitive performances in a neuroendocrine mouse model of depression based on a chronic CORT (David et al., 2009; Mendez-David et al., 2014). Given the multiple forms of learning and memory, we selected a range of cognitive behavioral paradigms that allows investigation of different memory systems including short-term episodic memory, associative memory and spatial reference learning and memory. Additional parameters such as cognitive flexibility and long-term memory were also evaluated in spatial memory tests.

MATERIALS AND METHODS

ANIMALS

Eight to 10-weeks old male C57BL/6J mice (Janvier Labs, France) were maintained on a 12L:12D schedule and were housed 5 per cage. Food and water were provided *ad libitum*. All behavioral testing occurred during the light phase between 7 am and 7 pm and were conducted in compliance with animal cares guidelines and with protocols approved by the Institutional Animal Care and Use Committee (Council Directive #87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la santé et de la Protection Animale, permission #92-256B to Denis J. David).

DRUGS

Corticosterone (4-pregnen-11b-diol-3 20-dione 21 hemisuccinate, CORT from Sigma-Aldrich, France) was dissolved in vehicle (0.45% hydroxypropyl-β-cyclodextrin, β-CD from Sigma-Aldrich, France). Corticosterone (35 μg/ml equivalent to 5 mg/kg/day) was delivered for 28 days in drinking water and continued when the behavioral tests were performed (David et al., 2009). Control animals received vehicle (β-CD) in drinking water during the entire experiment (**Figure 1**).

BEHAVIORAL TESTING

Four different cohorts of mice were used to assess learning and memory performances in anxio-depressive animals. To prevent any confounding effects between cognitive tasks, each animal was subjected to only one type of learning and memory test. Fur coat state of the animals was scored weekly during the whole treatment period. The anxio-depressive-like phenotype induced by chronic corticosterone among the different cohorts was evaluated using the Open Field and the Splash tests, before and after the cognitive task, respectively. Details of emotional assessment are mentioned in **Figure 1**.

Cognitive performances were evaluated using behavioral tests measuring episodic (novel object recognition test, NORT), associative (one-trial contextual fear conditioning, CFC) and visuo-spatial (MWM; Barnes maze, BM) learning/memory. Animals were placed in the experimental room 30 min before the start of the behavioral experiments.

ANXIETY AND DEPRESSION BEHAVIOR PARADIGMS

Fur coat state

The score corresponding to the state of the coat resulted from the sum of the score of five different body parts: head, neck, dorsal/ventral coat, tail and fore-/hind paws. For each body area, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat (Surget et al., 2008; David et al., 2009).

Open field

Motor activity was quantified in 43 × 43 cm plexiglas open field boxes (MED Associates, Georgia, VT). Two sets of 16 pulse-modulated infrared photobeams were placed on opposite walls 2–5 cm apart to record x-y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100 ms resolution. The computer defined grid lines that divided each open field into center and surround regions, with each of four lines being 11 cm from each wall. Time in the center was recorded for 30 min to evaluate anxiety-related behavior. The locomotor activity was quantified as total ambulatory distance.

Splash test

Splash test was performed as previously described (David et al., 2009). This test consisted in squirting a 10% sucrose solution on the mouse's snout. The grooming duration was then recorded over a 5 min period.

COGNITION BEHAVIORAL PARADIGMS

Episodic short-term memory: novel object recognition test

The procedure was adapted from the Sahay study (Sahay et al., 2011). The apparatus consisted in black plastic boxes (28 × 41 × 18 cm) slightly filled with sawdust (≈0.5–1 cm thickness) in a room with a low level of light. Locomotor activity was controlled during the entire experiment (parameter: ambulatory distance) using a videotracking procedure (ANY-maze Software, Bioseb, France). Objects exploration was hand-scored by an experimenter.

The NORT was divided into 4 training sessions and one test session. Each exposure lasted 5 min with a 3-min inter-trial interval. Between each trial, mice returned to their home cage, bedding of apparatus was changed and boxes were cleaned with 70% ethanol solution. During training sessions, two identical objects [cylindrical glassware (Ø:3 cm, height: 8 cm) filled with white cotton, (**Figure 2D**)] were present in the box. The mouse was placed in the middle of the box facing the wall and was allowed to freely explore the apparatus and the objects. During the test session, one of the familiar objects was removed from the cage and replaced by a novel object [Lego® rectangular structure (7 × 3 × 9 cm), (**Figure 2D**)]. The objects had been previously validated to ensure there was no inherent preference for either object (data not shown). The nature (Lego® vs. glass) and the position of the novel object (left vs. right) were chosen randomly.

Object exploration was defined as the orientation of the nose to the object at a distance ≤ 2 cm. Placing the forepaws on the objects was considered as exploratory behavior, but climbing on the objects was not. Objects were cleaned with 70% ethanol between trials to avoid olfactory cues. Results for this test were expressed as: (1) exploration of each object (in seconds) during

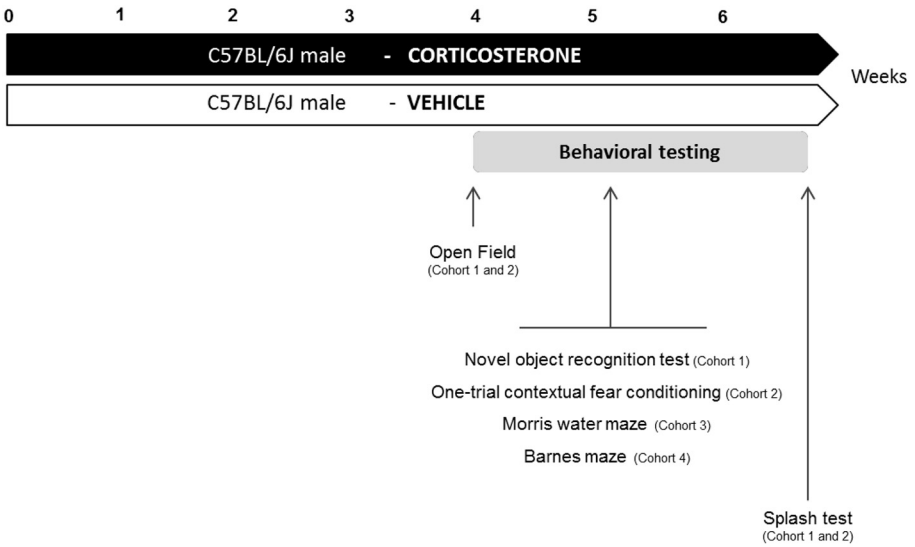


FIGURE 1 | Experimental design. In place of normal drinking water, grouped-housed male C57BL/6J mice were presented with vehicle (0.45% hydroxypropyl-β-cyclodextrin) or corticosterone (35 μg/mL). We performed a complete characterization of cognitive functions in four different cohorts of animals. The same animal was successively tested in the Open Field paradigm, the cognitive task (Novel object recognition test: cohort 1; One-trial contextual fear conditioning: cohort 2) and the Splash test. Cohort 3 and 4 were only tested for cognitive tasks (Morris water maze: cohort 3; Barnes maze: cohort 4).

training and test sessions(2) exploration (in percent) of each object during the test session, calculated as time spent exploring familiar or novel object divided by total time spent exploring both objects and (3) a discrimination index (DI) between objects during the test session, calculated as the difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects (DI = (N–F)/(N+F)).

Associative memory: one-trial contextual fear conditioning

Fear conditioning was conducted in StartFear Combined system from Harvard apparatus (Bioseb, France) in chambers with internal dimensions of 25 × 25× 25 cm. The chambers had metal walls on each side, clear plastic front and back walls and ceilings, and stainless steel bars on the floor. A house light mounted directly on the side of the chamber provided illumination. Each chamber was located inside a larger, insulated, plastic cabinet (67 × 53× 55 cm) that provided protection from outside light and noise. Each cabinet contained a ventilation fan that was operated during the sessions. Mice were held outside the experimental room in their home cages prior to testing and transported to the conditioning apparatus individually in standard mouse cages. Training chambers were cleaned with 70% ethanol solution before and after each trial to avoid any olfactive cues. The experimental design was adapted from previous studies (Drew et al., 2010) and ran over two consecutive days. On Day 1, mice were placed in the conditioning chamber and 3 min later received one shock (2 s, 0.75 mA). Mice were removed from the chamber 15 s after the shock. On Day 2, animals returned to the conditioning chamber for a 4-min period in the exact same conditions of Day 1, but without electrical shock, for a test of context-elicited freezing. Scoring was measured using Freezing software version 2.0.04 (Packwin, Harvard apparatus, Bioseb, France). The StartFear system allows recording and analyzing the signal generated by the animal movement through a high sensitivity weight transducer system. The behavior of mice was also recorded with digital video cameras mounted above the conditioning chamber.

Spatial reference learning and memory: Morris water maze and Barnes maze

Morris water maze. The MWM procedure was adapted from (Sahay et al., 2011). The apparatus consists in a circular pool (Ø: 122 cm, height: 50 cm) filled with 30 cm-depth water maintained at 22–23°C and made opaque by addition of white milk powder. The maze was divided into four quadrants (North, South, West, and East). Mice learned to locate an unmarked submerged platform in a pool devoid of intramaze cues. Geometrical extra cues were surrounding the maze to generate spatial learning. The escape platform (Ø: 10 cm; height: 30 cm) was placed in the target quadrant (North), 1 cm above the surface of water during the pre-training session and 1 cm below the water surface during the other sessions. The MWM task was performed with three successive steps (**Figure 4A**). The pre-training phase (1 session, 3 trials, Day 0) allowed mice to accustom to the pool and the visible platform placed in clear water. The acquisition phase was divided into 4 training sessions (Day 1–4) and one probe trial (Day 5). During training sessions, the platform was hidden to develop spatial learning. Each mouse received three 60-s trials per day with 60 s inter-trial intervals. The starting points were semi-randomized so that each trial started from a different quadrant. Between each trial, the mouse had to remain on the platform during 60 s. If the mouse did not find the platform within 60 s, the experimenter gently guided the animal to the platform. In this case, 60 s was recorded as the escape latency. A 60-s probe session (Day 5), during which the platform was

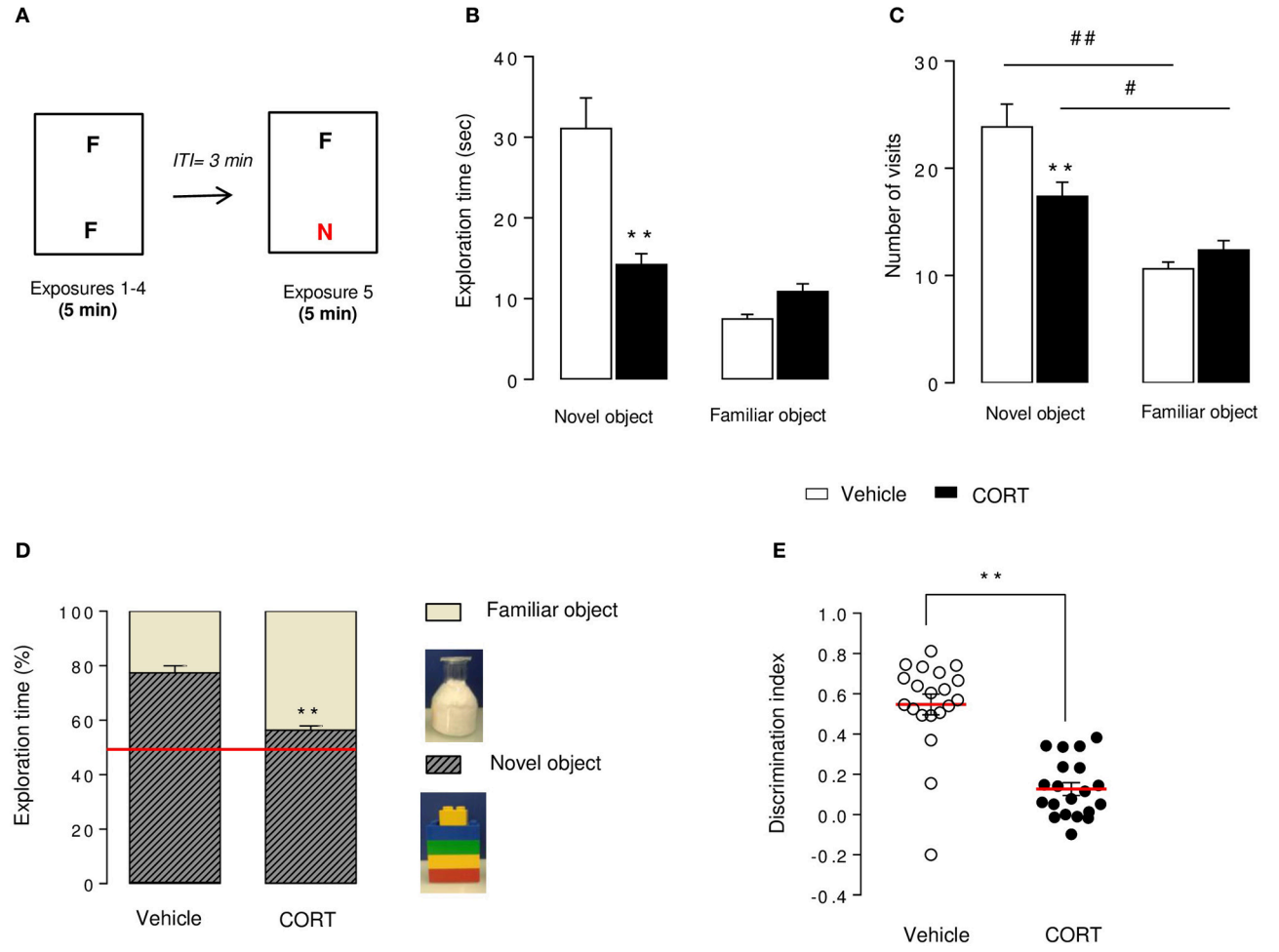


FIGURE 2 | Short-term episodic memory is altered in CORT-treated mice in the novel object recognition test. Schematic diagram of the NORT experimental time course **(A)**. Mice were exposed to 2 identical objects during 4 sessions (5 min each) with an intertrial interval (ITI) of 3 min. During the 5thexposure, one familiar object (F) was removed and replaced with a novel object (N) different in shape, color, and texture. Exploration time **(B)** and exploration frequency **(C)** of the novel and the familiar objects were measured during the test session. The exploration time was also expressed in percent of both novel object and familiar object **(D)**. The red line indicates the chance level. Discrimination index **(E)** of each animal was calculated (see Materials and Methods). Values are mean ± s.e.m., $n = 20$ animals per group $^{**}p < 0.01$ vs. vehicle group. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ vs. novel object of the group.

removed, was performed 24 h after the last trial of the learning period.

The same procedure was applied for the reversal phase in which the hidden platform was located in the opposite quadrant (South) during training sessions (Day 8–11). The platform was removed in the 2nd probe trial (Day 12) to assess spatial retention.

Time spent in target and opposite quadrants, latency to cross the platform zone for the first time and the number of entries in the platform zone were recorded. Mouse movement was measured using a video tracking system and analyzed by ANY-maze Software to record latency, distance and pathways to reach the escape platform through all the sessions.

Barnes maze. The BM procedure was modified from a previous work (Sunyer et al., 2007). The apparatus consisted in a clear gray circular platform (Ø: 92 cm, height: 100 cm; Bioseb, France) with 20 equally spaced holes (Ø: 5 cm) located 2 cm from the border. In this open environment, mice naturally seek a dark enclosed surrounding place, provided by a black goal box (20 × 9× 9 cm) located beneath one of the holes. During training sessions, the 19 other holes are closed. From the surface of the maze, the open escape hole looks identical to the closed holes so that the mice can locate the target box only with the spatial extra cues surrounding the maze. Similarly to the MWM test, the circular platform was virtually divided in 4 zones (including the target quadrant with the escape hole and the opposite quadrant). To reduce anxiety behavior, mice were habituated to the platform and the target box on the day before the beginning of the experiment.

Each trial began by placing the animal in a black starting cylinder (Ø: 8 cm, height: 12.5 cm) at the center of the platform that was removed after 10 s, allowing mice to freely explore the apparatus. Spatial acquisition was organized in 4 training sessions (Day 1–4, **Figure 5A**). Each training session consisted in four 3-min trials, with 20 min inter-trials interval during which animals

returned to their home cage. Mice that failed to find the target box within 3 min were gently guided to its location. For those mice, 180 s were recorded as the escape latency. All animals remained in the target box for 60 s after entering.

All trials were recorded by a camera and analyzed by ANY-maze Software. The following parameters were scored during all training trials: primary latency, latency to escape, primary errors and total errors. Primary latency was defined as the time required for mice to make initial contact with the target hole. Latency to escape was defined as the time it took animals to completely enter in the target box (all 4 paws out of the platform). Primary errors were defined as the number of holes visited before the first contact with the target hole and total errors were defined as the total number of holes visited during the trial that did not lead to the target box. A hole was considered visited when mice tilted their head over it (nose poke) or introduced their paws into the hole.

On Day 5, reference short-term memory was evaluated by a probe trial (90 s) during which the target box was removed and the target hole was closed. Mice were allowed to explore the maze and to visit the target hole and the adjacent holes. Latency to reach the target hole for the first time, number of errors before reaching the target hole, distribution of visits among all holes and time spent in each quadrant were recorded. On Day 12, mice were once again submitted to a probe trial (recall) in the same conditions as Day 5 to evaluate long-term retention. No training occurred between Days 5 and 12.

STATISTICS

Results from data analyses were expressed as mean ± s.e.m. Statistical analyses were processed with Statview 5.0® Software (SAS Institute, Cary, NC). For all experiments, comparisons between CORT-treated and control animals were performed by using *t*-tests. One-Way ANOVA with repeated-measures and Two-Way ANOVA were applied to the data when appropriate. Significant main effects and/or interactions were followed by Fisher's PLSD *post-hoc* analysis. One sample *t*-tests were used to compare the percent of time exploring the novel object vs. the chance level (50%) in the NORT and the time spent in the target quadrant vs. the chance level (25%) in the MWM and BM tests. For the latency to cross the platform in reversal phase, we used the Kaplan-Meier survival analysis due to the lack of normal distribution of the data. Mantel-Cox log-rank test was used to evaluate differences between experimental groups. Statistical significance was set at $p < 0.05$. A summary of statistical measures is included in Supplemental Table 1.

RESULTS

The consequences of an anxious/depressed-like state on episodic, associative or spatial memory were tested in the NORT, the CFC, the MWM, and the BM, respectively.

Long-term glucocorticoid exposure induced physiological changes such as an altered body weight (Figure S1A) ($p < 0.01$) and physical changes including deterioration of coat state (Figure S1B) similarly to chronic stress (Surget et al., 2008). This measure has been described as a reliable and well-validated index of a depressed-like state (Griebel et al., 2002; Santarelli et al., 2003). CORT-treated mice developed an anxiety-like phenotype in the Open Field test, characterized by a decrease in time spent in the center compared to control mice ($p < 0.05$, Figure S1C), but no modification of total ambulatory distance ($p > 0.1$, Figure S1D). We then investigated whether the deterioration of the coat state was linked to changes in grooming behavior. In the Splash test, we observed a decrease in grooming duration after squirting a 10% sucrose solution on the mouse's snout ($p < 0.01$, Figure S1E). Taken together, our results confirm through various behavioral readouts that mice displayed an anxiety/depression-like phenotype induced by an excess of glucocorticoids.

CHRONIC CORTICOSTERONE IMPAIRED EPISODIC MEMORY IN THE NOVEL OBJECT RECOGNITION TEST

The NORT procedure was conducted 4 weeks after the start of the corticosterone treatment (see **Figures 1**, **2A**). Control mice showed a novel object preference illustrated by an increase in exploration duration and exploration frequency (**Figures 2B,C**) of the novel object compared to the familiar one. Although the novel object investigation duration was not higher in CORT-treated mice compared to controls, the number of visits of the novel object was significantly increased in comparison to the familiar object (**Figures 2B,C**). Taking these data together, both experimental groups showed a novel object preference characterized by an increase in its relative exploration time ($p < 0.01$ vs. the chance level 50% for each group, **Figure 2D**), with a greater distinction for the novel object in control animals than CORT-treated animals ($p < 0.01$, **Figure 2D**). Consequently, CORT-treated mice showed a significant decrease in discrimination index compared to control mice ($p < 0.01$, **Figure 2E**), reflecting a reduction of episodic retention. It is unlikely that this effect was the consequence of a change in objects exploration time ($p < 0.05$, Figure S2A) because locomotor activity did not differ between groups across sessions ($p > 0.05$**,** Figure S2B).

Then, we assessed whether associative memory was active in CORT-treated mice using an hippocampal-dependent task, the one-trial CFC.

ALTERED ASSOCIATIVE MEMORY AFTER A CHRONIC CORTICOSTERONE ADMINISTRATION IN THE ONE-TRIAL CONTEXTUAL FEAR CONDITIONING

In the context-elicited fear following training, CORT-treated animals exhibited significantly less freezing than the control animals along the test ($p < 0.01$, **Figures 3A,B**). These data are in favor of an impairment of the associative memory in CORT-treated animals. As a validation of the experiment, freezing was measured before and after the shock on Day 1. The shock induced an increase in the freezing in both groups of animals ($p < 0.01$, Figure S3). Similarly, no difference occurred between CORT-treated and control animals before or after the shock ($p > 0.05$, Figure S3).

Then, to determine the consequences of a chronic CORT treatment on spatial learning and memory performances, mice were submitted the hippocampal-dependent MWM or the BM. Learning was evaluated in both tests through an acquisition phase (Day 1–4) followed by a probe trial (Day 5) to assess short-term retention (**Figures 4A**, **5A**).

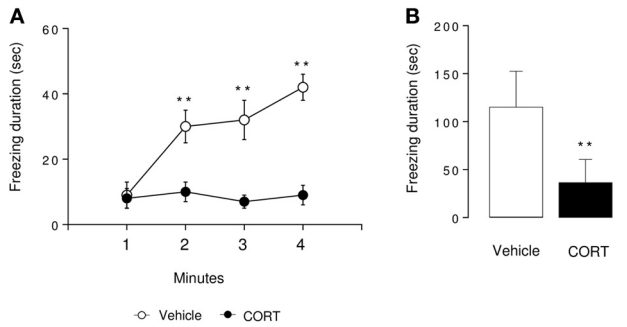


FIGURE 3 | Associative memory is impaired in CORT-treated mice in one-trial contextual fear conditioning. Contextual fear conditioning was produced by placing a mouse in the conditioning chamber and delivering one footshock 180 s later. Mice were returned to the conditioning chamber 24 h later to assess for context-elicited freezing. During Day 2, context-elicited freezing was analyzed for each minute of the test (ANOVA with repeated measures) **(A)** and during the whole session **(B)**. Values are mean ± s.e.m., $n = 5$–7 animals per group $^{**}p < 0.01$ vs. vehicle group.

CHRONIC CORTICOSTERONE IMPAIRED LEARNING, MEMORY AND COGNITIVE FLEXIBILITY IN THE MORRIS WATER MAZE

Morris water maze pre-training phase

Motivational behavior to swim was controlled by measuring latency and distance to reach the visible platform during the pre-training phase. The latency for control and CORT-treated mice i.e., 37.5 ± 2.59 and 38.99 ± 2.87 s, respectively, or the distance: 11.53 ± 1.43 and 10.46 ± 1.22 m for control and CORT-treated mice, respectively, did not differ between groups (*t*-test on latency $p = 0.70$; distance $p = 0.57$) indicating no difference in motivational behavior between these groups (Figures S4A,B).

Morris water maze acquisition

In the MWM, in contrast to the first 3 days of the acquisition, an increase in latency to reach the hidden platform ($p < 0.01$, **Figure 4B**) and an increase in path length to reach the platform ($p < 0.01$, **Figure 4C**) were observed on Day 4 in the CORT-treated animals in comparison to controls. Overall, an anxio/depressed-like state in mice affected spatial learning performance.

Morris water maze probe trial 1

Following training sessions, mice were subjected to a probe trial on Day 5 to assess short-term spatial memory. Both groups showed a preference for the target quadrant compared to the chance level ($p < 0.01$, **Figure 4D**), but CORT-treated mice showed a statistically significant increase in the time to cross the platform for the first time ($p < 0.05$, **Figure 4E**) and a decrease in the entries in the initial platform target zone ($p < 0.05$, **Figure 4F**) compared to control animals.

Taken together, these results reveal that CORT treatment affects short-term spatial retention in the MWM.

Morris water maze reversal

To test cognitive flexibility, the platform was moved from the target quadrant (North) to the opposite quadrant (South). Mice were then trained during 4 days (Day 8–11) to learn the new location of the hidden platform. Interestingly, from Day 9 to 11, the latency to reach the new location of the platform was significantly increased in CORT-treated mice compared to control group ($p < 0.01$, **Figure 4G**) without affecting the distance traveled to reach the platform ($p > 0.1$, **Figure 4H**). However, a significant interaction revealed a decrease in the latency to reach the platform in CORT-treated mice on Day 8 and an increase in this latency on Day 11 ($p < 0.05$ and $p < 0.01$, respectively). We also measured the time spent in the former target quadrant (North) during the 2 first days of the reversal protocol (Day 8–9) (Figure S4C). On Day 8, both groups spent significantly more time in the former target quadrant ($p < 0.05$ vs. the chance level 25%). In contrast, on Day 9, unlike control animals, CORT-treated mice still spent significantly more time in the former target quadrant ($p < 0.05$).

These data suggest that more than an impairment of spatial learning, cognitive flexibility is altered in CORT-treated mice as they failed to adapt their behavior to learn a new platform location.

Morris water maze probe trial 2

Following the reversal period, all animals were submitted to a probe trial on Day 12, during which CORT-treated mice failed to use extra cues to find the platform location. Unlike probe trial on Day 5, CORT-treated mice did not show preference for the target quadrant in comparison to control animals ($p < 0.01$, **Figure 4I**). Furthermore, an increase in the time to cross the platform for the first time ($p < 0.01$, **Figures 4J**, S4D) associated with a decrease in the number of entries in the new target zone was also observed in our anxio/depression-like mouse model ($p < 0.01$, **Figure 4K**).

CHRONIC CORTICOSTERONE IMPAIRED LEARNING, MEMORY AND LONG TERM RETENTION IN THE BARNES MAZE

The BM presents similarities to the MWM, assessing spatial learning performance, but without strong aversive stimuli.

Barnes maze acquisition

Previous spatial learning impairments observed in the MWM were confirmed in the BM. Indeed, an overall decrease in the primary latency (latency to first reach the target hole) was observed during the 4 training days in both groups, but CORT-treated animals segregate from control animals, starting at Day 2, with an increase in the time to identify the target hole ($p < 0.01$, **Figure 5B**), an increase in the time to escape into the target box ($p < 0.01$, Figure S5A) and a greater number of errors committed ($p < 0.01$, Figures S5B,C).

These data suggest that spatial learning performances are altered by a chronic CORT treatment in this spatial test.

Barnes maze probe trial

On Day 5, short-term spatial memory was evaluated by a probe trial. CORT-treated mice spent significantly less time in the target quadrant and more time in the opposite quadrant compared to vehicle animals ($p < 0.01$, **Figure 5C**). Similarly to what we observed in the MWM, an impairment of short-term memory was observed in the BM, with an increase in primary latency, a decrease in the number of visits in the target hole and an increase in primary errors ($p < 0.01$, **Figures 5D,E**, S5D). The analysis of

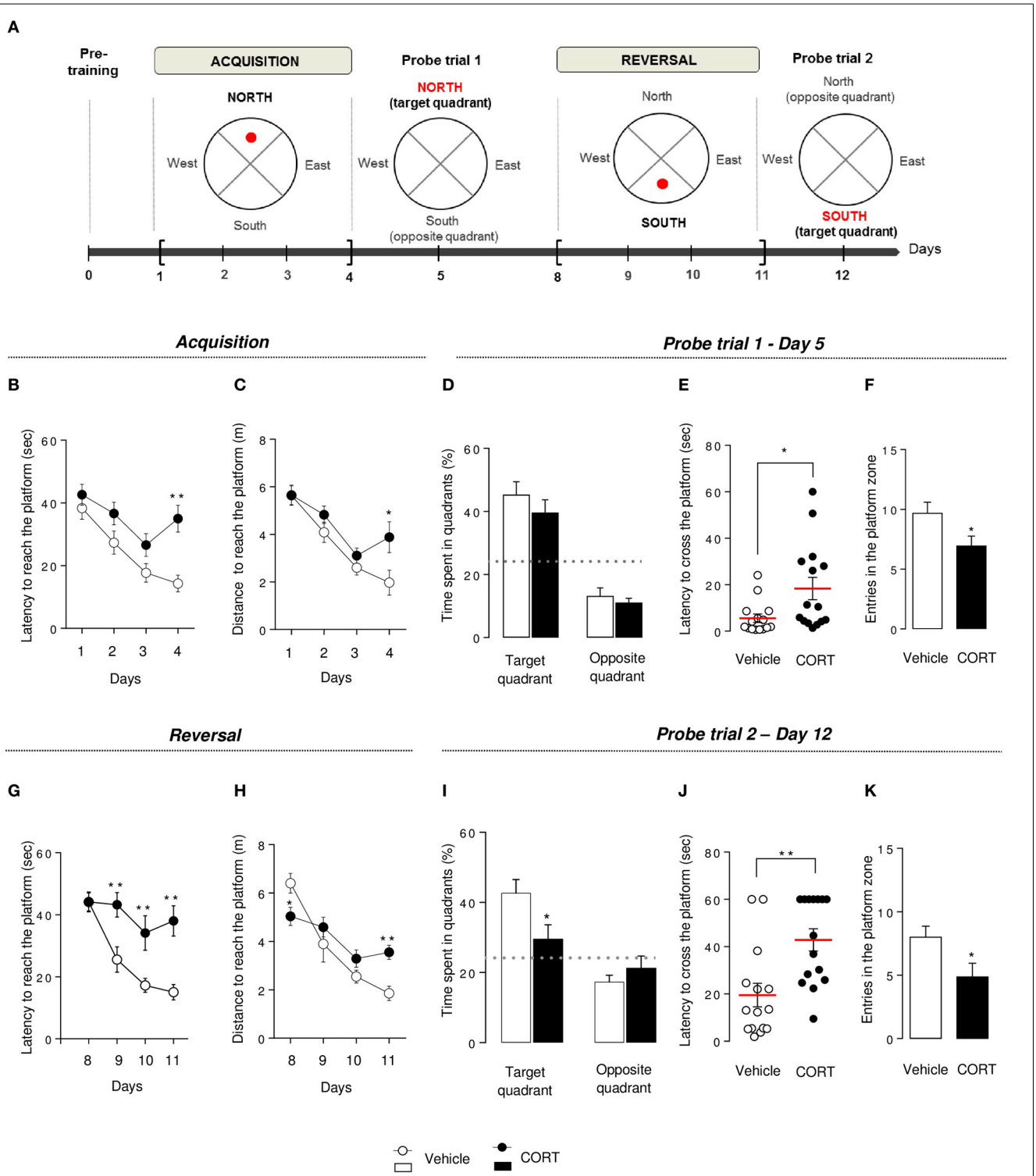


FIGURE 4 | Chronic corticosterone affects spatial learning performances, short-term memory and cognitive flexibility in the Morris water maze. Schematic diagram of the MWM experimental time course **(A)**. After a pre-training phase (day 0), the MWM protocol was divided into 2 phases. Each phase includes a 4-day learning period followed by a probe session without the platform. During acquisition, mice learned to locate the platform in the target quadrant (North) then have to locate the platform in the opposite quadrant (South) during reversal. During acquisition, learning was expressed as the latency **(B)** and the total distance traveled **(C)** to reach the hidden platform during training sessions (Day 1–4). Short-term memory was assessed during the probe test in Day 5 by measuring the time spent into the target and the opposite quadrants **(D)**, the latency to first cross the platform zone **(E)** and the number of entries in the platform zone **(F)**. During reversal,

(Continued)

FIGURE 4 | Continued
learning, and cognitive flexibility were expressed as the latency **(G)** and the total distance traveled **(H)** to reach the hidden platform during training sessions (Day 8–11). Cognitive flexibility was assessed during the probe trial in Day 12 by measuring the time spent into the target and the opposite quadrants **(I)**, the latency to first cross the platform zone **(J)** and the number of entries in the platform zone **(K)**. Values are mean ± s.e.m., $n = 10$–15 animals per group; $^{*}p < 0.05$; $^{**}p < 0.01$ vs. vehicle group. The dotted-line indicates the chance level.

the distribution pattern of visits finally confirmed that CORT-treated mice displayed lower spatial memory performances in this test compared to controls ($p < 0.01$, Figure S5E).

Barnes maze recall probe trial

Long-term memory was evaluated in CORT-treated and control mice, a week after the acquisition phase, in similar conditions than the first probe trial.

Unlike controls, CORT-treated mice were not able to find the location of the target hole. Indeed, the time spent in the target quadrant was significantly lower than controls ($p < 0.01$, **Figure 5F**) and not different from the chance level 25% ($p > 0.1$). A significant increase in primary latency and a significant decrease in the number of visits of the target hole ($p < 0.05$ and $p < 0.01$, respectively, **Figures 5G,H**) support that CORT-treated mice long-term retention could be affected, despite their acquisition learning difficulties previously demonstrated (**Figure 5B**).

DISCUSSION

Our study showed that in a neuroendocrine-based model of depressive-like behavior, not only emotion-related behavior is impacted, but chronic CORT also has a detrimental effect on cognitive performances, including short-term episodic memory, spatial reference learning and memory and associative memory. Indeed, these series of experiments yielded two main results: mice with an anxious-depressed-like phenotype showed (1) a deficit in learning and cognitive flexibility, (2) an impairment of short- and long-term memory.

IMPAIRED VISUO-SPATIAL LEARNING AND COGNITIVE FLEXIBILITY IN ANXIOUS-DEPRESSED-LIKE MICE

Visuo-spatial learning is impaired in anxious-depressed-like mice

Our study demonstrates that chronic administration of CORT in mice has a dramatic influence on learning in visuo-spatial paradigms such as the BM and the MWM. As shown in our prior studies (David et al., 2009; Mendez-David et al., 2014), no changes in the locomotor activity could account for this effect, as the total ambulatory distance swam in the MWM was similar between groups. The finding that chronic CORT administration causes spatial learning deficits is consistent with previous reports in rats (Bodnoff et al., 1995; McLay et al., 1998; Coburn-Litvak et al., 2003).

Associative learning was also affected after chronic CORT administration. Twenty-four hours after a first exposure to the stressor, CORT-treated animals froze less than controls in the CFC. The learning that underlies this test typically includes two distinct processes: (1) the acquisition of a mental representation of the context and (2) an association between the context representation and the unconditioned stimulus representation (Rudy et al., 2004). CFC with a single context-shock pairing is an adult hippocampal neurogenesis-dependent task, sensitive to the X-irradiation-induced blockade of neurogenesis (Denny et al., 2012). Our findings suggest that CORT-induced deficit in context conditioning may be related to a decrease in cell proliferation of progenitor cells in the dentate gyrus in adult hippocampus that may impede the acquisition of the context representation. Indeed, previous works demonstrated that chronic CORT exposure induces similar effects than a chronic stress on cell proliferation, decreasing the number of BrdU-positive cells in the dentate gyrus of the adult mouse hippocampus (Surget et al., 2008; David et al., 2009).

Other hippocampal subregions may participate to the deleterious effects of chronic CORT. For instance, CORT-treated animals also showed decrease in hippocampal CA3 branch points and total dendritic length in the apical tree that would be causally related with the learning impairment (Bisagno et al., 2000). This effect is shared with those of chronic stress procedures, which have been repeatedly found to produce an atrophy of apical dendrites of CA3 hippocampal pyramidal neurons (Watanabe et al., 1992; Magarinos and McEwen, 1995) as well as learning impairments in hippocampus-dependent tasks.

Cognitive flexibility is altered in anxious-depressed-like mice

Cognitive flexibility is an important executive function involving the ability to change a previously learned behavior in response to changes in environmental feedback. Rodents exposed to chronic stress also show reduced cognitive flexibility in attentional set-shifting and reversal learning tasks (Cerqueira et al., 2005, 2007; Bondi et al., 2008), an effect that can be alleviated after acute or subchronic antidepressant treatments (Bondi et al., 2008; Nikiforuk and Popik, 2011; Naegeli et al., 2013). In the MWM, we found similar evidence of loss of cognitive flexibility in our model. Indeed, CORT-treated animals failed to adapt their behavior to learn the new location of the platform, after switching it from the target to the opposite quadrant. Interestingly, in a mouse model of constitutively suppressed adult neurogenesis, the Cyclin D2 knockout mice, impairment in re-learning in the MWM was observed (Garthe et al., 2014). Moreover, re-learning the platform position in an already known general context after goal reversal could requires pattern separation (Wiskott et al., 2006), a process that can be stimulated with increased neurogenesis (Sahay et al., 2011). While this hypothesis was not tested here, we can hypothesize that reduced cognitive flexibility in CORT-treated animals may be linked to changes in adult hippocampal neurogenesis observed in this model.

IMPAIRED SHORT-TERM AND LONG-TERM MEMORY IN ANXIOUS-DEPRESSED-LIKE MICE

There has been a growing awareness that mood disorders are associated with distinct patterns of cognitive impairments (Clark,

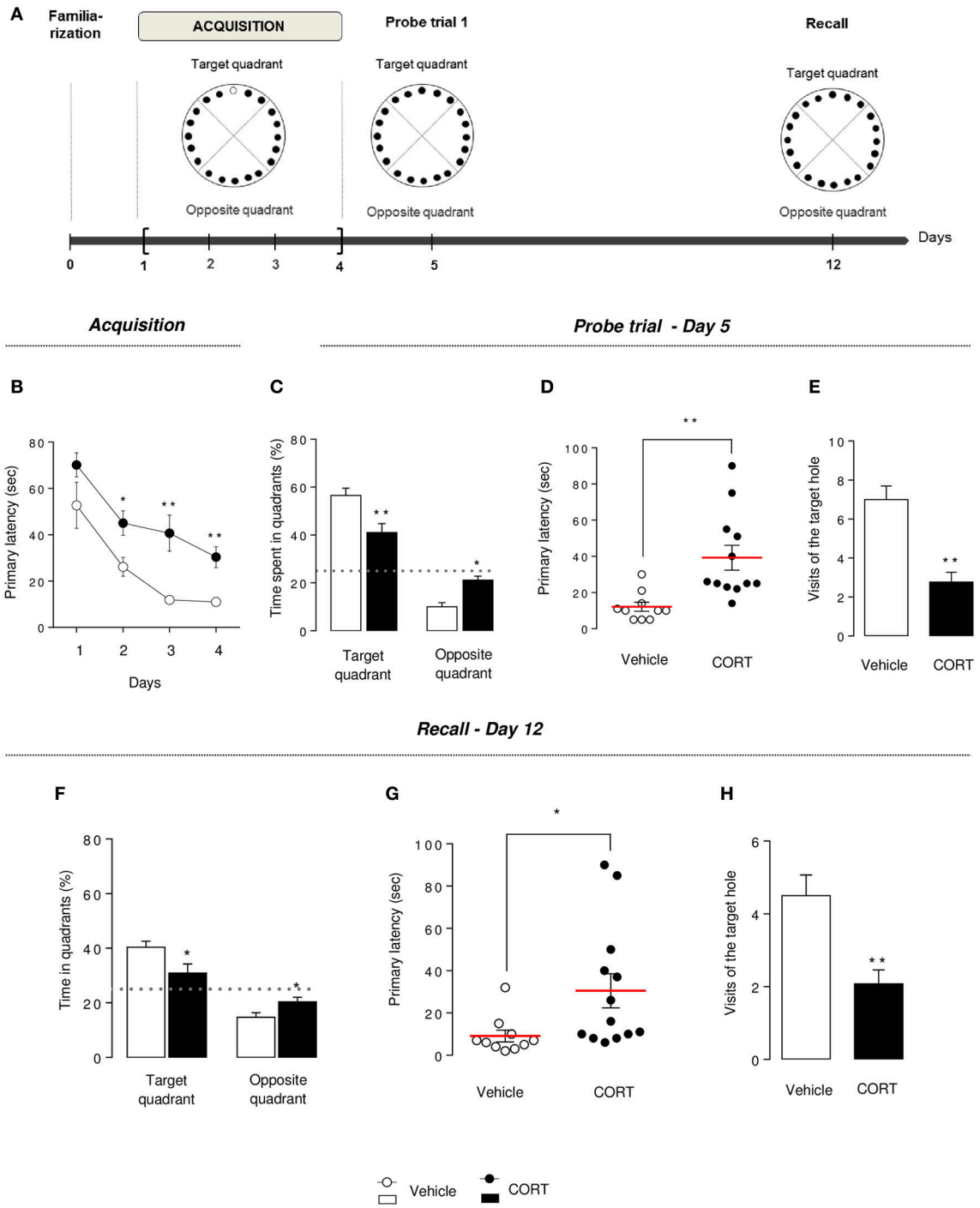


FIGURE 5 | Chronic corticosterone affects spatial learning performances and memory in the Barnes maze. Schematic diagram of the of the BM experimental time course **(A)**. Animals were trained during 4 days to learn the location of the target box. A first probe trial estimates short-term memory (Day 5). A second probe trial (recall) estimates long-term memory (Day 12). During acquisition (Day 1–4), learning was monitored by recording primary latency during the training sessions **(B)**. During the first probe trial (Day 5), the target box was removed and the target hole was closed. Short-term memory retention was evaluated by measuring the time spent into the target and the opposite quadrants **(C)**, the primary latency **(D)** and the number of visits in the target hole **(E)**. Mice were not trained from Day 6 to 11. During the recall trial, spatial long-term memory was assessed by measuring the time spent into the target and the opposite quadrants **(F)**, the primary latency **(G)** and the number of visits in the target hole **(H)**. Values are mean ± s.e.m., $n = 10$–13 animals per group; $^{*}p < 0.05$; $^{**}p < 0.01$ vs. vehicle group.

2009; Gotlib and Joormann, 2010). In this study, episodic (NORT), associative (CFC) and visuo-spatial (MWM and BM) memory were assessed in our model of anxiety-depression in mice.

HPA axis hyperactivation leads to negative effects on memory processes (Song et al., 2006; Aisa et al., 2007; Maccari and Morley-Fletcher, 2007). Specifically, a study demonstrated that mice submitted to learned helplessness paradigm or chronic mild stress procedures showed poor water maze performances (Song et al., 2006). Here, we observed that chronic CORT administration impaired spatial cognitive retention in both mazes. Moreover, because the BM evaluates cognitive function with minimal stress to the animal (vs. the MWM), we confirm that poor performances after chronic glucocorticoid treatment in both mazes are the result of cognitive impairment, rather than a possible effect of stress. Memory retention was also investigated in CORT-treated mice using the NORT. This test strongly relies on visual recognition memory and is based on rodent's exploratory behavior and spontaneous preference for novel objects (Ennaceur and Delacour, 1988). Notably, this task has been a model of short-term episodic memory as mice are able to recognize the familiar object up to a few hours post-training (Bertaina-Anglade et al., 2006). In our study, CORT-treated mice had no difficulties to distinguish between the two objects, but showed an important alteration on short-term memory characterized by a lower discrimination index compared to control mice. Non-spatial NORT is commonly described as a hippocampal-independent task. However, a recent report proposed that the hippocampus was required in this episodic short-term memory test (Cohen et al., 2013), thus raising the hypothesis that lower discrimination index of CORT-treated mice could be attributable to morphologic molecular and cellular changes in brain processes, especially in neurogenesis adaptation. Current studies investigating the relationship between non-spatial NORT and adult hippocampal neurogenesis led to conflicting findings: adult hippocampal neurogenesis alteration is linked either negatively (Jessberger et al., 2009; Cohen et al., 2013) or positively (Denny et al., 2012; Oury et al., 2013) to the novel object discrimination. It seems, at least that the functional integrity of the dentate gyrus is involved in the non-spatial NORT.

We also investigated long-term memory in the BM 1 week after the first probe trial, in similar conditions. In this spatial test, learning behavioral profile in CORT-treated mice makes difficult to interpret the long-term memory assessment. Although acquisition learning and memory were affected in CORT-treated mice, they still succeeded to learn the task. Comparing performances on Day 5 vs. 12 in CORT-treated mice, we showed that these animals lost their ability to locate the target hole a week after the last trial. This finding is in a favor of a change in long-term retention induced by the chronic CORT treatment. Similarities in long-term behavioral changes were observed in a study performed by El Hage et al. (2006), where mice exposed to a unique traumatic stress showed spatial disabilities in the radial maze that persisted beyond a long period, leading to long-term impaired memory.

TRANSLATIONAL APPLICABILITY OF OUR RESULTS

In humans, common physiological mechanisms involving hypothalamo-pituitary-adrenal (HPA) dysfunctions link stress-induced mood disorders and cognitive impairments. It has been shown that enhanced HPA axis activity induced adverse effects on cognitive performances in MDD subjects (Gomez et al., 2009; Hinkelmann et al., 2009). Substantial evidence shows that cognitive symptoms affect a large subset of patients with unipolar depression (for review Marazziti et al., 2010; Trivedi and Greer, 2014). Specifically, the characteristic cognitive profile includes impairments in pattern recognition memory, processing speed, visuo-spatial memory or executive function (McDermott and Ebmeier, 2009). Visuo-spatial learning deficits are well-documented through various automated neuropsychological test battery (Austin et al., 2001; Porter et al., 2003; Egerhazi et al., 2013). In a recent meta-analysis in early MDD, Lee et al. (2012) employed data from 644 patients from 13 different studies, and showed that patients with a first major depressive episode had significant visual learning impairments compared with healthy controls. Similarly, a clinical study assessing neuropsychological functioning showed that major depressive episode patients displayed spatial working memory alterations (Bourke et al., 2012). Few studies aimed at evaluate the cognitive flexibility in unipolar subjects. However, several studies showed that major depression can impair cognitive flexibility (Degl'innocenti et al., 1998; Deveney and Deldin, 2006; Murphy et al., 2012), an effect that could persist even after MDD remission (Hasselbalch et al., 2012).

STUDY LIMITATIONS

The mouse CORT model is a chronic exposure method optimized for use in modeling the persistent anxiety/depression-like state in rodents. Allowing multiple behavioral tests in the same animals, the CORT procedure is an etiologically relevant model of depression that is easily replicable between and within laboratories (Gourley and Taylor, 2009; Mendez-David et al., 2014). However, it does not fully replicate the core pathology of MDD, as animals in this model are not facing environmental stressors, or the greater female susceptibility observed in the disease (Guilloux et al., 2011). Although, it benefits from its reliability and repeatability compared to standard models of depression. Indeed, learned helplessness or chronic mild stress (CMS) procedures are hampered by protocol variability in rodents (Nestler et al., 2002), probably leading to the low reports of co-occurrence of anxiety and depression-like behaviors as well as learning-memory impairments in such a model (Gomez et al., 2013; Haridas et al., 2013).

CONCLUSION

Our results highlight that altered emotional phenotype after 4 weeks of chronic CORT treatment induced a cognitive deficit that affects all aspects of learning and memory, especially episodic, associative and visuo-spatial systems in mice. Because cognitive symptoms have a substantial impact on functional recovery and disability associated with depression, therapies are needed to improve or preserve cognition. Future research in this area should

evaluate potential cognitive properties of antidepressant in mice under stressful conditions. Considering the controversial results in the literature about beneficial (Song et al., 2006; Couto et al., 2012) or detrimental (Gumuslu et al., 2013) effects of chronic monoaminergic antidepressant drug treatment in cognition, it will be worth to investigate whether chronic antidepressant administration ameliorates cognitive performance in the chronic CORT model. The strong reliability of this animal model of anxiety/depression (see Mendez-David et al., 2013b, for review) will certainly allow the dissection of the mechanisms linking depression, cognitive impairments and antidepressant-treatment response.

AUTHOR CONTRIBUTIONS

Flavie Darcet, Jean-Philippe Guilloux, and Denis J. David designed research; Flavie Darcet, Indira Mendez-David performed research. Flavie Darcet, Indira Mendez-David, Laurent Tritschler, Alain M. Gardier, Jean-Philippe Guilloux and Denis J. David analyzed data and wrote the manuscript. Flavie Darcet, Indira Mendez-David, Laurent Tritschler, Alain M. Gardier, Jean-Philippe Guilloux and Denis J. David contributed to the preparation of the manuscript.

ACKNOWLEDGMENTS

We greatly acknowledge Valérie Domergue-Dupont and her staff from the animal care facility of the Institut Paris-Sud d'Innovation Therapeutique at the Université Paris Sud and Dr Marianne Léger (EA4259, Caen Basse-Normandie University, France) for advices regarding the experimental protocol.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fnbeh.2014.00136/abstract

REFERENCES

Aisa, B., Tordera, R., Lasheras, B., Del Rio, J., and Ramirez, M. J. (2007). Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology* 32, 256–266. doi: 10.1016/j.psyneuen.2006.12.013

Austin, M. P., Mitchell, P., and Goodwin, G. M. (2001). Cognitive deficits in depression: possible implications for functional neuropathology. *Br. J. Psychiatry* 178, 200–206. doi: 10.1192/bjp.178.3.200

Bertaina-Anglade, V., Enjuanes, E., Morillon, D., and Drieu La Rochelle, C. (2006). The object recognition task in rats and mice: a simple and rapid model in safety pharmacology to detect amnesic properties of a new chemical entity. *J. Pharmacol. Toxicol. Methods* 54, 99–105. doi: 10.1016/j.vascn.2006.04.001

Bisagno, V., Ferrini, M., Rios, H., Zieher, L. M., and Wikinski, S. I. (2000). Chronic corticosterone impairs inhibitory avoidance in rats: possible link with atrophy of hippocampal CA3 neurons. *Pharmacol. Biochem. Behav.* 66, 235–240. doi: 10.1016/S0091-3057(00)00265-3

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., and Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *J. Neurosci.* 15, 61–69.

Bondi, C. O., Rodriguez, G., Gould, G. G., Frazer, A., and Morilak, D. A. (2008). Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. *Neuropsychopharmacology* 33, 320–331. doi: 10.1038/sj.npp.1301410

Bourke, C., Porter, R. J., Carter, J. D., McIntosh, V. V., Jordan, J., Bell, C., et al. (2012). Comparison of neuropsychological functioning and emotional processing in major depression and social anxiety disorder subjects, and matched healthy controls. *Aust. N.Z. J. Psychiatry* 46, 972–981. doi: 10.1177/0004867412451502

Cerqueira, J. J., Mailliet, F., Almeida, O. F., Jay, T. M., and Sousa, N. (2007). The prefrontal cortex as a key target of the maladaptive response to stress. *J. Neurosci.* 27, 2781–2787. doi: 10.1523/JNEUROSCI.4372-06.2007

Cerqueira, J. J., Pego, J. M., Taipa, R., Bessa, J. M., Almeida, O. F., and Sousa, N. (2005). Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. *J. Neurosci.* 25, 7792–7800. doi: 10.1523/JNEUROSCI.1598-05.2005

Clark, D. A. (2009). Cognitive behavioral therapy for anxiety and depression: possibilities and limitations of a transdiagnostic perspective. *Cogn. Behav. Ther.* 38(Suppl. 1), 29–34. doi: 10.1080/16506070902980745

Coburn-Litvak, P. S., Pothakos, K., Tata, D. A., McCloskey, D. P., and Anderson, B. J. (2003). Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats. *Neurobiol. Learn. Mem.* 80, 11–23. doi: 10.1016/S1074-7427(03)00019-4

Cohen, S. J., Munchow, A. H., Rios, L. M., Zhang, G., Asgeirsdottir, H. N., and Stackman, R. W. Jr. (2013). The rodent hippocampus is essential for nonspatial object memory. *Curr. Biol.* 23, 1685–1690. doi: 10.1016/j.cub.2013.07.002

Couto, F. S., Batalha, V. L., Valadas, J. S., Data-Franca, J., Ribeiro, J. A., and Lopes, L. V. (2012). Escitalopram improves memory deficits induced by maternal separation in the rat. *Eur. J. Pharmacol.* 695, 71–75. doi: 10.1016/j.ejphar.2012.08.020

David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., et al. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* 62, 479–493. doi: 10.1016/j.neuron.2009.04.017

Degl'innocenti, A., Agren, H., and Backman, L. (1998). Executive deficits in major depression. *Acta Psychiatr. Scand.* 97, 182–188. doi: 10.1111/j.1600-0447.1998.tb09985.x

Denny, C. A., Burghardt, N. S., Schachter, D. M., Hen, R., and Drew, M. R. (2012). 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. *Hippocampus* 22, 1188–1201. doi: 10.1002/hipo.20964

Deveney, C. M., and Deldin, P. J. (2006). A preliminary investigation of cognitive flexibility for emotional information in major depressive disorder and non-psychiatric controls. *Emotion* 6, 429–437. doi: 10.1037/1528-3542.6.3.429

Drew, M. R., Denny, C. A., and Hen, R. (2010). Arrest of adult hippocampal neurogenesis in mice impairs single- but not multiple-trial contextual fear conditioning. *Behav. Neurosci.* 124, 446–454. doi: 10.1037/a0020081

Egerhazi, A., Balla, P., Ritzl, A., Varga, Z., Frecska, E., and Berecz, R. (2013). Automated neuropsychological test battery in depression – preliminary data. *Neuropsychopharmacol. Hung.* 15, 5–11.

El Hage, W., Griebel, G., and Belzung, C. (2006). Long-term impaired memory following predatory stress in mice. *Physiol. Behav.* 87, 45–50. doi: 10.1016/j.physbeh.2005.08.039

Elizalde, N., Gil-Bea, F. J., Ramirez, M. J., Aisa, B., Lasheras, B., Del Rio, J., et al. (2008). Long-lasting behavioral effects and recognition memory deficit induced by chronic mild stress in mice: effect of antidepressant treatment. *Psychopharmacology (Berl.)* 199, 1–14. doi: 10.1007/s00213-007-1035-1

Ennaceur, A., and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. *Behav. Brain Res.* 31, 47–59. doi: 10.1016/0166-4328(88)90157-X

Fava, M., Graves, L. M., Benazzi, F., Scalia, M. J., Iosifescu, D. V., Alpert, J. E., et al. (2006). A cross-sectional study of the prevalence of cognitive and physical symptoms during long-term antidepressant treatment. *J. Clin. Psychiatry* 67, 1754–1759. doi: 10.4088/JCP.v67n1113

Fossati, P., Coyette, F., Ergis, A. M., and Allilaire, J. F. (2002). Influence of age and executive functioning on verbal memory of inpatients with depression. *J. Affect. Disord.* 68, 261–271. doi: 10.1016/S0165-0327(00)00362-1

Garcia, R., Spennato, G., Nilsson-Todd, L., Moreau, J. L., and Deschaux, O. (2008). Hippocampal low-frequency stimulation and chronic mild stress similarly disrupt fear extinction memory in rats. *Neurobiol. Learn. Mem.* 89, 560–566. doi: 10.1016/j.nlm.2007.10.005

Garthe, A., Huang, Z., Kaczmarek, L., Filipkowski, R. K., and Kempermann, G. (2014). Not all water mazes are created equal: cyclin D2 knockout mice with

constitutively suppressed adult hippocampal neurogenesis do show specific spatial learning deficits. *Genes Brain Behav.* 13, 357–364. doi: 10.1111/gbb.12130

Gomez, J. L., Lewis, M. J., Sebastian, V., Serrano, P., and Luine, V. N. (2013). Alcohol administration blocks stress-induced impairments in memory and anxiety, and alters hippocampal neurotransmitter receptor expression in male rats. *Horm. Behav.* 63, 659–666. doi: 10.1016/j.yhbeh.2013.01.007

Gomez, R. G., Posener, J. A., Keller, J., Debattista, C., Solvason, B., and Schatzberg, A. F. (2009). Effects of major depression diagnosis and cortisol levels on indices of neurocognitive function. *Psychoneuroendocrinology* 34, 1012–1018. doi: 10.1016/j.psyneuen.2009.01.017

Gotlib, I. H., and Joormann, J. (2010). Cognition and depression: current status and future directions. *Annu. Rev. Clin. Psychol.* 6, 285–312. doi: 10.1146/annurev.clinpsy.121208.131305

Gourley, S. L., and Taylor, J. R. (2009). Recapitulation and reversal of a persistent depression-like syndrome in rodents. *Curr. Protoc. Neurosci.* Chapter 9, Unit 9 32. doi: 10.1002/0471142301.ns0932s49

Griebel, G., Simiand, J., Serradeil-Le Gal, C., Wagnon, J., Pascal, M., Scatton, B., et al. (2002). Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc. Natl. Acad. Sci. U.S.A.* 99, 6370–6375. doi: 10.1073/pnas.092012099

Guilloux, J. P., Seney, M., Edgar, N., and Sibille, E. (2011). Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: relevance to emotionality and sex. *J. Neurosci. Methods* 197, 21–31. doi: 10.1016/j.jneumeth.2011.01.019

Gumuslu, E., Mutlu, O., Sunnetci, D., Ulak, G., Celikyurt, I. K., Cine, N., et al. (2013). The effects of tianeptine, olanzapine and fluoxetine on the cognitive behaviors of unpredictable chronic mild stress-exposed mice. *Drug Res. (Stuttg).* 63, 532–539. doi: 10.1055/s-0033-1347237

Hammar, A., and Ardal, G. (2009). Cognitive functioning in major depression–a summary. *Front. Hum. Neurosci.* 3:26. doi: 10.3389/neuro.09.026.2009

Hammar, A., Lund, A., and Hugdahl, K. (2003). Long-lasting cognitive impairment in unipolar major depression: a 6-month follow-up study. *Psychiatry Res.* 118, 189–196. doi: 10.1016/S0165-1781(03)00075-1

Haridas, S., Kumar, M., and Manda, K. (2013). Melatonin ameliorates chronic mild stress induced behavioral dysfunctions in mice. *Physiol. Behav.* 119, 201–207. doi: 10.1016/j.physbeh.2013.06.015

Hasselbalch, B. J., Knorr, U., Hasselbalch, S. G., Gade, A., and Kessing, L. V. (2012). Cognitive deficits in the remitted state of unipolar depressive disorder. *Neuropsychology* 26, 642–651. doi: 10.1037/a0029301

Hinkelmann, K., Moritz, S., Botzenhardt, J., Riedesel, K., Wiedemann, K., Kellner, M., et al. (2009). Cognitive impairment in major depression: association with salivary cortisol. *Biol. Psychiatry* 66, 879–885. doi: 10.1016/j.biopsych.2009.06.023

Jessberger, S., Clark, R. E., Broadbent, N. J., Clemenson, G. D. Jr., Consiglio, A., Lie, D. C., et al. (2009). Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn. Mem.* 16, 147–154. doi: 10.1101/lm.1172609

Lampe, I. K., Sitskoorn, M. M., and Heeren, T. J. (2004). Effects of recurrent major depressive disorder on behavior and cognitive function in female depressed patients. *Psychiatry Res.* 125, 73–79. doi: 10.1016/j.psychres.2003.12.004

Landro, N. I., Stiles, T. C., and Sletvold, H. (2001). Neuropsychological function in nonpsychotic unipolar major depression. *Neuropsychiatry Neuropsychol. Behav. Neurol.* 14, 233–240.

Lee, R. S., Hermens, D. F., Porter, M. A., and Redoblado-Hodge, M. A. (2012). A meta-analysis of cognitive deficits in first-episode major depressive disorder. *J. Affect. Disord.* 140, 113–124. doi: 10.1016/j.jad.2011.10.023

Maccari, S., and Morley-Fletcher, S. (2007). Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. *Psychoneuroendocrinology* 32(Suppl. 1), S10–S15. doi: 10.1016/j.psyneuen.2007.06.005

Magarinos, A. M., and McEwen, B. S. (1995). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience* 69, 89–98. doi: 10.1016/0306-4522(95)00259-L

Marazziti, D., Consoli, G., Picchetti, M., Carlini, M., and Faravelli, L. (2010). Cognitive impairment in major depression. *Eur. J. Pharmacol.* 626, 83–86. doi: 10.1016/j.ejphar.2009.08.046

McDermott, L. M., and Ebmeier, K. P. (2009). A meta-analysis of depression severity and cognitive function. *J. Affect. Disord.* 119, 1–8. doi: 10.1016/j.jad.2009.04.022

McLay, R. N., Freeman, S. M., and Zadina, J. E. (1998). Chronic corticosterone impairs memory performance in the Barnes maze. *Physiol. Behav.* 63, 933–937. doi: 10.1016/S0031-9384(97)00529-5

Mendez-David, I., David, D. J., Darcet, F., Wu, M. V., Kerdine-Romer, S., Gardier, A. M., et al. (2014). Rapid anxiolytic effects of a 5-HT receptor agonist are mediated by a neurogenesis-independent mechanism. *Neuropsychopharmacology*. 39, 1366–1378. doi: 10.1038/npp.2013.332

Mendez-David, I., Hen, R., Gardier, A. M., and David, D. J. (2013b). Adult hippocampal neurogenesis: an actor in the antidepressant-like action. *Ann. Pharm. Fr.* 71, 143–149. doi: 10.1016/j.pharma.2013.02.006

Millan, M. J., Agid, Y., Brune, M., Bullmore, E. T., Carter, C. S., Clayton, N. S., et al. (2012). Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. *Nat. Rev. Drug Discov.* 11, 141–168. doi: 10.1038/nrd3628

Murphy, F. C., Michael, A., and Sahakian, B. J. (2012). Emotion modulates cognitive flexibility in patients with major depression. *Psychol. Med.* 42, 1373–1382. doi: 10.1017/S0033291711002418

Murrough, J. W., Iacoviello, B., Neumeister, A., Charney, D. S., and Iosifescu, D. V. (2011). Cognitive dysfunction in depression: neurocircuitry and new therapeutic strategies. *Neurobiol. Learn. Mem.* 96, 553–563. doi: 10.1016/j.nlm.2011.06.006

Naegeli, K. J., O'connor, J. A., Banerjee, P., and Morilak, D. A. (2013). Effects of milnacipran on cognitive flexibility following chronic stress in rats. *Eur. J. Pharmacol.* 703, 62–66. doi: 10.1016/j.ejphar.2013.02.006

Naismith, S. L., Hickie, I. B., Turner, K., Little, C. L., Winter, V., Ward, P. B., et al. (2003). Neuropsychological performance in patients with depression is associated with clinical, etiological and genetic risk factors. *J. Clin. Exp. Neuropsychol.* 25, 866–877. doi: 10.1076/jcen.25.6.866.16472

Nestler, E. J., Barrot, M., Dileone, R. J., Eisch, A. J., Gold, S. J., and Monteggia, L. M. (2002). Neurobiology of depression. *Neuron* 34, 13–25. doi: 10.1016/S0896-6273(02)00653-0

Nikiforuk, A., and Popik, P. (2011). Long-lasting cognitive deficit induced by stress is alleviated by acute administration of antidepressants. *Psychoneuroendocrinology* 36, 28–39. doi: 10.1016/j.psyneuen.2010.06.001

Orsetti, M., Colella, L., Dellarole, A., Canonico, P. L., and Ghi, P. (2007). Modification of spatial recognition memory and object discrimination after chronic administration of haloperidol, amitriptyline, sodium valproate or olanzapine in normal and anhedonic rats. *Int. J. Neuropsychopharmacol.* 10, 345–357. doi: 10.1017/S1461145706006705

Oury, F., Khrimian, L., Denny, C. A., Gardin, A., Chamouni, A., Goeden, N., et al. (2013). Maternal and offspring pools of osteocalcin influence brain development and functions. *Cell* 155, 228–241. doi: 10.1016/j.cell.2013.08.042

Patki, G., Solanki, N., Atrooz, F., Allam, F., and Salim, S. (2013). Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. *Brain Res.* 1539, 73–86. doi: 10.1016/j.brainres.2013.09.033

Porter, R. J., Gallagher, P., Thompson, J. M., and Young, A. H. (2003). Neurocognitive impairment in drug-free patients with major depressive disorder. *Br. J. Psychiatry* 182, 214–220. doi: 10.1192/bjp.182.3.214

Ravnkilde, B., Videbech, P., Clemmensen, K., Egander, A., Rasmussen, N. A., and Rosenberg, R. (2002). Cognitive deficits in major depression. *Scand. J. Psychol.* 43, 239–251. doi: 10.1111/1467-9450.00292

Richter, S. H., Zeuch, B., Lankisch, K., Gass, P., Durstewitz, D., and Vollmayr, B. (2013). Where have I been? Where should I go? Spatial working memory on a radial arm maze in a rat model of depression. *PLoS ONE* 8:e62458. doi: 10.1371/journal.pone.0062458

Rudy, J. W., Huff, N. C., and Matus-Amat, P. (2004). Understanding contextual fear conditioning: insights from a two-process model. *Neurosci. Biobehav. Rev.* 28, 675–685. doi: 10.1016/j.neubiorev.2004.09.004

Sahay, A., Scobie, K. N., Hill, A. S., O'carroll, C. M., Kheirbek, M. A., Burghardt, N. S., et al. (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* 472, 466–470. doi: 10.1038/nature09817

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301, 805–809. doi: 10.1126/science.1083328

Song, L., Che, W., Min-Wei, W., Murakami, Y., and Matsumoto, K. (2006). Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacol. Biochem. Behav.* 83, 186–193. doi: 10.1016/j.pbb.2006.01.004

Sunyer, B., Patil, S., Höger, H., and Lubec, G. (2007). Barnes maze, a useful task to assess spatial reference memory in the mice. *Protocol Exchange* doi: 10.1038/nprot.2007.390

Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., et al. (2008). Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biol. Psychiatry* 64, 293–301. doi: 10.1016/j.biopsych.2008.02.022

Trivedi, M. H., and Greer, T. L. (2014). Cognitive dysfunction in unipolar depression: implications for treatment. *J. Affect. Disord.* 152–154, 19–27. doi: 10.1016/j.jad.2013.09.012

Vythilingam, M., Vermetten, E., Anderson, G. M., Luckenbaugh, D., Anderson, E. R., Snow, J., et al. (2004). Hippocampal volume, memory, and cortisol status in major depressive disorder: effects of treatment. *Biol. Psychiatry* 56, 101–112. doi: 10.1016/j.biopsych.2004.04.002

Watanabe, Y., Gould, E., Cameron, H. A., Daniels, D. C., and McEwen, B. S. (1992). Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. *Hippocampus* 2, 431–435. doi: 10.1002/hipo.450020410

WHO. (2008). *World Health Organization - The Global Burden of Disease - 2004 Update.* Geneva: WHO Library.

Wiskott, L., Rasch, M. J., and Kempermann, G. (2006). A functional hypothesis for adult hippocampal neurogenesis: avoidance of catastrophic interference in the dentate gyrus. *Hippocampus* 16, 329–343. doi: 10.1002/hipo.20167

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 January 2014; accepted: 03 April 2014; published online: 01 May 2014.
Citation: Darcet F, Mendez-David I, Tritschler L, Gardier AM, Guilloux J-P and David DJ (2014) Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. Front. Behav. Neurosci. ***8****:136. doi: 10.3389/fnbeh.2014.00136*
This article was submitted to the journal Frontiers in Behavioral Neuroscience.

Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression

Flavie Darcet, Indira Mendez-David, Laurent Tritschler, Alain M. Gardier, Jean-Philippe Guilloux[*], Denis J. David[*,§]

Université Paris-Sud, EA3544, Faculté de Pharmacie,
5 rue Jean-Baptiste Clément, Châtenay-Malabry, France

*Last co-authorship.
§ To whom correspondence should be addressed.

Dr Denis DAVID
Univ Paris-Sud
Fac Pharmacie
5, rue J-B Clément, Tour D1, 2e etage
EA 3544 "Pharmacologie des troubles anxio-dépressifs et Neurogenèse"
F-92296 Chatenay-Malabry cedex
tel: (33) 1 46 83 59 68
Fax: (33) 1 46 83 53 55
E-mail: denis.david@u-psud.fr

SUPPLEMENTAL FIGURES

Supplemental Figure 1: Effects of chronic corticosterone treatment on the anxious/depressed-like phenotype

Effects of a chronic CORT treatment on the anxious/depressed-like phenotype (**A**) Weekly measures of mouse body weight during all the protocol showed a greater gain weight in CORT-treated mice compared to control mice. (**B**) Chronic CORT treatment induced a significant alteration of the fur coat state, reflected by an increasing coat state score from week 2 to week 6. Anxiety, evaluated in the Open Field test, was expressed as (**C-D**) time spent in the center, in seconds, for the entire session (**C**) and also as ambulatory distance in the center, in meters, for the entire session (**D**). Depressive-like phenotype assessed in the Splash test was expressed as mean duration of grooming after receiving a 10% sucrose solution on the snout. (**E**) Values are mean ± SEM (n=20 animals per group, Cohort 1) *p<0.05; **p<0.01 *versus* vehicle group.

Supplemental Figure 2: Effects of chronic corticosterone treatment on exploration duration and locomotor activity in the novel object recognition test.

Effects of chronic CORT treatment on both objects exploration time (**A**) and ambulatory distance (**B**) during training and test sessions. Values are mean ± SEM (n=20 animals per group) **p<0.01 *versus* vehicle group.

Supplemental Figure 3: Effects of a chronic corticosterone treatment on freezing duration during shock exposure in the one-trial contextual fear conditioning.

The percentage of freezing was measured before and after the shock during Day 1. A one-way ANOVA with repeated-measures was applied. Values are mean ± SEM (n=5-7 animals per group) **p<0.01 *versus* before the shock.

Supplemental Figure 4: Effects of a chronic corticosterone treatment on pre-training and reversal parameters in the Morris water maze.

Latency (**A**) and distance travelled (**B**) to reach the visible platform were recorded during the pre-training phase (Day 0). Relative time spent in the former target quadrant (North) was measured on Day 8 and Day 9 (**C**). A survival curve was performed for latency to first cross the platform during the reversal probe trial in Day 12 (**D**). Values are mean ± SEM (n=15 animals per group) **p<0.01 *versus* vehicle group.

Supplemental Figure 5: Effects of a chronic corticosterone treatment on various learning parameters and short-term memory retention in the Barnes maze.

Total latency (**A**), primary errors (**B**) and total errors (**C**) were recorded in the Barnes maze as learning indicators. During the probe trial on Day 5, the number of primary errors was calculated (**D**) and the number of visits among all holes of the maze were counted to compare patterns of distribution (**E**). Values are mean ± SEM (n=10-13 animals per group) *p<0.05; **p<0.01 *versus* vehicle group.

Supplemental Table 1: complete statistical summary analysis for behavioral data

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Novel object recognition test	Exploration time	Two-way ANOVA	Factor 1 Treatment	F=10.23	1,76	p<0.01**	2B
			Factor 2 Object	F=40.98	1,76	p<0.01**	
			Interaction (F1 X F2)	F=23.18	1,76	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Novel Object, Familiar Object)			p<0.01**	
	Exploration frequency	Two-way ANOVA	Factor 1 Treatment	F=4.18	1,76	p<0.05*	2C
			Factor 2 Object	F=51.12	1,76	p<0.01**	
			Interaction (F1 X F2)	F=12.43	1,76	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Novel object, Familiar Object)			p<0.01**	
			Familiar *vs* Novel object (Veh)			p<0.01**	
			Familiar *vs* Novel object (CORT)			p<0.05*	
	Exploration time	*t*-test	CORT *vs* Veh	F=48.44	38	p<0.01**	2D
		One sample *t*-test	CORT *vs* 50%	t=4.015	19	p<0.01**	
			Veh *vs* 50%	t=10.63	19	p<0.01**	
	Discrimination Index	*t*-test	CORT *vs* Veh	F=48.44	38	p<0.01**	2E
	Exploration time across sessions	One-way ANOVA with repeated measures	Factor 1 Treatment	F=15.37	1,114	p<0.01**	S2A
			Factor 2 Time	F=9.068	3,114	p<0.01**	
			Interaction (F1 X F2)	F=1.481	3,114	p>0.1	
		PLSD Post-hoc test	CORT *vs* Veh (Session 4)			p<0.01**	
	Total exploration time (Probe test)	*t*-test	CORT *vs* Veh	F=8.23	38	p<0.01**	
	Distance travelled across sessions	One-way ANOVA with repeated measures	Factor 1 Treatment	F=3.425	1,114	p>0.05	S2B
			Factor 2 Time	F=47.80	3,114	p<0.01**	
			Interaction (F1 X F2)	F=0.21	3,114	p>0.1	
	Distance travelled (Probe test)	*t*-test	CORT *vs* Veh	F=0.019	38	p>0.5	
One-trial contextual fear conditioning	Freezing duration	One-way ANOVA with repeated measures	Factor 1 Treatment	F=19.83	1,30	p<0.01**	3A
			Factor 2 Time	F=6.11	3,30	p<0.01**	
			Interaction (F1 X F2)	F=5.24	3,30	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh, (minute 2)			p<0.05*	
			CORT *vs* Veh, (minute 3 and 4)			p<0.01**	
	Freezing duration	*t*-test	CORT *vs* Veh	F=19.84	10	p<0.01**	3B
	Freezing duration during Day 1	Two-way ANOVA	Factor 1 Treatment	F=0.523	1,20	p>0.5	S3
			Factor 2 Time	F=20.35	1,20	p<0.01**	
			Interaction (F1 X F2)	F=0.073	1,20	p>0.1	

Morris water maze	Latency to reach the platform (Acquisition)	One-way ANOVA with repeated measures	Factor 1 Treatment	F=10.92	1,84	p<0.01**	**4B**
			Factor 2 Time	F=14.99	3,84	p<0.01**	
			Interaction (F1 X F2)	F=2.67	3,84	p=0.0525	
		PLSD Post-hoc test	CORT *vs* Veh (Day 4)			p<0.01**	
	Total distance (Acquisition)	One-way ANOVA with repeated measures	Factor 1 Treatment	F=5.57	1,84	p<0.05*	**4C**
			Factor 2 Time	F=24.84	1,84	p<0.01**	
			Interaction (F1 X F2)	F=2.24	1,84	p>0.05	
		PLSD Post-hoc test	CORT *vs* Veh (Day 4)			p<0.01**	
	Total distance during probe test-Day 12	*t*-test	CORT *vs* Veh	F=3.385	1,28	p>0.05	
	Time in quadrants (Probe test-Day 5)	Two-way ANOVA	Factor 1 Treatment	F=1.40	1,56	p>0.05	**4D**
			Factor 2 Quadrant	F=22.86	1,56	p<0.01**	
			Interaction (F1 X F2)	F=4.295	1,56	p<0.05*	
		One sample *t*-test	CORT *vs* 25%	t=8.479	14	p<0.01**	
			Veh *vs* 25%	t=8.971	14	p<0.01**	
	Latency to cross the platform (Probe test-Day 5)	*t*-test	CORT *vs* Veh	F=6.55	28	p<0.05*	**4E**
	Entries in the platform zone (Probe test-Day 5)	*t*-test	CORT *vs* Veh	F=4.47	28	p<0.05*	**4F**
	Latency to reach the platform (Reversal)	One-way ANOVA with repeated measures	Factor 1 Treatment	F=11.88	1,84	p<0.01**	**4G**
			Factor 2 Time	F=18.76	3,84	p<0.01**	
			Interaction (F1 X F2)	F=6.32	3,84	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Day 2 to Day 4)			p<0.01**	
	Total distance (Reversal)	One-way ANOVA with repeated measures	Factor 1 Treatment	F=1.95	1,84	p>0.1	**4H**
			Factor 2 Time	F=34.78	3,84	p<0.01**	
			Interaction (F1 X F2)	F=7.49	3,84	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Day 8)			p<0.05*	
			CORT *vs* Veh (Day 11)			p<0.01**	
	Total distance during probe test-Day 12	*t*-test	CORT *vs* Veh	F= 3.613	1,28	p>0.05	
	Time in quadrants (Probe test-Day 12)	Two-way ANOVA	Factor 1 Treatment	F=1.76	1,56	p>0.05	**4I**
			Factor 2 Quadrant	F=23.27	1,56	p<0.01**	

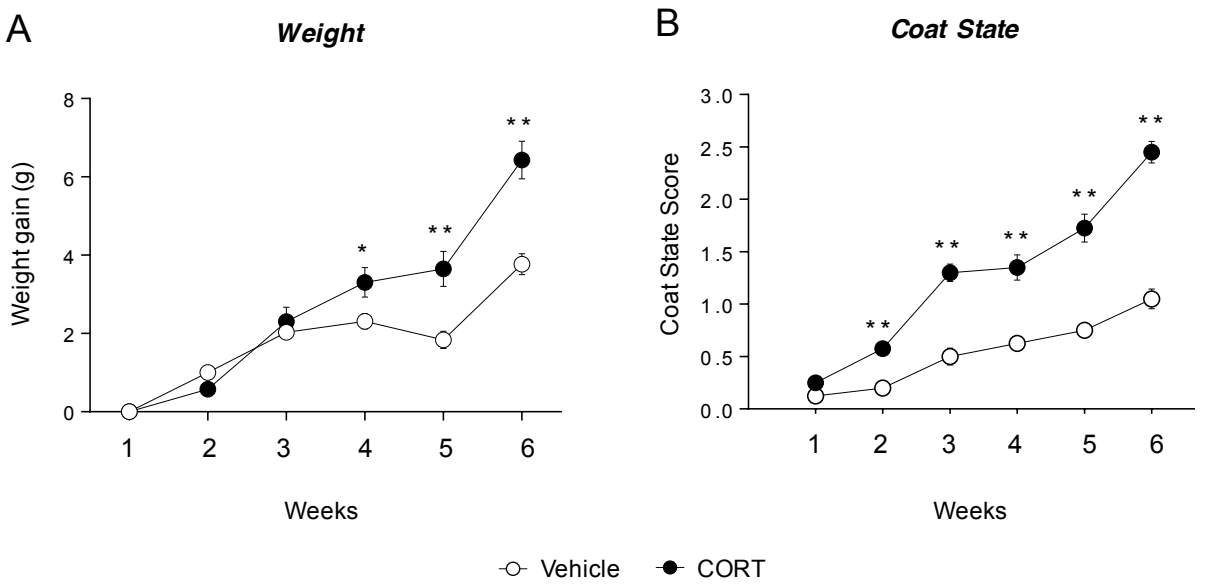
			Interaction (F1 X F2)	F=5.97	1,56	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Target quadrant)			p<0.05*	
		One sample *t*-test	CORT *vs* 25%	t=1.086	14	p>0.2	
			Veh *vs* 25%	t=4.496	14	p<0.01**	
	Latency to cross the platform (Probe test-Day 12)	*t*-test	CORT *vs* Veh	F=11.96	28	p<0.01**	**4J**
	Entries in the platform zone (Probe test-Day 12)	*t*-test	CORT *vs* Veh	F=5.15	28	p<0.05*	**4K**
	Latency to reach the visible platform	*t*-test	CORT *vs* Veh	F=0.386	28	p>0.5	**S4A**
	Distance to reach the visible platform	*t*-test	CORT *vs* Veh	F=0.569	28	p>0.1	**S4B**
	Time spent in former target quadrant (North)	One-way repeated measures ANOVA	Factor 1 Treatment	F=4.963	1,56	p<0.05*	**S4C**
			Factor 2 Time	F=2.946	1,56	p>0.5	
			Interaction (F1 X F2)	F=1.327	1,56	p>0.5	
		PLSD Post-hoc test	CORT *vs* Veh (Day 9)			p<0.05*	
		One sample *t*-test	CORT *vs* 25% (Day 8)	t=2.658	14	p<0.05*	
			Veh *vs* 25% (Day 8)	t=2.301	14	p<0.05*	
			CORT *vs* 25% (Day 9)	t=2.379	14	p<0.05*	
	Latency to cross the platform (Probe test-Day 12)	Mantel-Cox test	Kaplan-Meier Survival analysis			p<0.01**	**S4D**
Barnes maze	Primary latency	One-way repeated measures ANOVA	Factor 1 Treatment	F=13.74	1,60	p<0.01**	**5B**
			Factor 2 Time	F=26.69	3,60	p<0.01**	
			Interaction (F1 X F2)	F=0.60	3,60	p>0.6	
		PLSD Post-hoc test	CORT *vs* Veh (Day 2)			p<0.05*	
			CORT *vs* Veh (Day 3, Day 4)			p<0.01**	

	Time in quadrants (Day 5 probe test)	Two-way ANOVA	Factor 1 Treatment	F=0.73	1,42	p>0.05	**5C**
			Factor 2 Quadrant	F=151.0	1,42	p<0.01**	
			Interaction (F1 X F2)	F=19.41	1,42	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Opposite quadrant)			p<0.05*	
			CORT *vs* Veh (Target quadrant)			p<0.01**	
		One sample *t*-test	CORT *vs* 25%	t=4.79	12	p<0.01**	
			Veh *vs* 25%	t=10.26	9	p<0.01**	
	Primary latency (Day 5 probe trial)	*t*-test	CORT *vs* Veh	F=11.31	21	p<0.01**	**5D**
	Visits of the target hole (Day 5 probe trial)	*t*-test	CORT *vs* Veh	F=25.81	21	p<0.01**	**5E**
	Time in quadrants (Day 12 probe trial)	Two-way ANOVA	Factor 1 Treatment	F=0.57	1,42	p>0.05	**5F**
			Factor 2 Quadrant	F=53.67	1,42	p<0.01**	
			Interaction (F1 X F2)	F=9.43	1,42	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Target, opposite quadrant)			p<0.05*	
		One sample *t*-test	CORT *vs* 25%	t=1.76	12	p<0.01**	
			Veh *vs* 25%	t=6.81	9	p<0.01**	
	Primary latency (Day 12 probe trial)	*t*-test	CORT *vs* Veh	t=5.05	21	p<0.05*	**5G**
	Visits of the target hole (Day 12 probe trial)	*t*-test	CORT *vs* Veh	t=23.69	21	p<0.01**	**5H**
	Total latency (Acquisition)	One-way ANOVA with repeated measures	Factor 1 Treatment	F=16.21	1,60	p<0.01**	**S5A**
			Factor 2 Time	F=15.81	3,60	p<0.01**	
			Interaction (F1 X F2)	F=3.59	3,60	p<0.05*	
		PLSD Post-hoc test	CORT *vs* Veh (Day 2, Day 3, Day 4)			p<0.01**	
	Primary errors	One-way ANOVA with repeated measures	Factor 1 Treatment	F=46.48	1,60	p<0.01**	**S5B**
			Factor 2 Time	F=3.94	3,60	p<0.05*	
			Interaction (F1 X F2)	F=0.59	3,60	p>0.6	
		PLSD	CORT *vs* Veh (Day 1)			p<0.05*	

		Post-hoc test	CORT *vs* Veh (Day 2, Day 3, Day 4)			p<0.01**	
	Total errors	One-way ANOVA with repeated measures	Factor 1 Treatment	F=22.50	1,60	p<0.01**	**S5C**
			Factor 2 Time	F=2.96	3,60	p<0.05*	
			Interaction (F1 X F2)	F=5.00	3,60	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Day 2, Day 3, Day 4)			p<0.01**	
	Primary errors (Day 5 probe trial)	*t*-test	CORT *vs* Veh	F=8.95	21	p<0.01**	**S5D**
	Visits distribution	Two-way ANOVA	Factor 1 Treatment	F=25.9	1,42	p<0.01**	**S5E**
			Factor 2 Hole	F=32.46	19,42	p<0.01**	
			Interaction (F1 X F2)	F=10.22	19,42	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Target hole, +8 hole)			p<0.01**	
			CORT *vs* Veh (Opposite hole, -1/+1/+9 holes)			p<0.05*	
Weight	Weight gain	One-way ANOVA with repeated measures	Factor 1 Treatment	F=7.83	1,19	p<0.01**	**S1A**
			Factor 2 Time	F=181.79	5,19	p<0.01**	
			Interaction (F1 X F2)	F=19.77	5,19	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh, Week 4			p<0.05*	
			CORT *vs* Veh, Week 5 and 6			p<0.01**	
Coat state	Score	One-way ANOVA with repeated measures	Factor 1 Treatment	F=108.10	1,19	p<0.01**	**S1B**
			Factor 2 Time	F=109.36	5,19	p<0.01**	
			Interaction (F1 X F2)	F=16.47	5,19	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Week 2 to week 6)			p<0.01**	
Open Field	Time in center	*t*-test	CORT *vs* Veh	F=5.61	1,38	p<0.05*	**S1C**
	Ambulatory distance	*t*-test	Factor treatment	F=2.78	1,38	p>0.1	**S1D**
Splash test	Grooming duration	*t*-test	CORT *vs* Veh	F=18.63	1,38	p<0.01**	**S1E**

Legend: CORT: corticosterone; Veh: Vehicle

Figure S1


A
Weight
Weight gain (g)
Weeks
B
Coat State
Coat State Score
Weeks
Vehicle
CORT

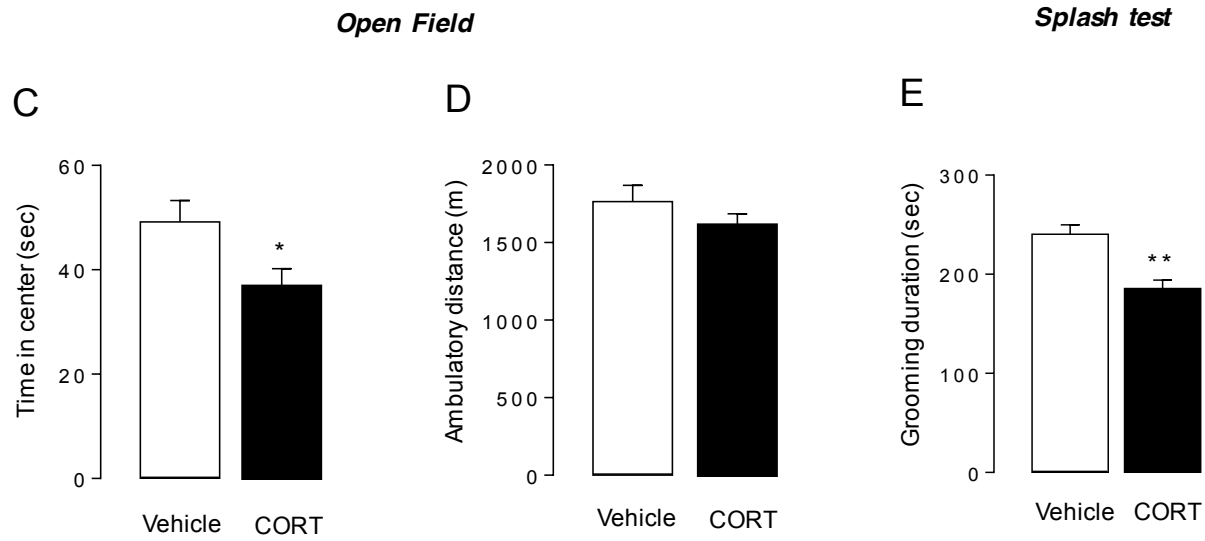

Open Field
C
Time in center (sec)
Vehicle
CORT
D
Ambulatory distance (m)
Vehicle
CORT
Splash test
E
Grooming duration (sec)
Vehicle
CORT

Figure S2

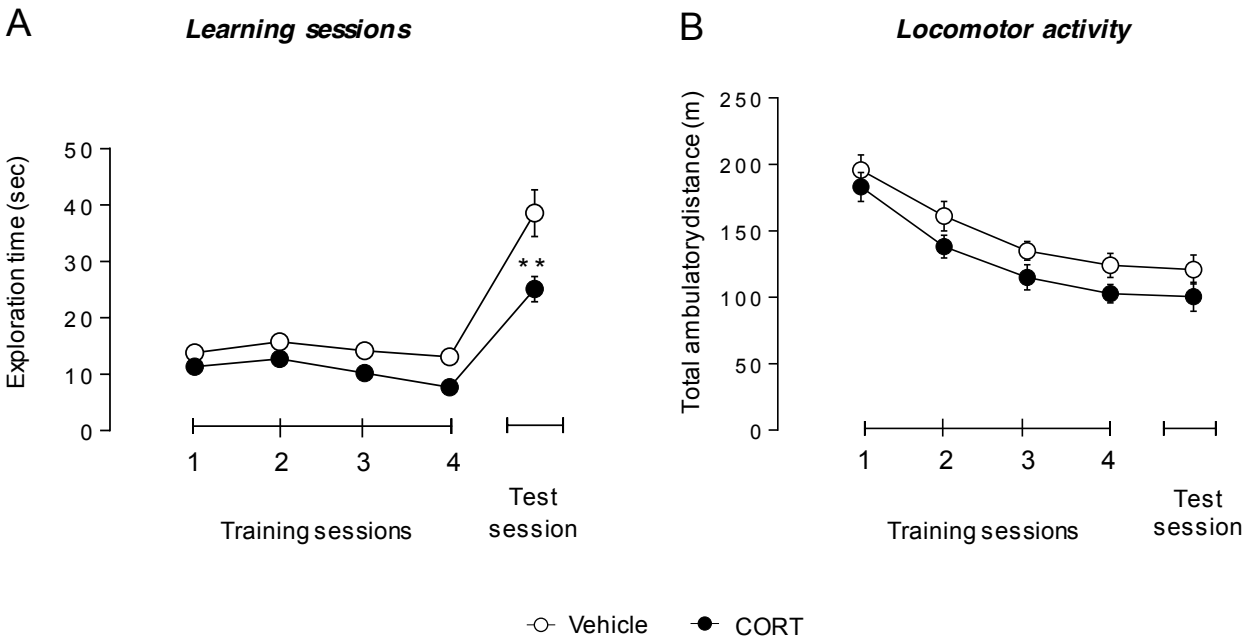

A
Learning sessions
Exploration time (sec)
50
40
30
20
10
0
**
1
2
3
4
Training sessions
Test session
B
Locomotor activity
Total ambulatorydistance (m)
250
200
150
100
50
0
1
2
3
4
Training sessions
Test session
Vehicle
CORT

Figure S3

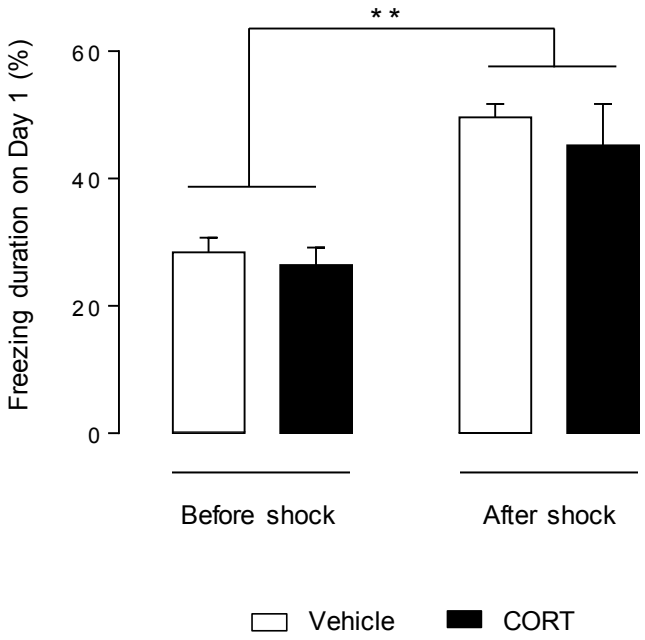

* *
Freezing duration on Day 1 (%)
60
40
20
0
Before shock
After shock
Vehicle
CORT

Figure S4

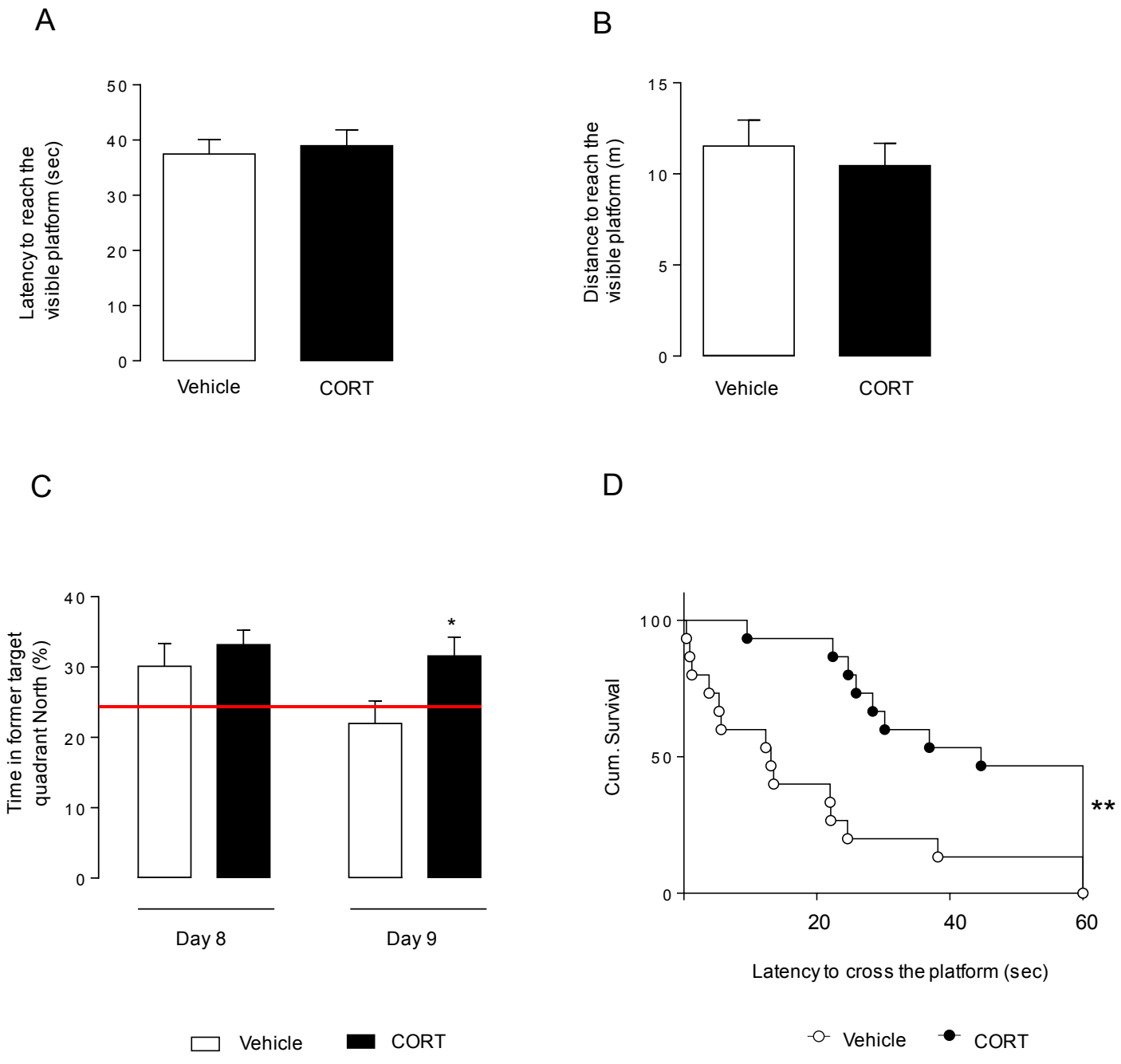
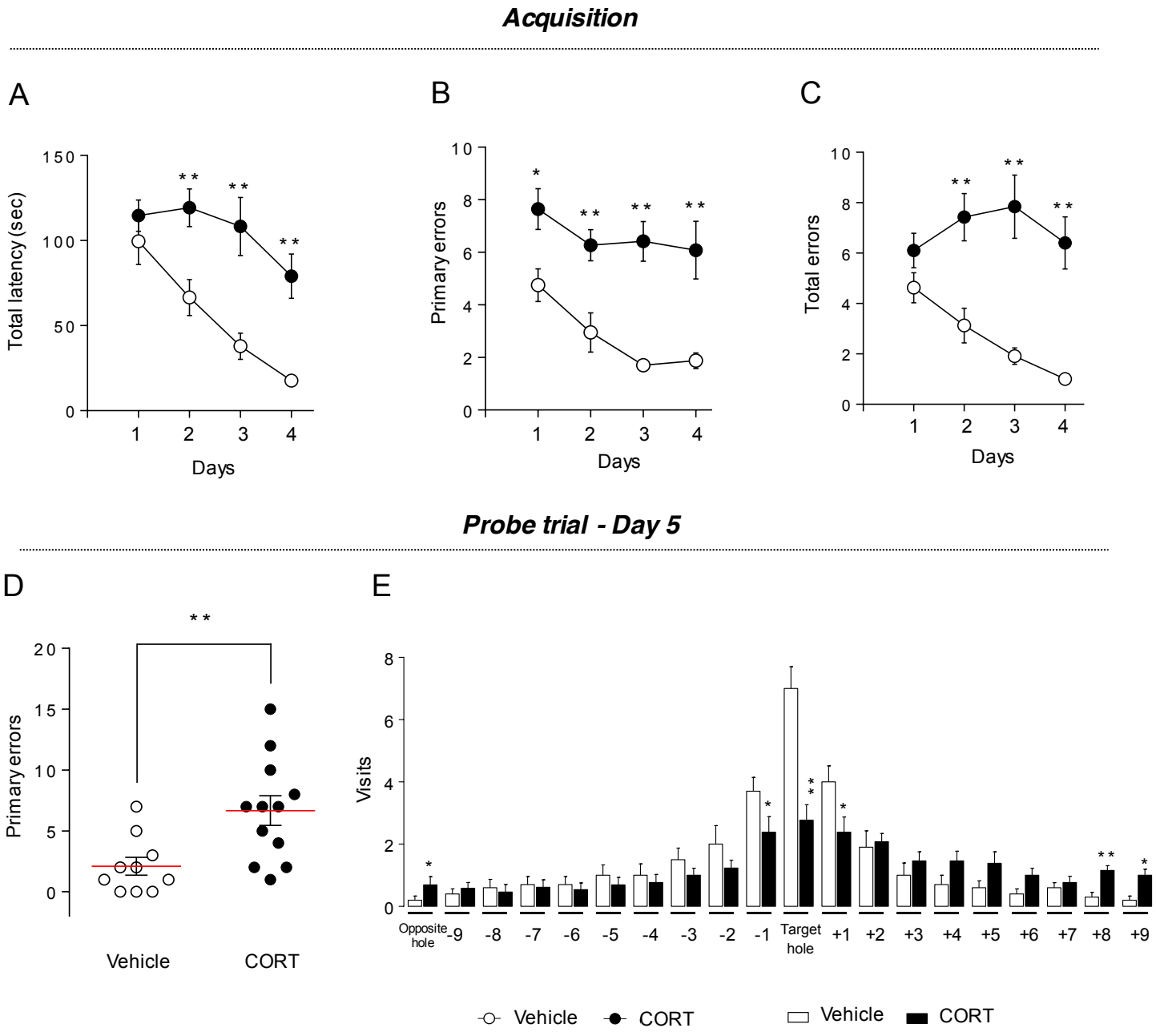

A
Latency to reach the visible platform (sec)
50
40
30
20
10
0
Vehicle
CORT
B
Distance to reach the visible platform (m)
15
10
5
0
Vehicle
CORT
C
Time in former target quadrant North (%)
40
30
20
10
0
*
Day 8
Day 9
Vehicle
CORT
D
Cum. Survival
100
50
0
**
20
40
60
Latency to cross the platform (sec)
Vehicle
CORT

Figure S5


Acquisition
A
Total latency (sec)
Days
B
Primary errors
Days
C
Total errors
Days
Probe trial - Day 5
D
Primary errors
Vehicle
CORT
E
Visits
Opposite hole
Target hole
Vehicle
CORT
Vehicle
CORT

Commentaires sur Article 1 :

L'ensemble de ces résultats suggère qu'une souris développant un phénotype anxio/dépressif, suite à l'exposition prolongée à une faible dose de corticostérone exogène, présente un **déficit cognitif global**, incluant une altération de la mémoire de type épisodique, une dégradation des performances spatiales dont la capacité de flexibilité mentale et une diminution de la mémoire associative.

D'autre part, si l'efficacité d'un traitement chronique par la fluoxetine pour restaurer le phénotype anxio/dépressif n'est plus à démontrer, ses activités sur les performances cognitives n'ont à ce jour pas encore évaluées dans ce modèle animal d'anxiété/dépression.

A l'inverse, l'efficacité des agonistes du récepteur 5-HT_4 dans les troubles cognitifs n'est plus à prouver. Mais si de nombreuses études ont évalué l'activité pro-cognitive et de type antidépressive des agonistes du récepteur 5-HT_4 chez des animaux naïfs, aucune n'a à ce jour examiné leur activité dans un modèle d'anxiété/dépression.

ARTICLE 2: Rapid anxiolytic effects of a 5-HT_4 receptor agonist are mediated by a neurogenesis-independent mechanism

Indira Mendez-David, Denis J David, Flavie Darcet, Melody V Wu, Saadia Kerdine-Römer, Alain M Gardier, René Hen

Publié dans Neuropsychopharmacology, Nov 28. doi: 10.1038/npp.2013.332 (2014)

Question posée :

Une administration subchronique et chronique d'un agoniste du récepteur 5-HT_4, le RS67333, est-elle capable de restaurer le phénotype anxio/dépressif induit par l'exposition prolongée de corticostérone ?

Résumé de l'étude :

Les inhibiteurs sélectifs de recapture de sérotonine (ISRSs) sont les médicaments les plus prescrits dans le traitement de la dépression caractérisée. Malheureusement, l'apparition des effets thérapeutiques des ISRSs est souvent retardée de 3 à 6 semaines après le début du traitement. D'autre part, seulement un tiers des patients ne répondent pas au traitement initialement prescrit. Ces problématiques accentuent la nécessité d'identifier des antidépresseurs agissant plus rapidement et qui soient plus efficace (Samuels et al., 2011). Il a été proposé que les agonistes du récepteurs 5-HT_4 tel que le RS67333 pourraient être un élément intéressant dans le traitement de la dépression (Lucas, 2009; Lucas et al., 2005; Lucas and Debonnel, 2002; Lucas et al., 2010; Lucas et al., 2007). Alors qu'un grand nombre d'études ont évalué l'activité de type antidépressive des agonistes des récepteurs 5-HT_4 chez des souris naïves, aucune n'a à ce jour examiné leur activité dans un modèle d'anxiété/dépression. Dans cette étude, nous avons donc examiné si l'activation du récepteur 5-HT_4 par un agoniste, le RS67333, pouvait corriger le phénotype « anxio/dépressif » induit par le modèle CORT chez les souris. Enfin, en utilisant ce même modèle d'anxiété/dépression combiné à une ablation de la neurogenèse hippocampique par irradiation, nous avons déterminé si l'action de type anxiolytique et antidépressive du RS67333 après 7 et 28 jours de traitement recrutait un mécanisme dépendant de la neurogenèse hippocampique.

Contribution personnelle :

Au cours de ce travail :

- J'ai participé au suivi des animaux incluant la préparation et administration des différents traitements pharmacologiques (traitement par la CORT et par la fluoxetine, préparation et implantation des mini-pompes pour RS67333 et GR125487).

- J'ai participé à l'ensemble des tests comportementaux pour la cohorte des animaux traités par la corticostérone.

- J'ai participé aux analyses immunohistochimiques des étapes de la neurogenèse hippocampique depuis la perfusion, le prélèvement et la coupe des cerveaux jusqu'aux marquages des cellules.

- J'ai participé à la rédaction des données statistiques de l'article.

Neuropsychopharmacology (2014), 1–13

www.neuropsychopharmacology.org

Rapid Anxiolytic Effects of a 5-HT_4 Receptor Agonist Are Mediated by a Neurogenesis-Independent Mechanism

Indira Mendez-David[1,5], Denis J David[1,5], Flavie Darcet[1], Melody V Wu[2,3], Saadia Kerdine-Römer[4], Alain M Gardier[1] and René Hen*[,2,3]

[1]Univ Paris Sud, EA3544, Faculté de Pharmacie, Châtenay-Malabry, France; [2]Departments of Neuroscience and Psychiatry, Columbia University, New York, NY, USA; [3]Department of Integrative Neuroscience, New York State Psychiatric Institute, New York, NY, USA; [4]Univ Paris Sud, INSERM UMR 996, Faculté de Pharmacie, Châtenay-Malabry, France

Selective serotonin reuptake inhibitors (SSRIs) display a delayed onset of action of several weeks. Past work in naive rats showed that 5-HT_4 receptor agonists had rapid effects on depression-related behaviors and on hippocampal neurogenesis. We decided to investigate whether 5-HT_4 receptor stimulation was necessary for the effects of SSRIs in a mouse model of anxiety/depression, and whether hippocampal neurogenesis contributed to these effects. Using the mouse corticosterone model of anxiety/depression, we assessed whether chronic treatment with a 5-HT_4 receptor agonist (RS67333, 1.5 mg/kg/day) had effects on anxiety- and depression-related behaviors, as well as on hippocampal neurogenesis in comparison with chronic fluoxetine treatment (18 mg/kg/day). Then, using our anxiety/depression model combined with ablation of hippocampal neurogenesis, we investigated whether neurogenesis was necessary for the behavioral effects of subchronic (7 days) or chronic (28 days) RS67333 treatment. We also assessed whether a 5-HT_4 receptor antagonist (GR125487, 1 mg/kg/day) could prevent the behavioral and neurogenic effects of fluoxetine. Chronic treatment with RS67333, similar to fluoxetine, induced anxiolytic/antidepressant-like activity and stimulated adult hippocampal neurogenesis, specifically facilitating maturation of newborn neurons. However, unlike fluoxetine, anxiolytic effects of RS67333 were already present after 7 days and did not require hippocampal neurogenesis. Chronic treatment with GR125487 prevented both anxiolytic/antidepressant-like and neurogenic effects of fluoxetine, indicating that 5-HT_4 receptor activation is necessary for these effects of SSRIs. 5-HT_4 receptor stimulation could represent an innovative and rapid onset therapeutic approach to treat depression with comorbid anxiety.
Neuropsychopharmacology advance online publication, 22 January 2014; doi:10.1038/npp.2013.332

Keywords: 5-HT_4 receptor; anxiolytic; neurogenesis; fast onset

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed drugs for the treatment of depression and several anxiety disorders. Unfortunately, the onset of action of SSRIs is often delayed by 3–6 weeks (Artigas, 2013). The existence of this delayed action combined with the fact that one-third of patients do not respond to treatment emphasizes the need for faster acting and more effective antidepressants (Samuels *et al*, 2011).

It has been proposed that 5-HT_4 receptor agonists such as RS67333 may bring new hope for treating depression (Lucas, 2009; Lucas *et al*, 2005; Lucas and Debonnel, 2002; Lucas *et al*, 2010; Lucas *et al*, 2007). Indeed, administration of 5-HT_4 agonists induced similar molecular and behavioral changes as common antidepressants in rodents (Bockaert *et al*, 2008; Lucas *et al*, 2007; Pascual-Brazo *et al*, 2012). Depressed-like state in the olfactory bulbectomy or chronic mild stress model was completely abolished after 10–14 days of RS67333 treatment in rats, suggesting a more rapid response mechanism in comparison with classical antidepressants (Lucas *et al*, 2007). A positive behavioral response in the Novelty-Suppressed Feeding (NSF) test in rat, a complete reversion of anhedonic-like state (sucrose consumption), and an increase in swimming behavior in defeated mice in the forced swim test were also observed after a short period of RS67333 treatment (Gomez-Lazaro *et al*, 2012; Pascual-Brazo *et al*, 2012). In addition to behavioral data, and in agreement with a previous report from Lucas *et al* (2007), a recent study performed in naive rats confirmed that a short period of treatment with RS67333 increased the number of newborn cells in the dentate gyrus (DG) (Pascual-Brazo *et al*, 2012). These results are interesting because hippocampal neurogenesis has been implicated in some of the behavioral effects of antidepressants in adult rodents (David *et al*, 2009; Santarelli *et al*, 2003). However, no direct evidence has yet linked the antidepressant-like effects of 5-HT_4 receptor activation and its neurogenic effects. Finally, it has been

*Correspondence: Professor René Hen, Departments of Neuroscience and Psychiatry, Columbia University, New York, NY 10032, USA, Tel: +1 212 543 5328, Fax: +1 212 543 5074, E-mail: rh95@columbia.edu
[5]These authors contributed equally to this work.
Received 6 September 2013; revised 16 November 2013; accepted 20 November 2013; accepted article preview online 28 November 2013

suggested that SSRIs and 5-HT$_4$ receptor agonists share common mechanisms of action. Indeed, the 5-HT$_4$ receptor agonist, RS67333, augmented the acute effect of paroxetine on extracellular 5-HT levels in rat ventral hippocampus, and after only 3 days of administration it increased basal hippocampal 5-HT levels (Licht *et al*, 2010). The coadministration of the SSRI citalopram and RS67333 strongly potentiated the antidepressant-like properties of the latter in several electrophysiological, molecular, and behavioral paradigms (Lucas *et al*, 2010).

Although a number of studies have assessed the antidepressant-like activity of 5-HT$_4$ receptor agonists, none have so far evaluated their anxiolytic-like profile. It is noteworthy that some SSRIs are often prescribed for the treatment of anxiety disorders (Burghardt and Bauer, 2013). Anxiety disorders have a lifetime prevalence of over 25%, thus making them the most common psychiatric disorders (Kheirbek *et al*, 2012). Moreover, a comorbidity between depression and anxiety disorders is commonly observed. Thus, this study aimed to investigate both antidepressant and anxiolytic-like effects of either subchronic or chronic administration of a 5-HT$_4$ receptor agonist in a model of anxiety/depression based on the elevation of glucocorticoids in mice (CORT model) (David *et al*, 2009). Standard models of depression that rely on environmental stress manipulations such as learned helplessness or the chronic mild stress are hampered by protocol variability and reported difficulties in replication, thus highlighted the need for a reliable, easily replicable depression model (Nestler *et al*, 2002). The corticosterone model is a chronic exposure method optimized for use in modeling the persistent anxiety/depression-like state in rodents, allowing for multiple behavioral tests in the same animals using an etiologically relevant model of depression that is easily replicable between and within laboratories (David *et al*, 2009; David *et al*, 2010; Gould, 2011; Mendez-David *et al*, 2013).

We also assessed whether chronic 5-HT$_4$ receptor stimulation can affect proliferation of newborn cells and maturation of newborn neurons. Finally, using our mouse model of anxiety/depression combined with ablation of hippocampal neurogenesis by X-irradiation, we assessed whether the anxiolytic/antidepressant action of RS67333 after 7 and 28 days of treatment recruits a neurogenesis-dependent mechanism.

MATERIALS AND METHODS

Subjects

Adult male C57BL/6Ntac mice were purchased from Taconic Farms (Lille Skensved, Denmark and Germantown, NY, USA for the pharmacological and the X-irradiation studies, respectively). All mice were 7–8 weeks old, weighed 23–25 g at the beginning of the treatment, and were maintained on a 12 L:12 D schedule (lights on at 0600 hours). They were housed in groups of five. Food and water were provided *ad libitum*. All testing was conducted in compliance with the laboratory animal care guidelines and with protocols approved by the Institutional Animal Care and Use Committee (Council directive no. 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions no. 92-256B to DJD).

Drugs

Corticosterone [4-pregnen-11b-DIOL-3 20-DIONE 21-hemisuccinate (35 μg/ml)] purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) was dissolved in a vehicle (0.45% hydroxypropyl-β-cyclodextrin (β-CD); Sigma-Aldrich). Fluoxetine hydrochloride (160 μg/ml, equivalent to 18 mg/kg/day) was purchased from Anawa Trading, (Zurich, Switzerland) and dissolved in 0.45% β-CD/corticosterone solution. 1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-butyl-4piperidinyl)-1-propanone hydrochloride (RS67333), and 5-Fluoro-2-methoxy-[1-[2-[(methylsulfonyl) amino]ethyl]-4-piperidinyl]-1H-indole-3-methylcarboxylate sulfamate (GR125487) were purchased from Tocris Bioscience (Bristol, UK) and dissolved in 0.9% saline solution. RS67333 and GR125487 were chosen based on previous work (Cachard-Chastel *et al*, 2007; Lucas *et al*, 2007).

RS67333 shows high-binding affinity for the 5-HT$_4$ receptor with a pKi of 8.7 (Bockaert *et al*, 2004; Eglen *et al*, 1995). Except for the sigma receptors, which are bound at affinities comparable to 5-HT$_4$ (sigma 1: pKi = 8.9 and sigma 2: pKi = 8.0), RS67333 has a pKi of <6.7 for other neurotransmitter receptors including 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{2A}$, 5-HT$_{2C}$, dopamine D$_1$, D$_2$, and muscarinic M$_1$–M$_3$ receptors. However, little is known about the function of sigma receptors'.

GR125487 is the most selective 5-HT$_4$ receptor antagonist with a pKi = 10.6 (Schiavi *et al*, 1994), presenting a selectivity more than 1000-fold over other 5-HT receptor (Gale *et al*, 1994). The dose of RS67333 and GR125487 used in this study were chosen based on previous works (Cachard-Chastel *et al*, 2007; Lucas *et al*, 2007).

Corticosterone Model and Treatment

Our model of elevated glucocorticoids (also named CORT model) is able to blunt the response of the hypothalamic–pituitary–adrenal axis as shown by the markedly attenuated stress-induced corticosterone levels observed in these mice (David *et al*, 2009). This is probably a consequence of the negative feedback exerted by corticosterone on the hypothalamic–pituitary–adrenal axis. This model displays hallmark characteristics of anxiety and depression.

The dose and duration of corticosterone treatment was selected based on previous studies (David *et al*, 2009; Rainer *et al*, 2011). Corticosterone (35 μg/ml, equivalent to about 5 mg/kg/day) or vehicle (0.45% β-CD) was available *ad libitum* in the drinking water in opaque bottles to protect it from light. Corticosterone-treated water was changed every 3 days in order to prevent any possible degradation. Thereafter, while administration with β-CD or corticosterone continued, mice were treated with vehicle (0.45% β-CD), fluoxetine, RS67333, GR125487 alone, or GR125487 in the presence of fluoxetine (Supplementary Figure 1). Both RS67333 and GR125487 were delivered by osmotic minipumps at a dose of 1.5 mg/kg/day and 1 mg/kg/day, respectively (Lucas *et al*, 2005). Fluoxetine (18 mg/kg/day) was delivered in the drinking water as previously described (David *et al*, 2009). Osmotic minipumps (42 days minipumps,

2006 model, Alzet, Cupertino, CA) were implanted subcutaneously under light anesthesia (ketamine/xylazine; (75/20 mg/kg) from Sigma-Aldrich. Control animals (vehicle/vehicle or corticosterone/vehicle groups) were also implanted with a minipump containing 0.9% saline (2006 model, Alzet). Treatment was always maintained until the end of the experiments. Corticosterone and fluoxetine dosages were calculated assuming an average fluid intake of about 5 ml/day (David *et al*, 2009).

Behavioral Tests

The same cohort of animals was tested in five different behavioral models of anxiety and depression. Each animal, over a week, was successively tested in the Open Field (OF), Elevated Plus Maze (EPM), NSF, Splash Test (ST), and Tail Suspension Test (Supplementary Material). Behavioral testing occurred during the light phase between 0700 and 1900 hours. Behavioral paradigms occurred after 7 or 28 days of drug treatment depending on the study (Supplementary Figure S1A and S1B).

Immunohistochemistry

The effects of chronic RS67333 treatment on cell proliferation or maturation of newborn neurons was assessed in corticosterone-treated animals. After anesthesia with ketamine and xylazine (100 mg/ml ketamine and 20 mg/ml xylazine), mice were perfused transcardially (cold saline for 2 min, followed by 4% cold paraformaldehyde at 4 °C). The brains were then removed and cryoprotected in 30% sucrose and stored at 4 °C. Serial sections (35 μm) were cryosectioned through the entire hippocampus and stored in PBS with 0.1% NaN_3.

Proliferation of newborn cells. We first looked at proliferation of newborn cells using Ki-67 immunohistochemistry as described previously (Xia *et al*, 2012). Sections were washed in PBS, blocked (PBS containing 0.3% Triton X-100 and 10% normal donkey serum (NDS)), and incubated with primary antibody overnight at 4 °C (Ki67 rabbit, 1 : 100, Vector, Burlingame, CA). Following washes in PBS, sections were incubated with fluorescence-coupled rabbit secondary antibody (Jackson ImmunoResearch, Beckman, France). Stereological quantification of Ki-67 labeling was performed using an Olympus BX51 microscope (Germany).

Maturation of newborn neurons. For doublecortin (DCX) staining, the procedure consisted of the following steps: 1 h incubation in 0.1 M TBS with 0.5% Triton X-100 and 10% NDS, followed by goat anti-DCX primary antibody (1 : 100) in TBS/Tx/NDS for 24 h at 4 °C. Biotinylated secondary donkey anti-goat antibody (1 : 500) in TBS/NDS for 1 h at room temperature was used, followed by a 1 h amplification step using an avidin–biotin complex (Vector). The immunohistochemistry protocol was adapted from David *et al* (2009). DCX-positive (DCX^+) cells were subcategorized according to their dendritic morphology: DCX^+ cells without and DCX^+ cells with tertiary (or higher order) dendrites. The maturation index was defined as the ratio of DCX^+ cells possessing tertiary dendrites to the total number of DCX^+ cells.

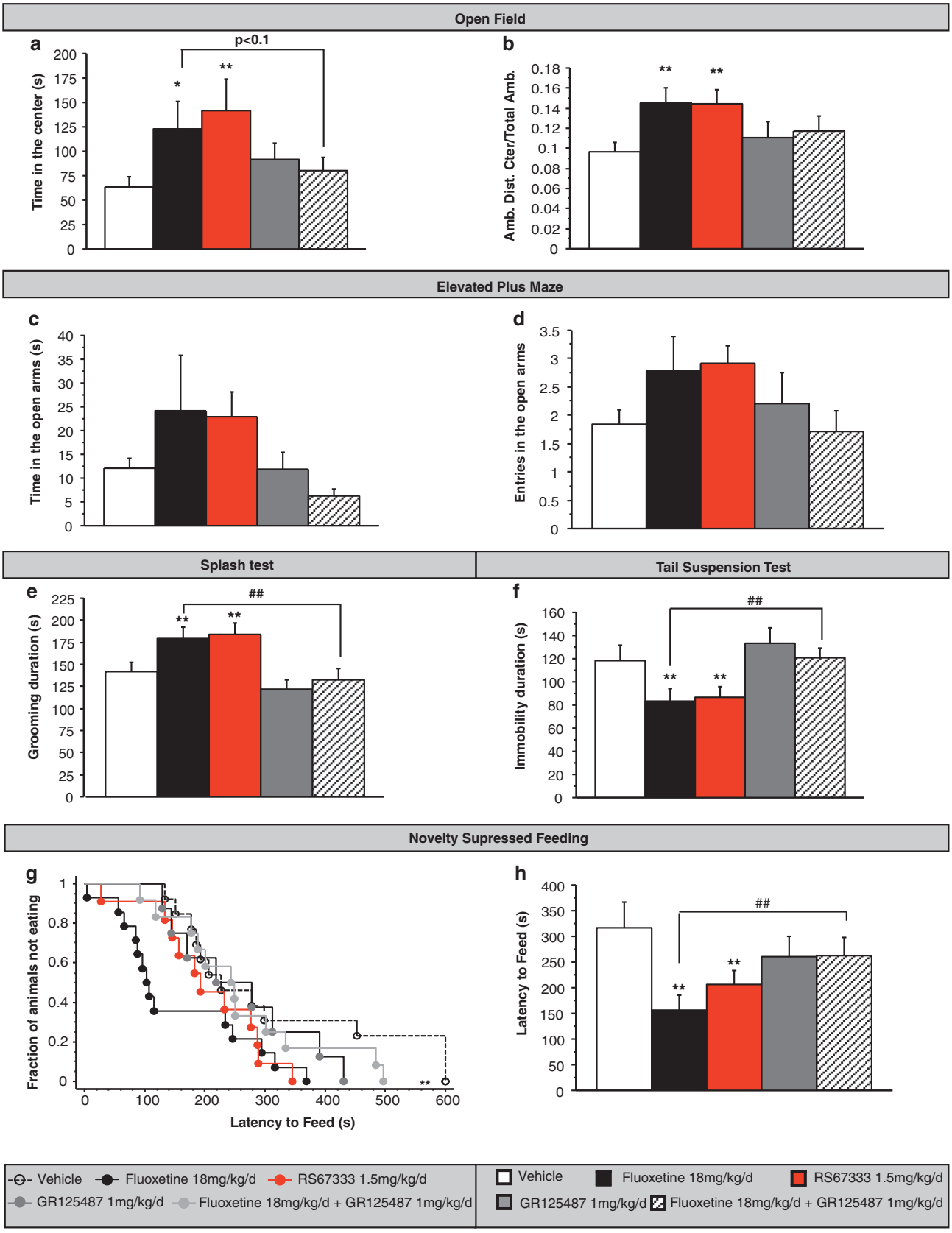
Sholl analysis. Sholl analysis was performed as described elsewhere (Guilloux *et al*, 2013). DCX^+ cells with tertiary or higher order dendrites were traced using Neurolucida software (MicroBrightField, Williston, VT) on an Olympus BX51 microscope equipped with a motorized stage device and ×100 immersion oil objective. Sholl analysis for dendritic complexity was performed using the accompanying software (NeuroExplorer; MicroBrightField, version 10) to determine dendritic length and number of intersections (branch points). One DCX^+ cell was traced for each 35-μm hippocampal slice; $n = 6$ cells/brain for DAB-stained sections).

X-irradiation

A separate batch of mice were anesthetized with ketamine and xylazine (75/20 mg/kg), placed in a stereotaxic frame, and exposed to cranial irradiation using a PXI X-RAD 320 X-ray system operated at 300 kV and 12 mA with a 2-mm Al filter. Animals were protected with a lead shield that covered the entire body, but left unshielded a 3.22 × 11-mm treatment field above the hippocampus (interaural 3.00 mm to 0.00 mm) exposed to X-ray, thus effectively preventing irradiation from targeting the rest of the brain (Santarelli *et al*, 2003). The corrected dose rate was ~0.95 Gy/min at a source to skin distance of 36 cm. The procedure lasted for 2 min and 39 s, delivering a total of 2.5 Gy. Three 2.5 Gy doses were delivered on days 1, 4, and 7 as previously described (Quesseveur *et al*, 2013). This 7.5 Gy cumulative dose was determined from prior pilot experiments to be the minimum dosage necessary to result in permanent ablation of adult-born neurons in the DG as assessed by expression of the immature neuronal marker DCX. The reason for using a fractionated paradigm rather than a single high dose of 7.5 Gy is that the ablation is not permanent after a single high dose. Histological staining for CD68 as a marker of inflammation throughout the brain revealed that irradiated mice were indistinguishable from sham animals 8 weeks post irradiation, indicating minimal nonspecific side effects of irradiation at time of behavioral testing (Meshi *et al*, 2006). Immunohistochemistry confirmed the ablation of adult hippocampal neurogenesis (Supplementary Figure 4).

Data Analysis and Statistics

Results from data analyses were expressed as mean ± SEM. Data were analyzed using StatView 5.0 software (SAS Institute, Cary, NC). For all experiments, one-way or two-way ANOVAs with repeated measures were applied to the data as appropriate. Significant main effects and/or interactions were followed by Fisher's PLSD *post hoc* analysis, unpaired *t*-tests. In the NSF test, we used the Kaplan–Meier survival analysis owing to the lack of normal distribution of the data. Mantel–Cox log rank test was used to evaluate differences between experimental groups. Statistical significance was set at $p < 0.05$. A summary of statistical measures is included in Supplementary Tables S1–S6, available online.


Open Field
a
p<0.1
Time in the center (s)
b
Amb. Dist. Cter/Total Amb.
Elevated Plus Maze
c
Time in the open arms (s)
d
Entries in the open arms
Splash test
e
Grooming duration (s)
Tail Suspension Test
f
Immobility duration (s)
Novelty Supressed Feeding
g
Fraction of animals not eating
Latency to Feed (s)
h
Latency to Feed (s)
Vehicle
Fluoxetine 18mg/kg/d
RS67333 1.5mg/kg/d
GR125487 1mg/kg/d
Fluoxetine 18mg/kg/d + GR125487 1mg/kg/d

RESULTS

5-HT$_4$ Receptor Stimulation Produces Anxiolytic-Like and Antidepressant-Like Effects in a Model of Anxiety/Depression

To induce an anxious/depressed-like state in C57BL/6Ntac mice, we administered a low dose of corticosterone (35 μg/ml) for 4 weeks as described in David *et al* (2009) ('CORT model'). After chronic corticosterone, we tested the effects of a 4-week treatment with the 5-HT$_4$ agonist RS67333 (1.5 mg/kg/day) in comparison with fluoxetine (18 mg/kg/day). To assess the selectivity of these effects, we also tested whether the 5-HT$_4$ antagonist GR125487 (1 mg/kg/day), alone or in combination with fluoxetine, affected the behavioral phenotype (see experimental design, Supplementary Figure S1). In the OF, the anxiety-like phenotype induced by chronic corticosterone was reversed by chronic fluoxetine and by the 5-HT$_4$ agonist RS67333 (one-way ANOVA, $^{*}p<0.05$, Figure 1a). Indeed, chronic fluoxetine and RS67333 treatment increased time spent in the center (Figure 1a). A trend for an increase in the number of entries in the center was also observed with both compounds (Supplementary Figures S2A and B). It is unlikely that this effect was the consequence of a change in locomotor activity, as the total ambulatory distance was not affected and the ratio of ambulatory distance in the center divided by total distance was increased for both treatments (one-way ANOVA, $^{*}p<0.05$, Figure 1b). Interestingly, while the 5-HT$_4$ antagonist GR125487 by itself did not affect any anxiety parameters, it prevented fluoxetine-induced anxiolytic-like effects. Indeed, the fluoxetine-induced increase in time spent in the center was prevented by chronic GR125487 administration. These data indicate that 5-HT$_4$ stimulation induces an anxiolytic-like effect and is necessary for the anxiolytic effect of chronic fluoxetine treatment.

To further validate these results, we next tested the effects of RS67333 and fluoxetine alone or in the presence of GR125487 in the same animals in another anxiety-related test, the EPM. We found that chronic RS67333 and fluoxetine induced a trend for an increase in time spent in and number of entries into the open arms, (Figure 1c and d). This anxiolytic-like effect of fluoxetine was completely abolished by treatment with the 5-HT$_4$ antagonist, GR125487.

We next assessed whether chronic treatment with the 5-HT$_4$ agonist RS67333 could also produce antidepressant-like effects. Thus, the same mice were tested in the ST and the tail suspension test. We observed that after squirting a 10% sucrose solution on the mouse's snout, increased grooming duration was observed in both the fluoxetine and the RS67333 groups (one-way ANOVA, $^{**}p<0.01$, Figure 1e). Chronic treatment with the 5-HT$_4$ antagonist GR125487 prevented the antidepressant-like activity of chronic fluoxetine. Similarly, in the tail suspension test, both fluoxetine and RS67333 had antidepressant-like effects and these effects of fluoxetine were blocked by GR125487 (one-way ANOVA, $^{**}p<0.01$; Figure 1f).

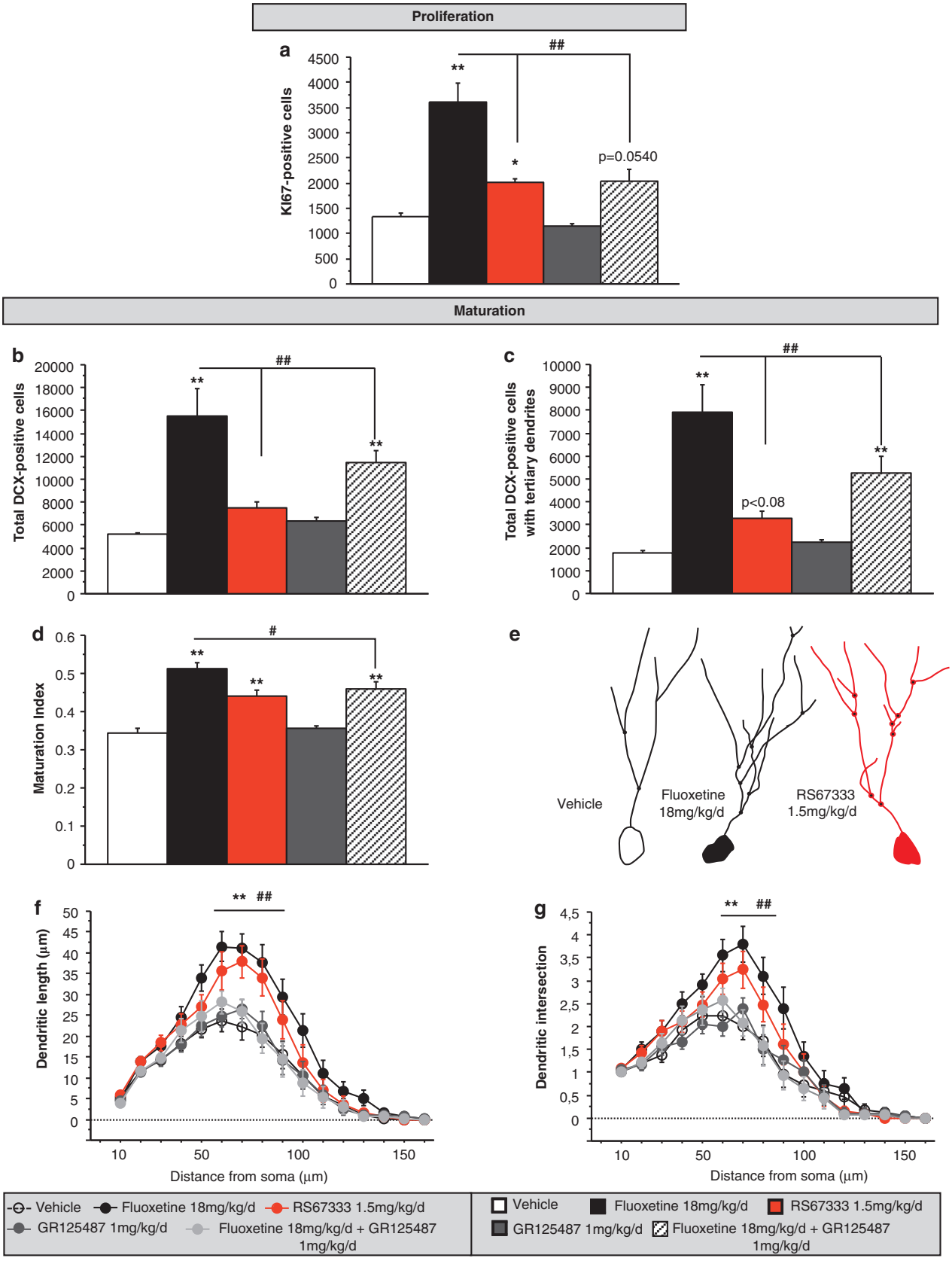
Finally, we tested these mice in the NSF test that is sensitive to both acute anxiolytics and chronic antidepressants (Guilloux *et al*, 2013) (Figure 1g and h). Similar to chronic fluoxetine, chronic RS67333 decreased the latency to feed (Kaplan–Meier survival analysis, Mantel–Cox log rank test, $^{**}p<0.01$) without affecting the home-cage food consumption (Supplementary Figure S2C). Chronic treatment with the 5-HT$_4$ antagonist (GR125487) prevented the effect of fluoxetine. Altogether, these data indicate that 5-HT$_4$ receptor activation produces both anxiolytic-like and antidepressant-like effects comparable to those of fluoxetine in the chronic corticosterone model of anxiety/depression. Furthermore, we show that 5-HT$_4$ activation is necessary for the anxiolytic and antidepressant effects of fluoxetine in this model.

5-HT$_4$ Receptor Activation Facilitates the Maturation of Newborn Neurons in the Adult Hippocampus

To investigate the potential cellular mechanisms underlying the behavioral effects of the 5-HT$_4$ agonist RS67333, we next evaluated changes in adult hippocampal neurogenesis that may be relevant to antidepressant action (Surget *et al*, 2011).

In agreement with previous observations (David *et al*, 2009; Rainer *et al*, 2011), chronic fluoxetine exposure resulted in an increase in the number of dividing neural precursors as assessed by the number of Ki67-positive cells in the subgranular zone of the DG (one-way ANOVA, $^{*}p<0.05$, Figure 2a). The 5-HT$_4$ agonist, RS67333, also increased the number of neural precursors, but to a lesser extent than fluoxetine ($+51\%$ *vs* $+170\%$). The 5-HT$_4$ antagonist partially blocked the effect of chronic fluoxetine. These results suggest that 5-HT$_4$ receptors contribute to the effects of fluoxetine on proliferation, but that other 5-HT receptors are likely to be also involved.

Figure 1 Effects of chronic 5-HT$_4$ receptor stimulation (28 days) on the anxious/depressed-like phenotype induced by chronic corticosterone exposure. (a and b) Effects of chronic treatment with 5-HT$_4$ ligands or fluoxetine, starting after 4 weeks of corticosterone (35 μg/ml), on anxiety behaviors in the open field (OF) test. Anxiety is measured as mean time spent in the center in seconds (a) or the ratio of ambulatory distance in the center/total ambulatory distance (b). Values plotted are mean ± SEM ($n=10$–15/group). $^{*}p<0.05$, $^{**}p<0.01$ *vs* corticosterone/vehicle group. (c and d) Effects of chronic treatment with 5-HT$_4$ ligands or fluoxetine, starting after 4 weeks of corticosterone (35 μg/ml), on anxiety behaviors in the elevated plus maze (EPM). Anxiety is expressed as mean time in (c) or entries into (d) the open arms. Values plotted are mean ± SEM ($n=10$–15/group). (e) Effect of chronic treatment with 5-HT$_4$ ligands or fluoxetine on corticosterone-induced depression-related behaviors in the splash test (ST). Results are expressed as mean duration of grooming after receiving a 10% sucrose solution on the snout. Values plotted are mean ± SEM ($n=10$–15/group). $^{**}p<0.01$, $^{\#\#}p<0.01$ *vs* corticosterone/vehicle group and corticosterone/fluoxetine group, respectively. (f) Effect of chronic treatment with 5-HT$_4$ ligands or fluoxetine in the tail suspension test following chronic corticosterone. Results are expressed as mean of immobility duration in seconds. Values plotted are mean ± SEM ($n=10$–15/group). $^{**}p<0.01$, $^{\#\#}p<0.01$ *vs* corticosterone/vehicle group and corticosterone/fluoxetine group, respectively. (g and h) Effects of chronic treatment with 5-HT$_4$ ligands or fluoxetine on anxiety- and depression-like behaviors in the novelty-suppressed feeding (NSF) paradigm after chronic corticosterone. Values plotted are cumulative survival of animals that have not eaten over 10 min ($n=10$–15/group) (g) or mean of latency to feed in seconds ± SEM (h). $^{**}p<0.01$, $^{\#\#}p<0.01$ *vs* corticosterone/vehicle group and corticosterone/fluoxetine group, respectively.


Proliferation
a
Ki67-positive cells
4500
4000
3500
3000
2500
2000
1500
1000
500
0
**
*
##
p=0.0540
Maturation
b
Total DCX-positive cells
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
**
**
##
c
Total DCX-positive cells with tertiary dendrites
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
**
**
##
p<0.08
d
Maturation Index
0.6
0.5
0.4
0.3
0.2
0.1
0
**
**
**
#
e
Vehicle
Fluoxetine 18mg/kg/d
RS67333 1.5mg/kg/d
f
Dendritic length (µm)
50
45
40
35
30
25
20
15
10
5
0
** ##
10
50
100
150
Distance from soma (µm)
g
Dendritic intersection
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
** ##
10
50
100
150
Distance from soma (µm)
Vehicle
Fluoxetine 18mg/kg/d
RS67333 1.5mg/kg/d
GR125487 1mg/kg/d
Fluoxetine 18mg/kg/d + GR125487 1mg/kg/d
Vehicle
Fluoxetine 18mg/kg/d
RS67333 1.5mg/kg/d
GR125487 1mg/kg/d
Fluoxetine 18mg/kg/d + GR125487 1mg/kg/d

We next assessed the number of young adult-born neurons in the DG that express DCX, a protein that is expressed for about a month after the birth of adult-born neurons (Couillard-Despres *et al*, 2005) (Supplementary Figure S3). We also subcategorized the DCX$^+$ cells according to their dendritic morphology: total number of DCX$^+$ cells and DCX$^+$ cells with complex, tertiary dendrites (Figure 2b–g). As previously described, chronic fluoxetine increased the number of DCX$^+$ cells with tertiary dendrites and the maturation index, defined as the ratio of DCX$^+$ cells possessing tertiary dendrites over the total DCX$^+$ cells in control animals (David *et al*, 2009) (one-way ANOVA, $^{**}p<0.01$, Figure 2b–d). Chronic treatment with RS67333 affected modestly both the total number of DCX$^+$ cells and also the number of DCX cells with tertiary dendrites.

The 5-HT$_4$ antagonist, GR125487, partially blocked the neurogenic effects of fluoxetine. However, while the effects of chronic fluoxetine on the number of DCX$^+$ cells with tertiary dendrites are larger than those of chronic 5-HT$_4$ receptor activation, the effect of these compounds on the maturation index is similar ($+51\%$ and 44% for fluoxetine and RS67333, respectively).

The dendrites of adult-born granule cells become progressively more complex during the first 4 weeks after their birth, a stage when the cells express DCX (Couillard-Despres *et al*, 2005). To further examine the effect of 5-HT$_4$ receptor activation on the dendritic morphology of newborn cells, we performed Sholl analyses on DCX$^+$ cells with tertiary dendrites. DCX$^+$ cells in chronic fluoxetine-treated and RS67333-treated animals displayed an increase in dendritic length (one-way ANOVA, $^{**}p<0.01$; Figure 2e and f) and in number of dendritic intersections (one-way ANOVA, $^{**}p<0.01$; Figure 2e and g). Fluoxetine-induced increase in dendritic complexity was abolished by a chronic treatment with the 5-HT$_4$ antagonist, GR125487.

Overall, these results suggest that 5-HT$_4$ receptor activation facilitates the maturation of newborn neurons in the adult hippocampus.

An Assessment of Causality Between the Neurogenic and Behavioral Effects of Short- and Long-Term Treatments with the 5-HT$_4$ Agonist in the Chronic CORT Model

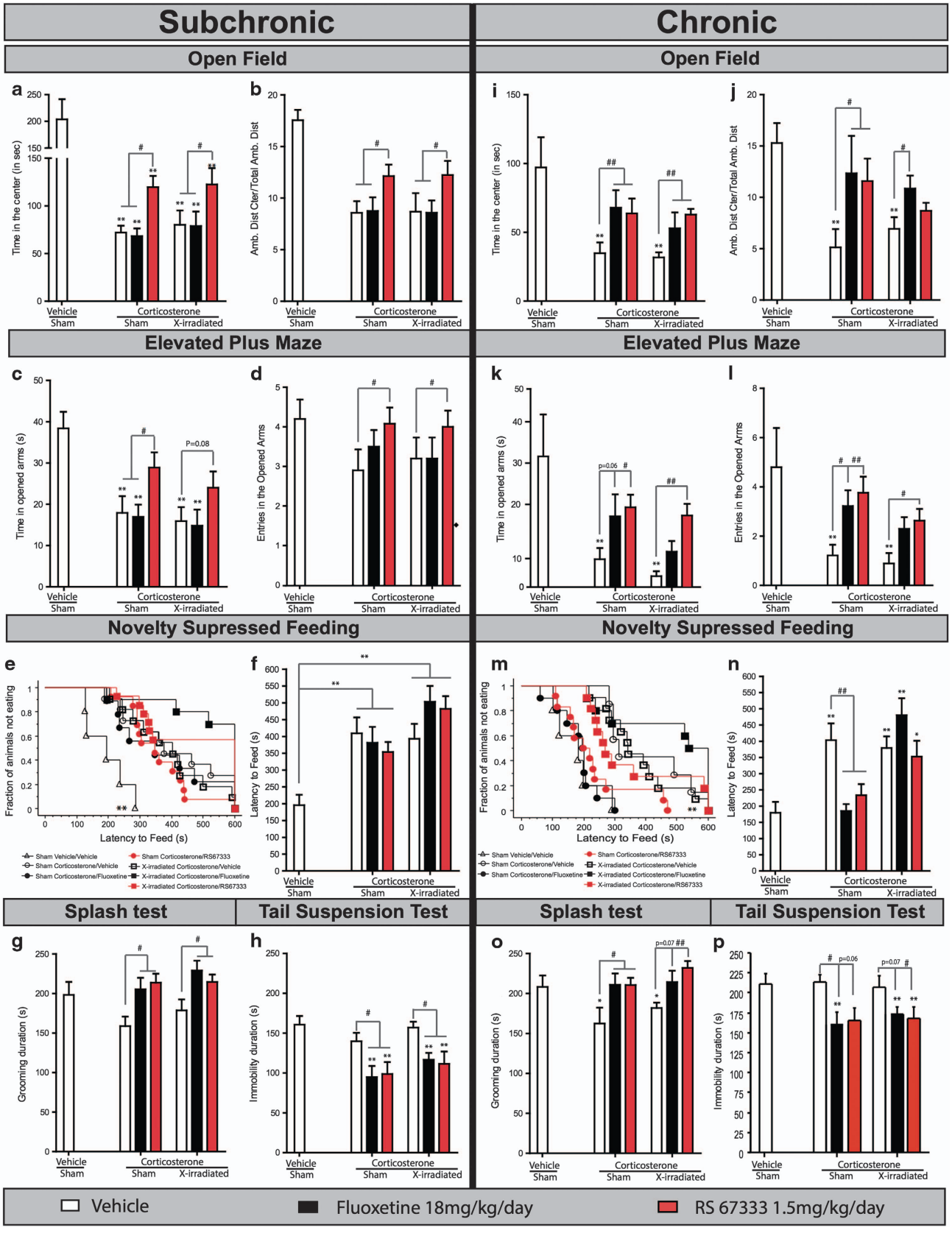
As we have shown that long-term 5-HT$_4$ activation induced anxiolytic/antidepressant-like effects and facilitated maturation of newborn neurons, we decided to test the requirement of hippocampal neurogenesis for the emergence of behavioral changes after 5-HT$_4$ receptor activation in our CORT model. Moreover, a recent study in rats reported that the behavioral and neurogenic (proliferation of newborn cells) effects of the 5-HT$_4$ receptor agonist RS67333 occur after short-term administration (3–7 days depending on the paradigm) (Pascual-Brazo *et al*, 2012). Thus, we also investigated whether subchronic RS67333 treatment induced a rapid onset of behavioral effects. To address these points, mice were submitted to focal hippocampal X-irradiation before the start of chronic corticosterone treatment alone or in combination with the 5-HT$_4$ agonist RS67333 (1.5 mg/kg/day) or fluoxetine (18 mg/kg/day) (Supplementary Figure S1B). These animals were subjected to anxiety- and depression-related tests first after 7 days of treatment and again after 28 days of treatment.

As previously described (David *et al*, 2009; Rainer *et al*, 2011), the chronic CORT paradigm resulted in an anxious/depressed-like phenotype. The efficacy of corticosterone model was assessed by comparing the behavioral phenotype of controls to corticosterone-treated mice (Figure 3). In anxiety-related tests, chronic corticosterone treatment had a marked effect on all anxiety parameters, resulting in decreased time spent in center and ratio of distance in center divided by total distance in the OF (one-way ANOVA, $^{*}p<0.05$ or $^{**}p<0.01$; Figure 3a, i and j), and in decreased time and entries in the open arms in the EPM (one-way ANOVA, $^{**}p<0.01$, Figure 3c, k and l). In the ST, which is a depression-related test, chronic CORT resulted in a decrease in grooming (one-way ANOVA, $^{**}p<0.01$; Figure 3o), and in the NSF test, which is related to both anxiety and depression, chronic CORT increased the latency to feed (Kaplan–Meier survival analysis, Mantel–Cox log rank test, $^{**}p<0.01$; Figure 3e, f, m and n). As previously observed in a similar paradigm, the forced swim test (David *et al*, 2009; Rainer *et al*, 2011), chronic corticosterone treatment did not affect the immobility duration in the TST (Figure 3h and p), suggesting distinct underlying mechanisms between these tests and the OF, EPM, NSF, or ST.

The Rapid Anxiolytic and Antidepressant-Like Effects of a Subchronic 5-HT$_4$ Agonist Treatment do not Require Hippocampal Neurogenesis

A 7-day treatment with RS67333 produced anxiolytic and antidepressant-like effects in a battery of behavioral tests (Figure 3a–h). In the OF and EPM paradigms, all anxiety-related parameters were impacted. The time spent in the center (Figure 3a), the number of entries in the center (Supplementary Figure S5A), the ratio of center distance/

Figure 2 Effects of chronic 5-HT$_4$ receptor stimulation (28 days) on proliferation and dendritic maturation of young neurons in the DG of the adult hippocampus. (a) Effect of chronic treatment with 5-HT4 ligands or fluoxetine, starting after 4 weeks of corticosterone (35 μg/ml), on cell proliferation. Cell proliferation is measured as mean number of Ki-67-positive cells (a). Values plotted are mean ± SEM ($n=3$–5/group). $^{*}p<0.05$, $^{**}p<0.01$, $^{\#\#}p<0.01$ *vs* corticosterone/vehicle group and corticosterone/fluoxetine group, respectively. (b) Effect of chronic treatment with 5-HT$_4$ ligands or fluoxetine on total number of doublecortin-positive cells (DCX$^+$; mean ± SEM; $n=4$–5 mice/group) was measured after chronic corticosterone. $^{**}p<0.01$, $^{\#\#}p<0.01$ *vs* corticosterone/vehicle group, and corticosterone/fluoxetine group, respectively. (c and d) DCX$^+$ cells were categorized as to whether they exhibited tertiary dendrites. Effects of fluoxetine treatment on the DCX$^+$ cells with tertiary dendrites (c) and maturation (d) of newborn granule cells were measured after chronic corticosterone. Values are mean ± SEM ($n=4$–5/group). $^{**}p<0.01$, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs* corticosterone/vehicle group and corticosterone/fluoxetine group, respectively. (e) Representative image and traces from Sholl analyses of DCX$^+$ cells with tertiary branches after vehicle, chronic fluoxetine, chronic RS67333, and GR125487 in presence or not of fluoxetine in corticosterone-treated animals ($n=3$–4 mice/group, six cells/mouse). (f and g) Effects of chronic treatment with the 5-HT$_4$ ligands RS67333 or fluoxetine on the dendritic length (f) or the number of intersections (g) following a Sholl analysis. Values are mean ± SEM ($n=4$–5 mice/group). $^{**}p<0.01$, $^{\#\#}p<0.01$ vs corticosterone/vehicle group and corticosterone/fluoxetine/GR125487 group, respectively.


Subchronic
Chronic
Open Field
Open Field
Elevated Plus Maze
Elevated Plus Maze
Novelty Supressed Feeding
Novelty Supressed Feeding
Splash test
Tail Suspension Test
Splash test
Tail Suspension Test
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
Time in the center (in sec)
Amb. Dist Cter/Total Amb. Dist
Time in opened arms (s)
Entries in the Opened Arms
Fraction of animals not eating
Latency to Feed (s)
Grooming duration (s)
Immobility duration (s)
Vehicle
Sham
Corticosterone
Sham
X-irradiated
P=0.08
p=0.06
p=0.07
Sham Vehicle/Vehicle
Sham Corticosterone/Vehicle
Sham Corticosterone/Fluoxetine
Sham Corticosterone/RS67333
X-irradiated Corticosterone/Vehicle
X-irradiated Corticosterone/Fluoxetine
X-irradiated Corticosterone/RS67333
Vehicle
Fluoxetine 18mg/kg/day
RS 67333 1.5mg/kg/day

total distance traveled (Figure 3b), the time spent in open arms (Figure 3c), and the number of entries in the open arms (Figure 3d) were increased after subchronic treatment with RS67333, regardless of whether the mice were exposed to X-irradiation or not (two-way ANOVA with significant treatment factor, $^{**}p<0.01$; Figure 3a–c). In contrast, subchronic treatment with fluoxetine did not have an anxiolytic effect in the OF and EPM paradigms (Figure 3a–d). These results indicate that the anxiolytic effects of RS67333 have a faster onset than those of fluoxetine, and that these effects do not require adult hippocampal neurogenesis.

Interestingly, in the NSF test, neither subchronic RS67333 nor subchronic fluoxetine had an effect on latency to feed in both sham and X-irradiated groups (Figure 3e and f), indicating that in this test, the anxiolytic/antidepressant activity of RS67333 and fluoxetine require a longer treatment.

In the ST and TST (Figure 3g and h), after 11 and 12 days of administration, both RS67333 and fluoxetine increased grooming duration and decreased immobility duration, respectively (two-way ANOVA with significant treatment factor, $^{**}p<0.01$). These antidepressant-like effects were not affected by focal hippocampal X-irradiation.

Altogether, these results demonstrate that, unlike fluoxetine, the 5-HT$_4$ agonist RS67333 elicits a rapid anxiolytic and antidepressant-like effect in all the paradigms tested (OF, EPM, ST, and TST) except the NSF. However, hippocampal neurogenesis is not required for these effects of RS67333.

In this study, we assessed the behavioral activity of RS67333 after both subchronic and chronic treatments. Thus, the same animals were tested after 7 and 28 days of treatment. It is therefore not surprising to observe changes in the basal values in control animals, as they have been exposed twice to behavioral tests. We have seen these effects of repeated testing routinely. For example, in the NSF paradigm, the latency to feed was decreased after a second exposure to the test (Wang *et al*, 2008). In the present study, we observed a decrease in the time spent in the center of the arena owing to the re-exposure to the test in all treated groups. However, the size of the anxiolytic-like effect of RS67333 remains the same between the first and the second exposure to the OF.

The Behavioral Effects of Long-Term 5-HT$_4$ Agonist Treatment are Mediated by Both Neurogenesis-Dependent and -Independent Mechanisms

As we previously demonstrated that chronic 5-HT$_4$ activation produced anxiolytic/antidepressant-like activity in the CORT model, we proceeded to investigate whether these behavioral effects require adult hippocampal neurogenesis.

The same battery of behavioral tests was performed again after 28 days of treatment with fluoxetine or RS67333 (Figure 3i–p). In the OF (Figure 3i and j) and the EPM (Figure 3k and l) paradigms, chronic RS67333 maintained the anxiolytic-like effect observed subchronically (two-way ANOVA with significant treatment factor, $^{*}p<0.05$; Figure 3i–l). Chronic fluoxetine also elicited an anxiolytic-like effect, whereas it had no effect after subchronic treatment (two-way ANOVA with significant treatment factor, $^{*}p<0.05$; Figure 3i and j). Moreover, these anxiolytic-like effects of RS67333 and fluoxetine were not affected by the ablation of adult hippocampal neurogenesis by X-irradiation.

In contrast, the anxiolytic/antidepressant-like effects of RS67333 and fluoxetine in the NSF paradigm were completely abolished by hippocampal X-irradiation (Figure 3m and n; Kaplan–Meier survival analysis, Mantel–Cox log rank test, $^{**}p<0.01$, two-way ANOVA with significant interaction between irradiation and treatment, $^{**}p<0.01$), indicating that these effects require adult hippocampal neurogenesis. Home-cage food consumption was not affected by drug treatment or irradiation (Supplementary Figure S5D).

In the ST and TST, long-term administration of RS67333 and fluoxetine induced an increase in grooming duration and a decrease in immobility duration that were not affected by focal X-irradiation (two-way ANOVA with significant treatment factor, $^{**}p<0.01$ for both tests; Figure 3o and p).

Altogether, these results indicate that the effects of chronic treatment with RS67333 and fluoxetine in the

Figure 3 Neurogenesis-dependent and -independent effects of subchronic (7 days) or chronic (28 days) 5-HT$_4$ agonist treatment on corticosterone-induced behavioral changes in mice. (a and b/i and j) Effects of subchronic (a and b) or chronic (i and j) treatment with RS67333, a 5-HT$_4$ agonist, after focal X-irradiation of the mouse hippocampus on corticosterone-induced anxiety-like behaviors in the open field (OF) test. Anxiety is expressed as mean time spent in the center, in seconds, for the entire session (a or i), and also as the mean of percentage ambulatory distance in the center over total ambulatory distance traveled (b or j). Values are mean ± SEM ($n=9$–15 mice/group for corticosterone-treated animals and $n=5$ for vehicle/vehicle). $^{**}p<0.01$, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs* control vehicle/vehicle group and corticosterone/RS67333 or corticosterone/vehicle group, respectively. (c and d/k and l) Effects of subchronic (c and d) or chronic (k and l) treatment with RS67333, a 5-HT4 agonist, after focal X-irradiation of the mouse hippocampus on corticosterone-induced anxiety-like behaviors in the elevated plus maze (EPM) paradigm. Anxiety is expressed as mean time in the open arms (c or k) and also as the mean entries in the open arms (d or l). Values are mean ± SEM ($n=9$–15 mice/group for corticosterone-treated animals and $n=5$ for vehicle/vehicle). $^{**}p<0.01$, $^{\#}p<0.05$ *vs* control vehicle/vehicle group and corticosterone/RS67333 or corticosterone/vehicle group, respectively. (e and f/m and n) Effects of subchronic (e and f) or chronic (m and n) treatment with RS67333, a 5-HT$_4$ agonist, after focal X-irradiation on corticosterone-induced anxiety- and depression-related behaviors in the novelty-suppressed feeding (NSF) paradigm. Results are cumulative survival of animals that have not eaten over 10 min (e or m) or mean ± SEM of latency to feed in seconds (f or n) ($n=9$–15 mice/group for corticosterone-treated animals and $n=5$ for vehicle/vehicle). $^{*}p<0.05$, $^{**}p<0.01$, $^{\#\#}p<0.01$ *vs* control vehicle/vehicle group and corticosterone/RS67333 or corticosterone/vehicle group, respectively. (g–o) Effects of subchronic (g) or chronic (o) treatment with RS67333, a 5-HT$_4$ agonist, after X-irradiation on behavior in the splash test (ST). Results are expressed as mean ± SEM duration of grooming after receiving a 10% sucrose solution on the snout ($n=9$–15/group for corticosterone-treated animals and $n=5$ for vehicle/vehicle). $^{*}p<0.05$, $^{\#}p<0.05$, $^{\#\#}p<0.01$, *vs* control vehicle/vehicle group and corticosterone/vehicle group, respectively. (h–p) Effects of subchronic (h) or chronic (p) treatment with RS67333, a 5-HT$_4$ agonist, after X-irradiation on behavior in the tail suspension test. Results are expressed as mean ± SEM immobility duration (in seconds) ($n=9$–15 mice/group for corticosterone-treated animals and $n=5$ for vehicle/vehicle). $^{**}p<0.01$, $^{\#}p<0.05$ *vs* control vehicle/vehicle group and corticosterone/vehicle group, respectively.

OF, EPM, ST, and TST are independent of hippocampal neurogenesis. In contrast, the anxiolytic/antidepressant-like effects of these compounds in the NSF test require neurogenesis.

DISCUSSION

Fast Anxiolytic Action of a 5-HT$_4$ Agonist

Most current antidepressant treatments are limited by a significant degree of nonresponsiveness among patients (Trivedi *et al*, 2006), delayed onset of therapeutic efficacy, and a number of side effects (Kato and Serretti, 2010). The development of new antidepressants is therefore of considerable importance (Wong *et al*, 2010), and understanding the mechanisms underlying the delayed onset should offer insights into new approaches. Recent studies as well as our present results indicate that 5-HT4 receptor agonists are faster acting than SSRIs (Lucas *et al*, 2007; Pascual-Brazo *et al*, 2012; Tamburella *et al*, 2009).

Although a 7-day treatment with fluoxetine or RS67333 induced antidepressant-like activity in the TST and ST, only the 5-HT$_4$ agonist RS67333 resulted in anxiolytic-like activity in the OF and the EPM. A longer duration of treatment (28 days) is required for fluoxetine to exert anxiolytic-like effects comparable to 7 days of RS67333 treatment. Although recent evidence indicates that 5-HT$_4$ receptors may represent a new target for antidepressant drugs (Bockaert *et al*, 2004; Lucas *et al*, 2007; Pascual-Brazo *et al*, 2012; Tamburella *et al*, 2009), the role of 5-HT$_4$ receptor ligands in anxiety is unclear. Discrepancies in results observed with 5-HT$_4$ receptor antagonists have been observed. In one study, the 5-HT$_4$ receptor antagonists, SDZ205-557, GR113808, and SB204070, administered acutely, failed to induce anxiolytic-like behavior in the light/dark choice test in mice (Costall and Naylor, 1997), whereas in two other studies, acute administration of the 5-HT$_4$ receptor antagonists, SB204070, GR113808 (Silvestre *et al*, 1996), and SB207266A (Kennett *et al*, 1997; Silvestre *et al*, 1996) had an anxiolytic-like effect in rats in the EPM. 5-HT$_4$ receptor knockout (KO) mice do not display an anxious or depressed-like phenotype, although an attenuated response to novelty may be relevant to mood disorders (Compan *et al*, 2004). In our hands, chronic treatment with GR125487 did not affect the anxiety-like phenotype induced by chronic corticosterone treatment. 5-HT$_4$ receptor agonists have mainly been tested in behavioral tests of antidepressant-like activity (see (Lucas, 2009) for review). Only one study investigated the effects of RS67333 in the OF paradigm over a 5-min period (Lucas *et al*, 2007). The authors showed that hyperlocomotion induced by olfactory bulbectomy was totally abolished after 14 days of RS67333 treatment in rats. To our knowledge, the present study is the first to clearly demonstrate fast anxiolytic-like activity of a 5-HT$_4$ receptor agonist in a mouse model of anxiety/depression. There is considerable evidence that RS67333 is a specific agonist of the 5-HT$_4$ receptor. Indeed, three studies have evaluated the effects of RS67333 in the presence of the selective 5-HT4 antagonist GR125487, and shown that the effects of RS67333 are blocked by GR125487. Lucas *et al* (2005) showed that the increase in 5-HT firing induced by RS67333 (1.5 mg/kg, acutely, during 3 or 21 days) is prevented by GR125487 administration. This effect of RS67333 on the firing of 5-HT neurons is likely to contribute to the phenotype we observe in the current study. Enhanced cognition induced by RS67333 was blocked by the 5-HT$_4$ receptor antagonist GR 125487 (1 mg/kg, intravenous) (Freret *et al*, 2012; Lamirault and Simon, 2001). Both studies tested the specificity of the effects of RS67333 in the Novel Object Recognition test by using the highly potent and selective 5-HT$_4$ receptor antagonist, GR125487. They found that 1 mg/kg of GR125487, which had no effect *per se* on the discrimination index, totally reversed the beneficial effects of RS67333, arguing thus that these effects of RS67333 are mediated by 5-HT$_4$ receptor.

Interestingly, in the NSF test, both fluoxetine and RS67333 had an anxiolytic/antidepressant-like effect only after chronic treatment, suggesting that the neurobiological mechanisms involved in this paradigm are different from those underlying the other tests (OF, EPM, TST, and ST). Indeed, we show that the effects of RS67333 and fluoxetine in the OF, EPM, TST, and ST are independent of hippocampal neurogenesis, whereas the effects of these compounds in the NSF test require neurogenesis. This is in agreement with our previous study showing both neurogenesis-dependent (NSF) and independent (OF, forced swim test) effects of fluoxetine in the CORT model (David *et al*, 2009). It is also noteworthy that the only test (NSF) that requires neurogenesis is also the one that requires a chronic administration. This observation is likely related to the fact that young adult-born neurons take several weeks to mature and that the critical period during which adult-born neurons contribute to behavior extends from 4 to 6 weeks after their birth (Denny *et al*, 2012). Interestingly, the effect of the 5-HT$_4$ agonist RS67333 on proliferation of neural precursors are weaker than those of fluoxetine, whereas the effects of RS67333 on the maturation of young neurons are similar to those of fluoxetine. Newborn neurons undergo an accelerated maturation after chronic fluoxetine treatment (Wang *et al*, 2008) and possibly also after 5-HT$_4$ receptor activation. These results suggest that the neurogenesis-dependent effect of RS67333 and fluoxetine in the NSF test is more likely to result from increased maturation than from increased proliferation.

The fast onset of action of the 5-HT$_4$ receptor agonist could be a consequence of an increase in serotonergic output to projection areas (Lucas *et al*, 2005; Lucas and Debonnel, 2002). Indeed, by measuring spontaneous electrical activity in mice lacking 5-HT$_4$ receptors, Conductier *et al* (2006) demonstrated that 5-HT$_4$ receptors exert a tonic positive influence on the firing activity of dorsal raphe nucleus 5-HT neurons, and previous studies have shown that 5-HT$_4$ receptor activation by selective agonists modulates central 5-HT neurotransmission, increasing the firing of dorsal raphe nucleus 5-HT neurons (Lucas and Debonnel, 2002). There is also accumulating evidence that cortical regions are involved in 5-HT4-induced anxiolytic/antidepressant-like activities (Lucas *et al*, 2005) (for review see also (Lucas, 2009)). 5-HT$_4$ receptors in the prefrontal cortex control the firing rate of midbrain serotonergic neurons via descending inputs (Lucas *et al*, 2005), and their activation leads to increases in serotonin release in projection sites including the hippocampus (Ge and Barnes, 1996).

Requirement of 5-HT$_4$ Receptors for the Behavioral and Neurogenic Effects of SSRIs

A blockade of 5-HT$_4$ receptors with the antagonist GR125487 prevented the anxiolytic and antidepressant-like effects of fluoxetine. These results show that 5-HT$_4$ receptor activation is necessary for the behavioral effects of SSRIs. Our results are consistent with a previous study showing a specific induction of 5-HT$_4$ expression after SSRI treatment (Schmidt *et al*, 2012). SSRIs are thought to act as indirect agonists of 5-HT$_4$ receptors rather than direct agonists. Using the NIMH Psychoactive Drug Screening Program database, we did not find any study looking at the binding affinity of fluoxetine at the mouse 5-HT$_4$ receptor. The only study looking at binding affinities of fluoxetine for serotoninergic receptor demonstrated negligible binding of fluoxetine to the 5-HT$_4$ receptor in pig striatal membranes (Lucchelli *et al*, 1995). In addition, quantitative autoradiography revealed that the binding of the 5-HT$_4$ receptor ligand [^{3}H]GR113808 was not significantly changed in fluoxetine-treated mice (Kobayashi *et al*, 2012). Thus, in the present study, the anxiolytic/antidepressant-like effects of fluoxetine likely resulted from an indirect activation of the 5-HT4 receptor through an increase in endogenous 5-HT levels in the synaptic cleft following the blockade of the selective serotonin transporter.

However, it is unlikely that 5-HT$_4$ receptor activation alone can be responsible for all SSRIs-mediated anxiolytic/antidepressant-like activity. Among the 14 known 5-HT receptor subtypes, the 5-HT$_{1A}$ receptor has been prominently implicated in the modulation of mood and anxiety-related behaviors (Santarelli *et al*, 2003). 5-HT$_{1A}$ receptor KO mice were insensitive to the behavioral effects of chronic fluoxetine, suggesting that activation of 5-HT$_{1A}$ receptors is also a critical component in the mechanism of action of SSRIs. Recent data also suggest a potential non-cell autonomous mechanism by which serotonin regulates neurogenesis and the response to antidepressants through 5-HT$_{1A}$ receptor (Samuels, personal communication). However, we cannot rule out adaptive changes in the serotoninergic system, including variations in 5-HT$_4$ receptor levels, which could explain the absence of behavioral effects of fluoxetine in 5-HT$_{1A}$ receptor KO mice. Indeed, decreases in the density of the serotonin transporter (5-HTT) were measured in several brain regions of these 5-HT$_{1A}$ mutant mice (Ase *et al*, 2001), and a recent study described that variation in serotonin transporter expression could cause adaptive changes in 5-HT$_4$ receptor levels in serotonin transporter overexpressing mice (Jennings *et al*, 2012).

SSRIs are potent stimulators of adult hippocampal neurogenesis (Klempin *et al*, 2010; Santarelli *et al*, 2003). However, the role of each serotoninergic receptor in the neurogenic effects of SSRIs is still a matter of investigation. We have showed that the 5-HT$_4$ agonist, RS67333, increased neurogenesis (proliferation and maturation) to a lesser extent than fluoxetine, and that the 5-HT$_4$ antagonist, GR125487, partially blocked neurogenic effects of chronic fluoxetine. These results suggest that 5-HT$_4$ receptors contribute to the effects of fluoxetine on proliferation and maturation of newborn neurons, but that other 5-HT receptors are likely to be involved. Pharmacological manipulations suggested that 5-HT$_{1A}$ receptors are involved in proliferation of precursor cells, whereas 5-HT$_2$ receptors affect both proliferation and promote neuronal differentiation (Klempin *et al*, 2010). Moreover, fluoxetine had no effect on neurogenesis (proliferation and survival) in 5-HT$_{1A}$ KO mice (Santarelli *et al*, 2003).

These results suggest that both 5-HT$_4$ and 5-HT$_{1A}$ receptors contribute to the effects of SSRIs on behavior and neurogenesis. Interestingly, both receptors are expressed in the DG, which may be the site responsible for their effects on neurogenesis. Recently, it has been suggested that 5-HT$_4$ receptor activation may also be involved in antidepressant-induced dematuration of mature dentate granule cells (Kobayashi *et al*, 2010). The exact mechanisms underlying in this phenomenon still needs further investigations. However, our results also show that most effects of SSRIs and 5-HT$_4$ agonists do not require hippocampal neurogenesis. Examining effects of tissue-specific manipulations of these receptors will be important to identify the circuits responsible for their fast acting anxiolytic and antidepressant actions.

CONCLUSIONS

Taken together, our results show, for the first time, in a mouse model of anxiety/depression, that a 5-HT$_4$ receptor agonist may be a fast-acting anxiolytic agent, and that 5-HT$_4$

Table 1 Neurogenesis-dependent and Independent Mechanism Involved in the Behavioral Effects of Subchronic and Chronic 5-HT$_4$ Agonist Treatment

Fluoxetine (18 mg/kg/day)		RS67333 (1.5 mg/kg/day)	
Subchronic	**Chronic**	**Subchronic**	**Chronic**
Open Field			
ϕ	+	+	+
/	Neurogenesis-independent	Neurogenesis-independent	Neurogenesis-independent
Elevated Plus Maze			
ϕ	+	+	+
/	Neurogenesis-independent	Neurogenesis-independent	Neurogenesis-independent
Novelty Suppressed Feeding			
ϕ	+	ϕ	+
/	Neurogenesis-dependent	/	Neurogenesis-dependent
Tail Suspension Test			
+	+	+	+
Neurogenesis-independent	Neurogenesis-independent	Neurogenesis-independent	Neurogenesis-independent
Splash Test			
+	+	+	+
Neurogenesis-independent	Neurogenesis-independent	Neurogenesis-independent	Neurogenesis-independent

Summary of effects seen in multiple behavioral tests throughout the study: ϕ, no effect; +, anxiolytic/antidepressant-like effects.

stimulation is necessary for the behavioral and neurogenic effects (proliferation and maturation) of fluoxetine, a classic SSRI antidepressant. Furthermore, we showed that, with the exception of the NSF test, the anxiolytic and antidepressant-like effects of the 5-HT$_4$ agonist were independent of hippocampal neurogenesis (Table 1).

The present study is encouraging for the development of RS-67333 as an anxiolytic/antidepressant compound for use in patients. However, the use of the 5-HT$_4$ receptor as a novel antidepressant target may be hampered by the fact that it also has important roles outside the central nervous system, for example, in the heart, gastrointestinal tract, adrenal gland, and urinary bladder (Tonini and Pace, 2006), which may prevent its development as an effective anxiolytic/antidepressant drug (Bockaert *et al*, 2004, 2008). Thus, signaling molecules that interact with the 5-HT$_4$ receptor such as P11 (Egeland *et al*, 2011; Warner-Schmidt *et al*, 2009) may represent novel targets for fast-acting anxiolytic/antidepressant treatments. There is indeed recent evidence that cortical neurons that express both P11 and 5-HT$_4$ receptors are involved in the behavioral effects of SSRIs (Schmidt *et al*, 2012), and that chronic treatment with fluoxetine results in an increase in 5-HT$_4$ receptor expressions in cortical neurons (Schmidt *et al*, 2012).

FUNDING AND DISCLOSURE

This work was also supported by Young Investigator Award to DJD (The Brain & Behavior Research Foundation). Dr DJD currently received investigator-initiated research support from Lundbeck and served as a consultant in the areas of target identification and validation and new compound development to Lundbeck, Roche, and Servier. Dr RH receives compensation as a consultant for Roche, Lundbeck and Servier in relation to the generation of novel antidepressants.

ACKNOWLEDGEMENTS

This work was supported by the technical assistance of Valerie Dupont-Domergue and staff from the animal care facility of the Institut Federatif de Recherche-IFR141 of the Paris-Sud University. We also thank Dr Jean-Philippe Guilloux for his help designing Figure 2.

REFERENCES

Artigas F (2013). Developments in the field of antidepressants, where do we go now? *Eur Neuropsychopharmacol* (e-pub ahead of print; doi:10.1016/j.euroneuro.2013.04.013).

Ase AR, Reader TA, Hen R, Riad M, Descarries L (2001). Regional changes in density of serotonin transporter in the brain of 5-HT1A and 5-HT1B knockout mice, and of serotonin innervation in the 5-HT1B knockout. *J Neurochem* **78**: 619–630.

Bockaert J, Claeysen S, Compan V, Dumuis A (2004). 5-HT4 receptors. *Curr Drug Targets CNS Neurol Disord* **3**: 39–51.

Bockaert J, Claeysen S, Compan V, Dumuis A (2008). 5-HT(4) receptors: history, molecular pharmacology and brain functions. *Neuropharmacology* **55**: 922–931.

Burghardt NS, Bauer EP (2013). Acute and chronic effects of selective serotonin reuptake inhibitor treatment on fear conditioning: Implications for underlying fear circuits. *Neuroscience* **247**: 253–272.

Cachard-Chastel M, Lezoualc'h F, Dewachter I, Delomenie C, Croes S, Devijver H *et al* (2007). 5-HT4 receptor agonists increase sAPPalpha levels in the cortex and hippocampus of male C57BL/6j mice. *Br J Pharmacol* **150**: 883–892.

Compan V, Zhou M, Grailhe R, Gazzara RA, Martin R, Gingrich J *et al* (2004). Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT4 receptor knock-out mice. *J Neurosci* **24**: 412–419.

Conductier G, Dusticier N, Lucas G, Cote F, Debonnel G, Daszuta A *et al* (2006). Adaptive changes in serotonin neurons of the raphe nuclei in 5-HT(4) receptor knock-out mouse. *Eur J Neurosci* **24**: 1053–1062.

Costall B, Naylor RJ (1997). The influence of 5-HT2 and 5-HT4 receptor antagonists to modify drug induced disinhibitory effects in the mouse light/dark test. *Br J Pharmacol* **122**: 1105–1118.

Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N *et al* (2005). Doublecortin expression levels in adult brain reflect neurogenesis. *Eur J Neurosci* **21**: 1–14.

David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I *et al* (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* **62**: 479–493.

David DJ, Wang J, Samuels BA, Rainer Q, David I, Gardier AM *et al* (2010). Implications of the functional integration of adult-born hippocampal neurons in anxiety-depression disorders. *Neuroscientist* **16**: 578–591.

Denny CA, Burghardt NS, Schachter DM, Hen R, Drew MR (2012). 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. *Hippocampus* **22**: 1188–1201.

Egeland M, Warner-Schmidt J, Greengard P, Svenningsson P (2011). Co-expression of serotonin 5-HT(1B) and 5-HT(4) receptors in p11 containing cells in cerebral cortex, hippocampus, caudate-putamen and cerebellum. *Neuropharmacology* **61**: 442–450.

Eglen RM, Bonhaus DW, Johnson LG, Leung E, Clark RD (1995). Pharmacological characterization of two novel and potent 5-HT4 receptor agonists, RS 67333 and RS 67506, *in vitro* and *in vivo*. *Br J Pharmacol* **115**: 1387–1392.

Freret T, Bouet V, Quiedeville A, Nee G, Dallemagne P, Rochais C *et al* (2012). Synergistic effect of acetylcholinesterase inhibition (donepezil) and 5-HT(4) receptor activation (RS67333) on object recognition in mice. *Behav Brain Res* **230**: 304–308.

Gale JD, Grossman CJ, Whitehead JW, Oxford AW, Bunce KT, Humphrey PP (1994). GR113808: a novel, selective antagonist with high affinity at the 5-HT4 receptor. *Br J Pharmacol* **111**: 332–338.

Ge J, Barnes NM (1996). 5-HT4 receptor-mediated modulation of 5-HT release in the rat hippocampus *in vivo*. *Br J Pharmacol* **117**: 1475–1480.

Gomez-Lazaro E, Garmendia L, Beitia G, Perez-Tejada J, Azpiroz A, Arregi A (2012). Effects of a putative antidepressant with a rapid onset of action in defeated mice with different coping strategies. *Prog Neuropsychopharmacol Biol Psychiatry* **38**: 317–327.

Gould TD (2011). Mood and Anxiety Related Phenotypes in Mice Characterization Using Behavioral TestsVolume II. *Neuromethods Ser*. Humana Press Springer Distributor, Secaucus, New York, NY, USA.

Guilloux JP, David I, Pehrson A, Guiard BP, Reperant C, Orvoen S *et al* (2013). Antidepressant and anxiolytic potential of the multimodal antidepressant vortioxetine (Lu AA21004) assessed by behavioral and neurogenesis outcomes in mice. *Neuropharmacology* **73**: 147–159.

Jennings KA, Licht CL, Bruce A, Lesch KP, Knudsen GM, Sharp T (2012). Genetic variation in 5-hydroxytryptamine transporter expression causes adaptive changes in 5-HT(4) receptor levels. *Int J Neuropsychopharmacol* **15**: 1099–1107.

Kato M, Serretti A (2010). Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. *Mol Psychiatry* **15**: 473–500.

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V *et al* (1997). SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. *Neuropharmacology* **36**: 609–620.

Kheirbek MA, Klemenhagen KC, Sahay A, Hen R (2012). Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. *Nat Neurosci* **15**: 1613–1620.

Klempin F, Babu H, De Pietri Tonelli D, Alarcon E, Fabel K, Kempermann G (2010). Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. *Front Mol Neurosci* **3**: pii: 14.

Kobayashi K, Haneda E, Higuchi M, Suhara T, Suzuki H (2012). Chronic fluoxetine selectively upregulates dopamine D(1)-like receptors in the hippocampus. *Neuropsychopharmacology* **37**: 1500–1508.

Kobayashi K, Ikeda Y, Sakai A, Yamasaki N, Haneda E, Miyakawa T *et al* (2010). Reversal of hippocampal neuronal maturation by serotonergic antidepressants. *Proc Natl Acad Sci USA* **107**: 8434–8439.

Lamirault L, Simon H (2001). Enhancement of place and object recognition memory in young adult and old rats by RS 67333, a partial agonist of 5-HT4 receptors. *Neuropharmacology* **41**: 844–853.

Licht CL, Knudsen GM, Sharp T (2010). Effects of the 5-HT(4) receptor agonist RS67333 and paroxetine on hippocampal extracellular 5-HT levels. *Neurosci Lett* **476**: 58–61.

Lucas G (2009). Serotonin receptors, type 4: a new hope? *Curr Drug Targets* **10**: 1085–1095.

Lucas G, Compan V, Charnay Y, Neve RL, Nestler EJ, Bockaert J *et al* (2005). Frontocortical 5-HT4 receptors exert positive feedback on serotonergic activity: viral transfections, subacute and chronic treatments with 5-HT4 agonists. *Biol Psychiatry* **57**: 918–925.

Lucas G, Debonnel G (2002). 5-HT4 receptors exert a frequency-related facilitatory control on dorsal raphe nucleus 5-HT neuronal activity. *Eur J Neurosci* **16**: 817–822.

Lucas G, Du J, Romeas T, Mnie-Filali O, Haddjeri N, Pineyro G *et al* (2010). Selective serotonin reuptake inhibitors potentiate the rapid antidepressant-like effects of serotonin4 receptor agonists in the rat. *PLoS One* **5**: e9253.

Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S *et al* (2007). Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* **55**: 712–725.

Lucchelli A, Santagostino-Barbone MG, Barbieri A, Candura SM, Tonini M (1995). The interaction of antidepressant drugs with central and peripheral (enteric) 5-HT3 and 5-HT4 receptors. *Br J Pharmacol* **114**: 1017–1025.

Mendez-David I, Hen R, Gardier AM, David DJ (2013). Adult hippocampal neurogenesis: an actor in the antidepressant-like action. *Ann Pharm Fr* **71**: 143–149.

Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L *et al* (2006). Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nature Neuroscience* **9**: 729–731.

Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK *et al* (2002). Preclinical models: status of basic research in depression. *Biol Psychiatry* **52**: 503–528.

Pascual-Brazo J, Castro E, Diaz A, Valdizan EM, Pilar-Cuellar F, Vidal R *et al* (2012). Modulation of neuroplasticity pathways and antidepressant-like behavioural responses following the short-term (3 and 7 days) administration of the 5-HT(4) receptor agonist RS67333. *Int J Neuropsychopharmacol* **15**: 631–643.

Quesseveur G, David DJ, Gaillard MC, Pla P, Wu MV, Nguyen HT *et al* (2013). BDNF overexpression in mouse hippocampal astrocytes promotes local neurogenesis and elicits anxiolytic-like activities. *Trans Psychiatry* **3**: e253.

Rainer Q, Xia L, Guilloux JP, Gabriel C, Mocaer E, Hen R *et al* (2011). Beneficial behavioural and neurogenic effects of agomelatine in a model of depression/anxiety. *Int J Neuropsychopharmacol* 1–15 (e-pub ahead of print).

Samuels BA, Leonardo ED, Gadient R, Williams A, Zhou J, David DJ *et al* (2011). Modeling treatment-resistant depression. *Neuropharmacology* **61**: 408–413.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S *et al* (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**: 805–809.

Schiavi GB, Brunet S, Rizzi CA, Ladinsky H (1994). Identification of serotonin 5-HT4 recognition sites in the porcine caudate nucleus by radioligand binding. *Neuropharmacology* **33**: 543–549.

Schmidt EF, Warner-Schmidt JL, Otopalik BG, Pickett SB, Greengard P, Heintz N (2012). Identification of the cortical neurons that mediate antidepressant responses. *Cell* **149**: 1152–1163.

Silvestre JS, Fernandez AG, Palacios JM (1996). Effects of 5-HT4 receptor antagonists on rat behaviour in the elevated plus-maze test. *Eur J Pharmacol* **309**: 219–222.

Surget A, Tanti A, Leonardo ED, Laugeray A, Rainer Q, Touma C *et al* (2011). Antidepressants recruit new neurons to improve stress response regulation. *Mol Psychiatry* **16**: 1177–1188.

Tamburella A, Micale V, Navarria A, Drago F (2009). Antidepressant properties of the 5-HT4 receptor partial agonist, SL65.0155: behavioral and neurochemical studies in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **33**: 1205–1210.

Tonini M, Pace F (2006). Drugs acting on serotonin receptors for the treatment of functional GI disorders. *Dig Dis* **24**: 59–69.

Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L *et al* (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry* **163**: 28–40.

Wang JW, David DJ, Monckton JE, Battaglia F, Hen R (2008). Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *J Neurosci* **28**: 1374–1384.

Warner-Schmidt JL, Flajolet M, Maller A, Chen EY, Qi H, Svenningsson P *et al* (2009). Role of p11 in cellular and behavioral effects of 5-HT4 receptor stimulation. *J Neurosci* **29**: 1937–1946.

Wong EH, Yocca F, Smith MA, Lee CM (2010). Challenges and opportunities for drug discovery in psychiatric disorders: the drug hunters' perspective. *Int J Neuropsychopharmacol* **13**: 1269–1284.

Xia L, Delomenie C, David I, Rainer Q, Marouard M, Delacroix H *et al* (2012). Ventral hippocampal molecular pathways and impaired neurogenesis associated with 5-HT(1)A and 5-HT(1)B receptors disruption in mice. *Neurosci Lett* **521**: 20–25.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)

SUPPLEMENTAL DATA

Rapid anxiolytic effects of a 5-HT_4 receptor agonist are mediated by a neurogenesis-independent mechanism

Authors: Indira Mendez-David[1*], Denis J. David[1*], Flavie Darcet[1], Melody V. Wu[3], Saadia Kerdine-Romer[2], Alain M. Gardier[1], René Hen[3,§]

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Behavioral tests

Open Field (OF)

This test was performed as described by David and colleagues (David *et al*, 2009). Motor activity was quantified in four 43 x 43 cm^2 Plexiglas open field boxes (MED Associates, Georgia, VT). Two sets of 16 pulse-modulated infrared photobeams were placed on opposite walls 2.5-cm apart to record x–y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100-ms resolution. The computer defined grid lines that divided each open field into center and surround regions, with each of four lines being 11 cm from each wall. Dependent measures in the center were the total time and the number of entries over a 30-min test period. The activity in the center was quantified as distance traveled in the center divided by total distance traveled.

Elevated Plus Maze (EPM)

This test was performed as described by David and colleagues (David *et al*, 2009). The maze is a plus-cross-shaped apparatus, with two open arms and two arms closed by walls linked by a central platform 50 cm above the floor. Mice were individually put in the center of the maze facing an open arm and were allowed to explore the maze during 5 min. The time spent in and the

number of entries into the open arms were used as an anxiety index. All parameters were measured using a videotracker (EPM3C, Bioseb, Vitrolles, France).

Novelty-Suppressed-Feeding (NSF)

The NSF is a conflict test that elicits competing motivations: the drive to eat and the fear of venturing into the center of a brightly lit arena. The latency to begin eating is used as an index of anxiety/depression-like behavior, because classical anxiolytic drugs as well as chronic antidepressants decrease this measure. The NSF test was carried out during a 10-min period as previously described (David *et al*, 2009). Briefly, the testing apparatus consisted of a plastic box (50x50x20 cm), the floor of which was covered with approximately 2 cm of wooden bedding. Twenty-four hours prior to behavioral testing, all food was removed from the home cage. At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the center of the box. Each animal was placed in a corner of the box, and a stopwatch was immediately started. The latency to eat (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was timed. Immediately afterwards, the animal was transferred to its home cage, and the amount of food consumed by the mouse in the subsequent 5 min was measured, serving as a control for change in appetite as a possible confounding factor.

Tail Suspension Test (TST)

The TST is an antidepressant activity screening test (Steru *et al*, 1985) often used to test compounds that are expected to affect depression related behaviors. Mice are suspended by their tails with tape, in such a position that they cannot escape or hold on to nearby surfaces. During this test, typically 6 minutes in duration, the resulting escape oriented behaviors are quantified

using an automated tail suspension test apparatus (Bioseb, Vitrolles, France). A specific strain gauge linked to a computer quantifies the time spent by the animal trying to escape.

Splash Test

This test consisted of squirting 200 µl of a 10% sucrose solution on the mouse's snout. The grooming duration was assessed at the end of the corticosterone regimen in the presence or absence of 4-weeks of drug treatment according to a protocol used elsewhere (David *et al*, 2009).

Immunohistochemistry

Doublecortin-immunostaining consisted of the following steps: slices were washed with 3 times with PBST (PBS 1X + 0.3% triton) for 15 min, blocked with 10%NDS (normal donkey serum) + PBST during 2hrs and incubated with by goat anti-doublecortin primary antibody (1:100) in PBS/Triton/NDS for 24 hrs at 4°C. After secondary antibody incubation, sections were developed using CY2 (Donkey anti-rabbit) 1/250 diluted (dilute in PBS) for 2hrs.

References

David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, *et al* (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* **62**(4): 479-493.

Steru L, Chermat R, Thierry B, Simon P (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* **85**(3): 367-370.

SUPPLEMENTAL FIGURES
Supplemental figure 1: Experimental timeline.

(A) In a first set of experiments, in place of normal drinking water, grouped-housed male C57BL/6Ntac mice were presented with vehicle (0.45% hydroxypropyl-β-cyclodextrin) or corticosterone (35 µg/ml) in the presence or absence of a 5-HT_4 agonist (RS67333, 1.5

mg/kg/day, Alzet® mini pump model 2006 implanted subcutaneously), fluoxetine, 18 mg/kg/day, or a 5-HT_4 antagonist alone (GR125487, 1 mg/kg/day, Alzet® mini pump model 2006 implanted subcutaneously) or in combination with fluoxetine. We investigated whether the behavioral changes induced by chronic corticosterone were reversed by chronic 5-HT_4 ligands, fluoxetine alone, or fluoxetine in combination with 5-HT_4 receptor antagonist treatment. The same animal was successively tested in the OF paradigm, the EPM, the NSF, the ST, and the TST and then sacrificed for neurogenesis

(B) In another set of experiments, focal X-irradiation of the hippocampus was employed to assess whether the mechanisms underlying RS67333 mediated rescue of corticosterone-induced anxiety/depressive-like behavior were neurogenesis-dependent. X-irradiation (2.5 Gy) was delivered five weeks before the start of corticosterone treatment. All animals (Sham or X-irradiated) received corticosterone (35 μg /ml) in the presence of vehicle, RS67333 (1.5 mg/kg/day), or fluoxetine (18 mg/kg/day). The anxiety/depressive-like phenotype of chronic corticosterone was assessed by comparing a chronic corticosterone/vehicle group *versus* a vehicle/vehicle group. The same animals were successively tested in the OF paradigm, the EPM, the NSF, the ST, and the TST after subchronic (days 7 to 11) or chronic (days 28 to 33) drug treatment.

Supplemental figure 2: Effects of chronic 5-HT_4 receptor stimulation on entries in the center in the open field paradigm and home food consumption in the NSF test in anxious/depressive mice.

(A-B) Effect of chronic 5-HT_4 ligands or fluoxetine treatment, started after 4 weeks of corticosterone (35 μg/ml), on the number of entries in the center (A) and on total ambulatory distance in the open field test (B). Values plotted are mean ± SEM (n= 10–15 per group).

(C) Effect of chronic 5-HT_4 ligands or fluoxetine treatment, started after 4 weeks of corticosterone (35 μg/ml), on home food consumption in the NSF test. Values plotted are mean ± SEM (n= 10–15 per group).

Supplemental figure 3: Images of doublecortin staining following corticosterone for 8 weeks ± chronic RS67333 (1.5 mg/kg/day), GR125487 (1 mg/kg/day), or fluoxetine (18 mg/kg/day) alone or in combination with GR125487 treatment.

Images of coronal sections of mouse hippocampal dentate gyrus stained for doublecortin (10x magnification).

Supplemental figure 4: Images of doublecortin staining following corticosterone for 8 weeks in Sham/vehicle or X-irradiated/vehicle animals.

Images of coronal sections of mouse hippocampal dentate gyrus stained for doublecortin (10x magnification) in sham/vehicle (A) or in Xray/vehicle–treated animals (B).

Supplemental figure 5: Effects of subchronic or chronic 5-HT_4 receptor stimulation on entries in the center in the open field paradigm and home food consumption in the NSF test in anxious/depressive X-irradiated mice.

(A) Effect of subchronic 5-HT_4 agonist or fluoxetine treatment, started after 4 weeks of corticosterone (35 μg/ml), on entries in the center in the open field test in X-irradiated mice. Values plotted are mean ± SEM (n = 9–15 per group for corticosterone-treated animals and n=5 for vehicle/vehicle). **$p<0.01$, #$p<0.05$, versus control vehicle/vehicle group and corticosterone/vehicle group, respectively.

(B) Effect of chronic 5-HT_4 agonist or fluoxetine treatment, started after 4 weeks of corticosterone (35 μg/ml), on home food consumption in the NSF test in X-irradiated mice.

Values plotted are mean ± SEM (n = 9–15 per group for corticosterone-treated animals and n=5 for vehicle/vehicle). **p<0.01, #p<0.05, versus control vehicle/vehicle group and corticosterone/vehicle group, respectively.

(C) Effect of subchronic 5-HT_4 agonist or fluoxetine treatment, started after 4 weeks of corticosterone (35 μg/ml), on food consumption in the novelty suppressed feeding test in X-irradiated mice. Values plotted are mean ± SEM (n = 9–15 per group for corticosterone-treated animals and n=5 for vehicle/vehicle).

(D) Effect of chronic 5-HT_4 agonist or fluoxetine treatment, started after 4 weeks of corticosterone (35 μg/ml), on food consumption in the novelty suppressed feeding test in X-irradiated mice. Values plotted are mean ± SEM (n = 9–15 per group for corticosterone-treated animals and n=5 for vehicle/vehicle).

SUPPLEMENTAL DATA

Supplemental Table 1: complete statistical summary analysis for behavioral data after chronic treatment

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Open Field	Total Time in the center	One-way ANOVA	Factor treatment	F=2.48	4,59	<0.05*	**1A**
		PLSD Post-hoc test	Cort/Veh vs Cort/Flx			<0.05*	
			Cort/Veh vs Cort/RS			<0.01**	
	Ratio Amb. Dist Cter/Total Amb Dist.	One-way ANOVA	Factor treatment	F=2.70	4,59	<0.05*	**1B**
		PLSD Post-hoc test	Cort/Veh vs Cort/Flx			<0.01**	
			Cort/Veh vs Cort/RS			<0.01**	
	Entries in the center	One-way ANOVA	Factor treatment	F=0.70	4,59	>0.5	**Supp 2A**
	Total Am	One-way ANOVA	Factor treatment	F=0.66	4,59	>0.5	**Supp 2B**
Elevated Plus Maze	Total Time in the open arms	One-way ANOVA	Factor treatment	F=1.6	4,59	>0.05	**1C**
		PLSD Post-hoc test	Cort/Flx vs Cort/FLX+GR			<0.05#	
	Entries in the open arms	One-way ANOVA	Factor treatment	F=1.78	4,59	>0.05	**1D**
Splash Test	Grooming duration	One-way ANOVA	Factor treatment	F=4.95	4,59	<0.01**	**1E**
		PLSD Post-hoc test	Cort/Veh vs Cort/Flx			<0.01**	
			Cort/Veh vs Cort/RS			<0.01**	
			Cort/Flx vs Cort/FLX+GR			<0.01##	
Tail Suspension Test	Immobility duration	One-way ANOVA	Factor treatment	F=3.76	4,59	<0.01**	**1F**
		PLSD Post-hoc test	Cort/Veh vs Cort/Flx			<0.01**	
			Cort/Veh vs Cort/RS			<0.01**	
			Cort/Flx vs Cort/FLX+GR			<0.01##	
Novelty Suppressed Feeding	Latency to Feed	Kaplan-Meier Survival analysis				<0.01**	**1G**
		One-way ANOVA	Factor treatment	F=2.83	4,59	<0.05*	**1H**
		PLSD Post-hoc test	Cort/Veh vs Cort/Flx			<0.01**	
			Cort/Veh vs Cort/RS			<0.01**	
			Cort/Flx vs Cort/FLX+GR			<0.01##	
	Food Cons.	One-way ANOVA	Factor treatment	F=1.18	4,59	>0.05	**Supp 2B**

Legend: CORT:corticosterone; Flx:fluoxetine; Veh:Vehicle; RS:RS67333; GR:GR125487

Supplemental table 2: complete statistical summary analysis for neurogenic data after chronic treatment

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Proliferation	KI-67 positive cells	One-way ANOVA	Factor treatment	F=20.07	4,13	<0.05*	**2A**
		PLSD Post-hoc test	CORT/Veh *vs* CORT/Flx			<0.01**	
			CORT/Veh *vs* CORT/RS			<0.05*	
			CORT/Flx *vs* CORT/RS			<0.01##	
Maturation	DCX-positive cells	One-way ANOVA	Factor treatment	F=9.36	4,13	<0.01**	**2B**
		PLSD Post-hoc test	CORT/Veh vs CORT/Flx			<0.01**	
			CORT/Veh vs CORT/FLX+GR			<0.01**	
			CORT/Flx vs CORT/FLX+GR			<0.01##	
			CORT/Flx *vs* CORT/RS			<0.01##	
	DCX-positive cells with tertiary dendrites	One-way ANOVA	Factor treatment	F=12.90	4,13	<0.01**	**2C**
		PLSD Post-hoc test	CORT/Veh *vs* CORT/Flx			<0.01**	
			CORT/Veh *vs* CORT/FLX+GR			<0.01**	
			Cort/Flx *vs* Cort/FLX+GR			<0.01##	
			CORT/Flx *vs* CORT/RS			<0.08	
	Maturation Index	One-way ANOVA	Factor treatment	F=34.95	4,13	<0.01**	**2D**
		PLSD Post-hoc test	CORT/Veh *vs* CORT/Flx			<0.01**	
			CORT/Veh *vs* CORT/RS			<0.01**	
			CORT/Veh *vs* CORT/FLX+GR			<0.01**	
			CORT/Flx *vs* CORT/FLX+GR			<0.01#	
	Dendritic length	One-way ANOVA	Factor treatment	F=8.94	4,91	<0.01**	**2F**
		PLSD Post-hoc test	CORT/Veh *vs* CORT FLX **(50, 60,70, 80, 90, 100 µm)**			<0.01**	
			CORT/Veh *vs* CORT/RS **(70 µm)**			<0.01**	
			CORT/Flx *vs* CORT Flx+GR **(50, 60,70, 80, 90, 100 µm)**			<0.01##	
	Dendritic intersection	One-way ANOVA	Factor treatment	F=7.49	4,91	<0.01**	**2G**
		PLSD Post-hoc test	CORT/Veh *vs* CORT Flx **(60,70, 80, 90 µm)**			<0.01**	
			CORT/Veh *vs* CORT/RS **(70, 80µm)**			<0.01**	
			CORT/Flx *vs* CORT Flx+GR **(60,70, 80,**			<0.01##	

			90, 100 µm)				

Legend: CORT:corticosterone; Flx:fluoxetine; Veh:Vehicle; RS:RS67333; GR:GR125487

Supplemental Table 3: complete statistical summary analysis for behavioral data after subchronic treatment in sham or X-irradiated mice

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Open Field	Total Time in the center	One-way ANOVA	Factor treatment	F=6.33	6,65	<0.01**	**3A**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* SHAM/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* SHAM/CORT/RS			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/RS			<0.01**	
	Entries in the center	One-way ANOVA	Factor treatment	F=3.90	6,65	<0.01**	**Supp 4A**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* SHAM/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Flx			<0.01**	
Elevated Plus Maze	Total Time in the open arms	One-way ANOVA	Factor treatment	F=3.35	6,65	<0.01**	**3C**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* SHAM/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Flx			<0.01**	
Novelty Suppressed Feeding	Latency to feed	Kaplan-Meier Survival analysis				<0.01**	**3E**
		One-way ANOVA	Factor treatment	F=3.79	6,65	<0.01**	**3F**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* SHAM/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* SHAM/CORT/RS			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/RS			<0.01**	
	Food cons.	One-way ANOVA	Factor treatment	F=2.20		<0.06	**Supp 4C**
Tail Suspension Test	Immobility duration	One-way ANOVA	Factor treatment	F=2.38	6,65	<0.05*	**3H**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* SHAM/CORT/RS			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/RS			<0.01**	

Legend : CORT:corticosterone; Flx:fluoxetine; Veh:Vehicle; RS:RS67333; XRAY:X-irradiated

Supplemental table 4: complete statistical summary analysis for behavioral data after subchronic treatment in sham or X-irradiated mice (two-way ANOVA)

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Open Field	Total Time in the center	2-way ANOVA	Factor 1 Pre-treatment	F=0.38	1,61	>0.5	3A
			Factor 2 Treatment	F=8.0	2,61	<0.01**	
			Interaction (F1 x F2)	F=0.039	2,61	>0.5	
		PLSD Post-hoc test	SHAM/CORT/RS *vs* SHAM/CORT/Veh			<0.05#	
			SHAM/CORT/RS *vs* SHAM/CORT/Flx			<0.05#	
			XRAY/CORT/RS *vs* XRAY/CORT/Veh			<0.05#	
			XRAY/CORT/RS *vs* XRAY/CORT/Flx			<0.05#	
	Ratio Amb. Dist Cter/Total Amb Dist.	2-way ANOVA	Factor 1 Pre-treatment	F< 0.0001	1,61	>0.5	3B
			Factor 2 Treatment	F=4.91	2,61	<0.05*	
			Interaction (F1 x F2)	F=0.005	2,61	>0.5	
		PLSD Post-hoc test	SHAM/CORT/RS *vs* SHAM/CORT/Veh			<0.05#	
			SHAM/CORT/RS *vs* SHAM/CORT/Flx			<0.05#	
			XRAY/CORT/RS *vs* XRAY/CORT/Veh			<0.05#	
			XRAY/CORT/RS *vs* XRAY/CORT/Flx			<0.05#	
	Entries in the center	2-way ANOVA	Factor 1 Pre-treatment	F=0.27	1,61	>0.5	Supp 4A
			Factor 2 Treatment	F=10.41	2,61	<0.01**	
			Interaction (F1 x F2)	F=0.120	2,61	>0.5	
		PLSD Post-hoc test	SHAM/CORT/RS *vs* SHAM/CORT/Veh			<0.05#	
			SHAM/CORT/RS *vs* SHAM/CORT/Flx			<0.05#	
			XRAY/CORT/RS *vs* XRAY/CORT/Veh			<0.13	
			XRAY/CORT/RS *vs* XRAY/CORT/Flx			<0.13	
Elevated Plus Maze	Total Time in the open arms	2-way ANOVA	Factor 1 Pre-treatment	F=0.86	1,61	>0.3	3C
			Factor 2 Treatment	F=4.96	2,61	<0.01**	
			Interaction (F1 x F2)	F=0.28	2,61	>0.5	

		PLSD Post-hoc test	SHAM/CORT/RS *vs* SHAM/CORT/Veh			<0.05#	
			SHAM/CORT/RS *vs* SHAM/CORT/Veh			<0.05#	
			XRAY/CORT/RS *vs* XRAY/CORT/Veh			<0.09	
	Entries in the open arms	2-way ANOVA	Factor 1 Pre-treatment	F=0.14	1,61	>0.5	**3D**
			Factor 2 Treatment	F=3.09	2,61	<0.053	
			Interaction (F1 x F2)	F=0.92	2,61	<0.41	
		PLSD Post-hoc test	SHAM/CORT/RS *vs* SHAM/CORT/Veh			<0.05#	
			XRAY/CORT/RS *vs* XRAY/CORT/Veh			<0.05#	
Splash test	Grooming duration	2-way ANOVA	Factor 1 Pre-treatment	F=2.28	1,61	>0.5	**3G**
			Factor 2 Treatment	F=7.16	2,61	<0.01**	
			Interaction (F1 x F2)	F=0.022	2,61	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Flx *vs* SHAM/CORT/Veh			<0.05#	
			SHAM/CORT/RS *vs* SHAM/CORT/Veh			<0.05#	
			XRAY/CORT/Flx *vs* XRAY/CORT/Veh			<0.05#	
			XRAY/CORT/RS *vs* XRAY/CORT/Veh			<0.05#	
Tail Suspension Test	Immobility duration	2-way ANOVA	Factor 1 Pre-treatment	F=2.28	1,61	<0.14	**3H**
			Factor 2 Treatment	F=7.16	2,61	<0.01**	
			Interaction (F1 x F2)	F=0.02	2,61	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.05#	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.05#	
			XRAY/CORT/Veh *vs* XRAY/CORT/Flx			<0.05#	
			XRAY/CORT/Veh *vs* XRAY/CORT/RS			<0.01##	

Factor 1 Pre-treatment: Sham or X-irradiation; Factor 2 Treatment: Vehicle or Corticosterone; CORT:corticosterone; Flx:fluoxetine; Veh:Vehicle; RS:RS67333; XRAY:X-irradiated

Supplemental Table 5: complete statistical summary analysis for behavioral data after chronic treatment

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Open Field	Total Time in the center	One-way ANOVA	Factor treatment	F=3.94	6,59	<0.01**	**3I**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
	Amb. Dist Center/Total Amb. Dist	One-way ANOVA	Factor treatment	F=2.48	6,59	<0.05*	**3J**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
	Entries in the center	One-way ANOVA	Factor treatment	F=2.94	6,59	<0.05*	**Supp 4B**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
Elevated Plus Maze	Total Time in the open arms	One-way ANOVA	Factor treatment	F=5.32	6,59	<0.01**	**3K**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
	Entries in the open arms	One-way ANOVA	Factor treatment	F=3.91	6,50	<0.01**	**3L**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
Novelty Suppressed Feeding	Latency to feed	Kaplan-Meier Survival analysis				<0.01**	**3M**
		One-way ANOVA	Factor treatment	F=7.31	6,59	<0.01**	**3N**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/Flx			<0.01**	
			SHAM/Veh/Veh *vs* XRAY/CORT/RS			<0.05*	

	Food cons.	One-way ANOVA	Factor treatment	F=1.28	6,59	<0.3	**Supp 4D**
Splash Test	Grooming duration	One-way ANOVA	Factor treatment	F=3.40	6,59	<0.01**	**3O**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Veh			<0.05*	
			SHAM/Veh/Veh *vs* XRAY/CORT/Veh			<0.26	
Tail Suspension Test	Immobility duration	One-way ANOVA	Factor treatment	F=2.36	6,59	<0.05*	**3P**
		PLSD Post-hoc test	SHAM/Veh/Veh *vs* SHAM/CORT/Flx			<0.05*	
			SHAM/Veh/Veh *vs* XRAY/CORT/Flx			<0.05*	
			SHAM/Veh/Veh *vs* SHAM/CORT/RS			<0.06	
			SHAM/Veh/Veh *vs* XRAY/CORT/RS			<0.07	

Legend: Factor 1 Pre-treatment: Sham or X-irradiation; Factor 2 Treatment: Vehicle or Corticosterone; CORT:corticosterone; Flx:fluoxetine; Veh:Vehicle; RS:RS67333; XRAY:X-irradiated

Supplemental Table 6: complete statistical summary analysis for behavioral data after chronic treatment in sham or X-irradiated mice (two-way ANOVA)

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Open Field	Total Time in the center	2-way ANOVA	Factor 1 Pre-treatment	F=0.64	1,55	>0.4	**3I**
			Factor 2 Treatment	F=5.37	2,55	<0.01**	
			Interaction (F1 x F2)	F=0.32	2,55	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.01##	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.01##	
			XRAY/CORT/Veh *vs* XRAY/CORT/Flx			<0.1	
			XRAY/CORT/Veh *vs* XRAY/CORT/RS			<0.05#	
	Ratio Amb. Dist Cter/Total Amb Dist.	2-way ANOVA	Factor 1 Pre-treatment	F=0.24	1,55	>0.5	**3J**
			Factor 2 Treatment	F=3.17	2,55	<0.05*	
			Interaction (F1 x F2)	F=0.60	2,55	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.01##	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.05#	
			XRAY/CORT/Veh *vs* XRAY/CORT/Flx			<0.2	
	Entries in the center	2-way ANOVA	Factor 1 Pre-treatment	F=0.052	1,55	>0.5	**Supp 4B**
			Factor 2 Treatment	F=5.47	2,55	<0.01**	
			Interaction (F1 x F2)	F=0.40	2,55	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.05#	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.05#	
			XRAY/CORT/Veh *vs* XRAY/CORT/Flx			<0.07	
			XRAY/CORT/Veh *vs* XRAY/CORT/RS			<0.01##	
Elevated Plus Maze	Total Time in the open arms	2-way ANOVA	Factor 1 Pre-treatment	F=3.45	1,55	<0.07	**3K**
			Factor 2 Treatment	F=9.28	2,55	<0.01**	

			Interaction (F1 x F2)	F=0.56	2,55	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.06	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.05#	
			XRAY/CORT/Veh *vs* XRAY/CORT/Flx			<0.09	
			XRAY/CORT/Veh *vs* XRAY/CORT/RS			<0.01##	
	Entries in the open arms	2-way ANOVA	Factor 1 Pre-treatment	F=2.98	1,55	<0.09	**3L**
			Factor 2 Treatment	F=7.91	2,55	<0.001***	
			Interaction (F1 x F2)	F=0.27	2,55	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.05#	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.01##	
			XRAY/CORT/Veh *vs* XRAY/CORT/Flx			<0.13	
			XRAY/CORT/Veh *vs* XRAY/CORT/RS			<0.042#	
Novelty Suppressed Feeding	Latency to feed	2-way ANOVA	Factor 1 Pre-treatment	F=14.42	1,55	<0.01**	**3N**
			Factor 2 Treatment	F=2.73	2,55	<0.08	
			Interaction (F1 x F2)	F=6.86	2,55	<0.01**	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.05#	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.01##	
	Food Cons.	2-way ANOVA	Factor 1 Pre-treatment	F=1.48	1,55	>0.2	**Supp 4D**
			Factor 2 Treatment	F=0.67	2,55	>0.5	
			Interaction (F1 x F2)	F=1.42	2,55	>0.2	
Splash test	Grooming duration	2-way ANOVA	Factor 1 Pre-treatment	F=2.22	1,55	>0.1	**3O**
			Factor 2 treatment	F=8.85	2,55	<0.01**	
			Interaction (F1 x F2)	F=0.35	2,55	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.05#	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.01##	
			XRAY/CORT/Veh *vs* XRAY/CORT/Flx			<0.07	
			XRAY/CORT/Veh *vs* XRAY/CORT/RS			<0.01##	

Tail Suspension Test	Immobility duration	2-way ANOVA	Factor 1 Pre-treatment	F=0.055	1,55	>0.5	**3P**
			Factor 2 Treatment	F=5.43	2,55	<0.01**	
			Interaction (F1 x F2)	F=0.18	2,55	>0.5	
		PLSD Post-hoc test	SHAM/CORT/Veh *vs* SHAM/CORT/Flx			<0.05#	
			SHAM/CORT/Veh *vs* SHAM/CORT/RS			<0.06	
			XRAY/CORT/Veh *vs* XRAY/CORT/Flx			<0.07	
			XRAY/CORT/Veh *vs* XRAY/CORT/RS			<0.05#	

Legend: Factor 1 Pre-treatment: Sham or X-irradiation; Factor 2 Treatment: Vehicle or Corticosterone; CORT:corticosterone; Flx=fluoxetine; Veh:Vehicle; RS:RS67333; XRAY:X-irradiated

Commentaires sur Article 2 :

Cette étude montre pour la première fois, dans un modèle d'anxiété/dépression, qu'un agoniste du récepteur 5-HT_4 peut agir comme un composé anxiolytique et antidépresseur de façon rapide et que la stimulation du récepteur 5-HT_4 est nécessaire pour les effets comportementaux et neurogéniques (prolifération et maturation) de la fluoxetine. De plus, nous avons démontré qu'à l'exception du test du NSF, les effets de type anxiolytiques et antidépresseurs de l'agoniste 5-HT_4 sont indépendants de la neurogenèse hippocampique.

Ce travail est encourageant pour le développement des agonistes du récepteur 5-HT_4 tel que le RS67333 comme un médicament anxiolytique et antidépresseur pour les patients atteints de dépression majeure avec une co-morbidité anxieuse. Toutefois, d'autres propriétés pharmacologiques importantes de ces molécules tant au niveau du système nerveux central (cognition, satiété) qu'au niveau périphérique (cœur, intestin, vessie) doivent être prises en compte pour une éventuelle utilisation de cette famille d'agonistes du récepteur 5-HT_4 en clinique.

Devant l'efficacité du composé RS67333 dans la correction du phénotype anxio/dépressif induit par le modèle CORT et connaissant les propriétés pro-cognitives des agonistes du récepteur 5-HT_4 chez des animaux naïfs, nous nous sommes interrogés sur la capacité du RS67333 à restaurer les troubles cognitifs induits par l'administration de corticostérone à long-terme.

ARTICLE 3: Chronic 5-HT$_4$ receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression

Flavie Darcet, Alain M Gardier, Denis J David, Jean-Philippe Guilloux

Publié dans le journal Neuroscience Letters (Février 2016)

Question posée :

Les différents troubles de l'apprentissage et de la mémoire induits par le modèle CORT sont-ils corrigés par une administration chronique d'un antidépresseur monoaminergique classique (fluoxetine) ou d'un agoniste des récepteurs 5-HT$_4$ (RS67333)?

Résumé de l'étude

Les troubles cognitifs sont souvent reportés comme des symptômes invalidants chez les patients souffrant de dépression majeure. De plus, plusieurs études ont montré que certaines des difficultés cognitives observées pendant des épisodes dépressifs persistaient malgré la rémission des patients. Il est donc nécessaire d'identifier de nouvelles stratégies thérapeutiques qui permettent de prendre en charge les symptômes émotionnels liés à la pathologie de la dépression au même titre que les troubles cognitifs associés.

Récemment, de nombreuses études se sont intéressées aux propriétés antidépressives des agonistes des récepteurs 5-HT$_4$ (Lucas et al 2007 ; Pascual-Brazo et al 2012). Cependant, peu d'entre elles conduisent leurs tests sur des animaux anxio/dépressifs (Mendez-David et al 2014) et moins encore se sont concentrées sur les propriétés pro-cognitives des agonistes des récepteurs 5-HT$_4$ dans un contexte d'anxiété/dépression.

C'est pourquoi nous avons testé l'efficacité du RS67333, un agoniste du récepteur 5-HT$_4$, dans notre modèle d'anxiété/dépression basé sur une exposition chronique à la corticostérone sur les performances cognitives des animaux, en comparaison à un traitement chronique par un antidépresseur monoaminergique classique, la fluoxetine.

Comme nous l'avons précédemment démontré, (**Article 1**), un trouble cognitif global chez les animaux traités à la CORT est observé. D'un point de vue pharmacologique, les traitements chroniques par la fluoxetine et par le RS67333 ont permis de restaurer le déficit de mémoire de type épisodique observé chez les animaux anxio/dépressifs, en rétablissant l'index de

discrimination au niveau des animaux contrôles. Dans le test de conditionnement par la peur, alors que l'agoniste du récepteur 5-HT_4 améliore l'altération de la mémoire associative induite par la CORT, le traitement par la fluoxetine ne modifie pas le déficit observé chez les animaux CORT. En outre, le nombre d'épisodes de freezing est même significativement inférieur à celui des animaux traités par la CORT. Enfin, dans le Barnes maze, le traitement par le RS67333 améliore aussi bien le déficit d'apprentissage que de mémoire chez les animaux CORT. Le traitement chronique par la fluoxetine, pour sa part, a permis de corriger uniquement le déficit d'apprentissage.

Dans l'ensemble, un traitement chronique par le RS67333 est capable de corriger entièrement le déficit cognitif induit par la corticostérone. En revanche, le traitement chronique par la fluoxetine ne permet d'obtenir qu'une restauration partielle des fonctions cognitives, dépendante du type de mémoire testé et des paramètres d'apprentissage et de mémoire eux-mêmes. Ces résultats suggèrent que ces deux stratégies thérapeutiques pourraient mettre en jeu des mécanismes d'action différents (mémoire associative et spatiale) ou parfois similaires (mémoire type épisodique) en fonction du type de mémoire engagé.

Contribution personnelle :

Au cours de ce travail :

- J'ai réalisé le suivi des animaux incluant la préparation et l'administration des traitements pharmacologiques (traitement par la CORT et par la fluoxetine, préparation et implantation des mini-pompes pour RS67333).

- J'ai mené l'ensemble des tests comportementaux émotionnels et cognitifs.

- J'ai analysé les résultats et rédigé l'intégralité de l'article sous la supervision du Pr. Denis David et du Dr Jean-Philippe Guilloux

Neuroscience Letters 616 (2016) 197–203

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Chronic 5-HT$_4$ receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression

Flavie Darcet, Alain M. Gardier, Denis J. David*, Jean-Philippe Guilloux*

Université Paris-Saclay, Univ. Paris-Sud, Faculté de Pharmacie, CESP, INSERM UMRS1178, Chatenay-Malabry 92296, France

HIGHLIGHTS

- A chronic corticosterone administration induces emotional and cognitive impairments.
- Chronic fluoxetine only partially reversed these cognitive alterations.
- A 5-HT4 receptor agonist restored CORT-induced deficits in all cognitive domains.

ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form
22 December 2015
Accepted 26 January 2016
Available online 3 February 2016

Keywords:
Cognitive deficits
Animal models of anxiety-depression
Fluoxetine
5-HT$_4$ receptor agonist

ABSTRACT

Cognitive disturbances are often reported as serious invalidating symptoms in patients suffering from major depression disorders (MDD) and are not fully corrected by classical monoaminergic antidepressant drugs. If the role of 5-HT$_4$ receptor agonists as cognitive enhancers is well established in naïve animals or in animal models of cognitive impairment, their cognitive effects in the context of stress need to be examined. Using a mouse model of anxiety/depression (CORT model), we reported that a chronic 5-HT$_4$ agonist treatment (RS67333, 1.5 mg/kg/day) restored chronic corticosterone-induced cognitive deficits, including episodic-like, associative and spatial learning and memory impairments. On the contrary, a chronic monoaminergic antidepressant drug treatment with fluoxetine (18 mg/kg/day) only partially restored spatial learning and memory deficits and had no effect in the associative/contextual task. These results suggest differential mechanisms underlying cognitive effects of these drugs. Finally, the present study highlights 5-HT$_4$ receptor stimulation as a promising therapeutic mechanism to alleviate cognitive symptoms related to MDD.

1. Introduction

Increasing evidence suggests that cognitive disturbances are closely associated with depressive symptoms observed in major depression disorders (MDD) [1,2]. In addition to the emotional and behavioral alterations characterizing this pathology, patients suffering from MDD display a broad range of cognitive deficits, varying from executive functions, attention, processing speed, working memory to visual learning and memory domains [3,4]. Cognitive processes such as attention and memory may not only be correlates of depressive episodes; they may also play a critical role in increasing individuals' vulnerability for the first onset and recurrence of depression (for review see Ref. [5]). Currently, these cognitive signs are not fully corrected by classical monoaminergic antidepressant drugs [6,7]. New antidepressant drug strategies that target mood-related symptoms as well as cognitive symptoms are needed to improve long-term outcomes, and particularly functional recovery [8].

It has been proposed that not only 5-HT$_4$ receptor agonists such as RS67333 may bring new hope for treating depression ([9,10] for review), but also represent a promising drug candidate to treat cognitive impairments [11]. During the past few years, the role of 5-HT$_4$ receptor agonists as cognitive enhancers has been well-established under basal conditions in rodents [12,13], in young or aged macaques [14] or in animal models of cognitive impairment [11,15,16], but their pro-cognitive effects in the context of anxiety/depression-like state has not been examined yet. A variety of studies have assessed cognitive disorders in different anxiety

* Corresponding authors at: UMR-S 1178, Univ. Paris Sud, Fac Pharmacie, 5, rue Jean-Baptiste Clément, 92296 Chatenay-Malabry, France.
E-mail addresses: denis.david@u-psud.fr (D.J. David), jean-philippe.guilloux@u-psud.fr (J.-P. Guilloux).

http://dx.doi.org/10.1016/j.neulet.2016.01.055
0304-3940/

or depression models in adult rodents [17–19]. Specifically, we previously showed that chronic corticosterone exposure in mice (CORT model) induces an anxiety/depressive-like phenotype [20], associated with episodic-like, associative and spatial learning and memory impairments [21]. Moreover, sub-chronic and chronic 5-HT_4 receptor stimulations reversed the CORT-induced higher emotional state [9,22]. The aim of the present study was to determine whether chronic activation of the 5-HT_4 receptor (RS67333), in comparison to fluoxetine, a chronic serotonergic antidepressant drug treatment, could reverse CORT-induced cognitive deficits. To this end, we selected a range of cognitive behavioral paradigms that allows investigation of different memory systems including episodic-like memory, associative/contextual memory and spatial reference learning and memory.

2. Materials and methods

2.1. Animals

Eight to 10-weeks old male C57BL/6JRj mice (Janvier Labs, France) were maintained on a 12L:12D schedule (Lights ON at 7AM) and were housed 5 per cage. Food and water were provided *ad libitum*. All testing were conducted in compliance with the laboratory animal care guidelines, with protocols approved by the Institutional Animal Care and Use Committee (CEE26 authorization 2012–099) and with the European directive 2010/63/EU.

2.2. Drugs and treatments

Corticosterone (CORT, 4-pregnen-11b-DIOL-3 20-DIONE 21-hemisuccinate (35 μg/ml equivalent to 5 mg/kg/day) from Sigma-Aldrich (France) was dissolved in vehicle (0.45% hydroxypropyl-β-cyclodextrin, β-CD), Sigma-Aldrich, France). Fluoxetine hydrochloride (Anawa trading, Switzerland) was dissolved in 0.45% β-CD [20]. RS67333 hydrochloride (1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-butyl-4piperidinyl)-1-propanone) (Tocris Bioscience, United Kingdom) was dissolved in 0.9% saline solution. Corticosterone was delivered for 28 days in drinking water to induce the anxio-depressed phenotype in mice (Supplementary Fig. 1). Thereafter, while administration with β-CD or corticosterone continued, mice were treated with vehicle (0.45% β-CD), fluoxetine (160 mg/ml delivered in drinking water, equivalent to 18 mg/kg/day) or RS67333 (delivered by osmotic mini-pumps at a dose of 1.5 mg/kg/day). The dose, route and duration of fluoxetine or RS67333 treatments were selected based on our previous report [9], to ensure a similar anxiolytic/antidepressant-like effect of treatment. Osmotic minipumps (42 days, model 2006, Alzet, USA) were implanted subcutaneously under light anesthesia (ketamine/xylazine: 75/20 mg/kg, Sigma Aldrich, France). Vehicle, CORT and CORT/Fluoxetine groups were also implanted with a mini-pump containing 0.9% saline. Treatment was maintained until the end of the experiments.

2.3. Behavioral testing

Cognitive and anxio/depressive effects of pharmacological treatments were conducted within the same cohort. Each animal was successively subjected to the behavioral tests as shown in Supplementary Fig. 1. At least one day resting was given to animals between each test. Animals were placed in the experimental room 30 min before the start of the behavioral experiments. A detailed description of materials and methods can be found in supplemental information.

2.3.1. Anxiety and depression behavior paradigms

Anxiety parameters and motor activity were quantified during 10 or 30 min respectively in Open Field (OF) boxes as described in [9]. Depressive-like behavior was assessed in the Splash test (ST) as previously described [20].

2.3.2. Cognition behavioral paradigms

2.3.2.1. Episodic memory: novel object recognition test (NORT). The procedure was adapted from the Sahay study [23] and was performed as previously described in [21]. Briefly, the NORT was divided into 4 training sessions and one test session. During training sessions, two identical objects were present in the box and the mouse was allowed to freely explore the apparatus and the objects. Results for this test were expressed as: 1) exploration (in percent) of each object during the test session and 2) a discrimination index (DI) between objects during the test session, calculated as the difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects ($DI = (N - F)/(N + F)$).

2.3.2.2. Associative/contextual memory: one-trial contextual fear conditioning. Associative/contextual memory was evaluated as previously described in [21], adapted from previous studies [24]. The experimental design ran over two consecutive days. On Day 2, animals returned to the conditioning chamber for a 4-min period, for a test of context-elicited freezing. Scoring was measured using Freezing software version 2.0.04 (Packwin, Harvard apparatus, Bioseb, France).

2.3.2.3. Spatial reference learning and memory: Barnes maze. The Barnes maze (BM) procedure was conducted as previously described [21]. Briefly, spatial acquisition was organized in 4 training sessions (Day 1 to Day 4) followed on day 5 by a retention probe trial (90 s) during which the target box was removed and the target hole was closed. Primary latency and primary errors to identify the target hole were manually scored. Latency to reach the target hole for the first time, number of errors before reaching the target hole, number of visits in the target hole and time spent in the target quadrant were recorded using ANY-maze Software version 4.99 (Stoelting, Bioseb, France).

2.4. Statistics

Results from data analyses were expressed as mean ± SEM. Statistical analyses were processed with Statview 5.0® Software (SAS Institute, Cary, NC). Detailed statistical methodology and results are provided in Supplementals.

3. Results

In the present study, we confirmed previous reports showing chronic corticosterone-induced anxiety/depression-like phenotype in mice [20]. Physiological (weight) and physical (fur coat) changes are indicators of depressed-like state in animals: here, a significant increase in body weight and coat state score in CORT-treated animals compared to controls was observed ($p < 0.01$, Fig. S2A and B). Moreover, CORT-treated mice displayed anxiety- and depressive-like phenotypes in the OF and the ST compared to controls. Interestingly, both chronic fluoxetine and 5-HT_4 receptor activation with RS67333 were able to significantly reverse chronic CORT-induced increase in body weight and deterioration of coat state ($p < 0.05$ and $p < 0.01$). In the OF, a trend and a significant increase in time spent in the center were observed in chronic R67333 or fluoxetine-treated mice, respectively ($p = 0.09$; $p < 0.05$), without affecting the total ambulatory distance ($p > 0.1$) (Fig. S2C and D). In the ST, both pharmacological treatments significantly

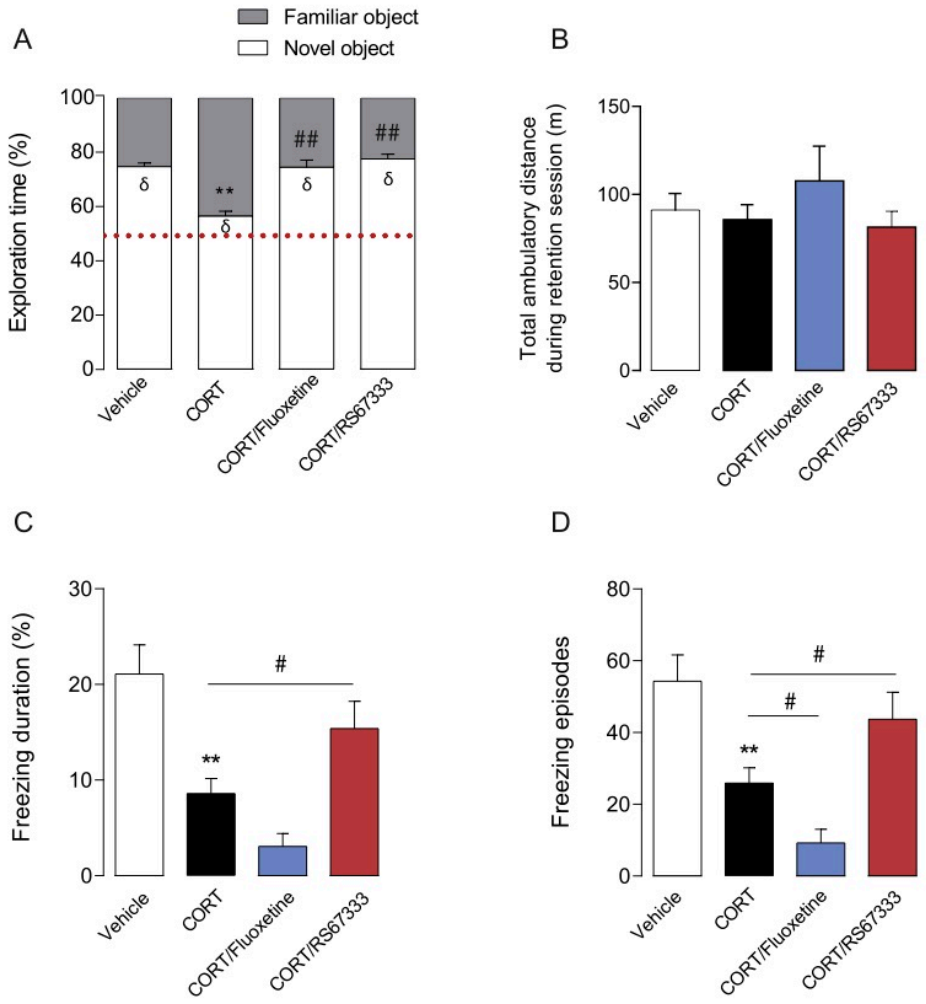


Fig. 1. Effects of chronic fluoxetine or 5-HT_4 receptor agonist treatments on CORT-induced episodic-like memory impairment and associative/contextual memory deficit. Novel Object Recognition test (A and B): the exploration time was expressed in percent of both novel object and familiar object with the red dot line indicating the chance level (A). Total ambulatory distance during the retention session was monitored (B). One-trial Contextual Fear Conditioning (C and D): contextual fear conditioning was induced by placing a mouse in the conditioning chamber and delivering a 2-s footshock 180 s later. Mice were returned to the conditioning chamber 24 h later to assess for context-elicited freezing. During Day 2, context-elicited freezing (C) and the number of freezing episodes (D) were analyzed during the whole session. Values are mean ± SEM, n = 10–15 animals per group; **p < 0.01 *versus* vehicle group; #p<0.05, ##p < 0.01 *versus* CORT-treated group, δ p < 0.05 compared to chance level 50%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reversed the chronic CORT treatment-induced decrease in grooming duration ($p < 0.01$) (Fig. S2E). Altogether, these data confirmed that 5-HT_4 receptor activation produces both anxiolytic-like and antidepressant-like effects comparable to those of fluoxetine in the chronic CORT model [9].

3.1. *Effects of chronic fluoxetine or 5-HT_4 receptor agonist treatment on episodic-like memory in mice chronically treated with corticosterone*

In the NORT, all experimental groups were able to discriminate the novel object from the familiar one, indicated by a significant increase in relative exploration time ($p < 0.01$ *versus* the chance level 50% for each groups, Fig. 1A). However, CORT-treated animals displayed a lower distinction for the novel object than vehicle animals, as suggested by a decrease in discrimination index (Fig. S3A, see also Ref. [21]). Both chronic fluoxetine or RS67333 treatments reversed CORT-induced episodic-like impairment by increasing novel object exploration time and consequently increasing the discrimination index ($p < 0.01$, Figs. 1 A and S3A). No significant difference was found between groups on control parameters (exploration duration across sessions and ambulatory distances) ($p > 0.1$, Figs. 1 B and S3A–C).

3.2. *Effects of chronic fluoxetine and 5-HT_4 receptor agonist treatment on associative/contextual memory in mice chronically treated with corticosterone*

During the Day 2 trial, CORT-treated mice exhibited a lower freezing duration than controls, associated with a decrease in freezing episodes ($p < 0.01$, Fig. 1C and D). RS67333 treatment restored CORT-induced associative/contextual memory deficit, by increasing freezing time and episodes ($p < 0.05$, Fig. 1C and D). On the contrary, fluoxetine failed to improve the freezing duration during the context-elicited fear trial and even showed a decrease in freezing episodes compared to CORT-treated mice ($p < 0.05$, Fig. 1D).

3.3. Effects of chronic fluoxetine and 5-HT$_4$ receptor agonist treatment on spatial learning and memory performances in mice chronically treated with corticosterone

Motivational behavior to move and search for the target hole was controlled by measuring mean speed during the training session of Day 1, and did not differ between groups ($p > 0.1$) (Fig. S4D).

3.3.1. Acquisition

Learning occurred in all groups, as latency to identify the target hole was decreased across learning sessions ($p < 0.01$, Fig. 2A). However, an increase in primary latency and errors in CORT-treated mice compared to controls confirmed the altered spatial learning performances in these animals compared to controls ($p < 0.01$, Figs. 2 A and S4C, see also [21]). Interestingly, both CORT/Fluox and CORT/RS67333 animals were able to partially recover of learning abilities, as indicated by a progressive significant decrease in primary latency across time compared to CORT-treated mice ($p < 0.01$, Fig. 2A). Paradoxically, while CORT/RS67333 showed a classical simultaneous decrease in primary errors across trials ($p < 0.01$), CORT/Fluox animals failed to improve this parameter, suggesting that research strategy of the target hole is different between treatments (Fig. S4A and B).

3.3.2. Retention trial

On Day 5, reference memory was assessed by a retention trial in which the target hole was removed and replaced by a false hole. Increases in primary errors and primary latency in CORT-treated mice compared to controls ($p < 0.01$, Fig. 2B and C) associated with a decrease in time spent in the target quadrant ($p < 0.05$, Fig. 2D) and a decrease in the number of visits in the target hole ($p < 0.01$, Fig. 2E) suggested that anxio/depressive-like animals have difficulties to remember the location of the target quadrant. Interestingly, a chronic RS67333 treatment improved all of the retention parameters when compared to CORT-treated mice ($p < 0.01$, Fig. 2B–E), suggesting that a chronic 5-HT$_4$ receptor stimulation can restore CORT-induced learning and retention spatial deficits. Intriguingly, whereas learning performances were reversed after a chronic fluoxetine treatment, most of the retention parameters remained impaired in CORT/Fluox animals (Fig. 2B–D), except the number of visits into the target hole ($p < 0.05$, Fig. 2E).

4. Discussion

The present study reveals the cognitive consequences of chronic fluoxetine or 5-HT$_4$ receptor agonist treatments in a neuroendocrine-based mouse model of anxiety/depression. Because memory functions are not limited to a single aspect, episodic-like (novel object recognition test), associative/contextual (one-trial contextual fear conditioning) and spatial (Barnes maze) learning and memory tasks were tested. Our results highlight that altered emotional phenotype after chronic CORT treatment induced a cognitive deficit that affects all aspects of learning and memory, especially episodic (NORT), associative/contextual (CFC) and visuo-spatial systems in mice (BM) [21]. A chronic 5-HT$_4$ receptor agonist treatment restored all the cognitive CORT-induced deficits. By contrast, a chronic fluoxetine treatment was able to reverse deficits in episodic-like memory (NORT) and some of learning parameters in the Barnes maze, but failed to improve the CORT-induced decrease in freezing behavior in the associative/contextual test and most of retention parameters in a spatial task.

The majority of preclinical research focused on the effects of 5-HT$_4$ receptor agonists in the cognitive domain (acute: [12,25]; chronic: [13]). Likewise, numerous studies described RS67333 as a potential antidepressant drug [9,22,26,27], and that fluoxetine antidepressant effects are blocked by a 5-HT$_4$ receptor antagonist, GR125487 [9]. This is the first report of the cognitive effects of a chronic systemic RS67333 treatment in a model of anxiety/depression. Identification of neuronal circuits involved in the cognitive effects elicited by activation of 5-HT$_4$ receptor under anxiety/depression-like phenotype still need further investigations. Although RS67333 displays a partial agonist activity on 5-HT$_4$ receptors, previous studies showed its similar behavioral effects compared to other full receptor agonists [16,26]. Additionally, the agonist efficacy of a drug also depends on the system in which it is evaluated as the receptor density or coupling efficiency can differ [28]. RS67333 shows high binding affinity for the 5-HT$_4$ receptor with a pKi of 8.7 [29,30]. Except for the sigma receptors, which are bound at affinities comparable to 5-HT$_4$ receptors (σ1: pKi = 8.9; and σ2: pKi = 8.0), RS67333 has a pKi of less than 6.7 for other neurotransmitters' receptors [31]. While we cannot rule out a role of the sigma receptor, GR125487 a 5-HT$_4$ receptor antagonist with no sigma receptor affinity blocked the RS67333-induced precognitive effects [32,33], supporting our hypothesis that 5-HT$_4$ receptor is prominently implicated in the modulation of mood and cognitive behaviors of RS67333.

In the novel object recognition test, RS6733 and fluoxetine had similar effects. Interestingly, neurochemical and behavioral studies suggest that 5-HT$_4$ receptors may play an important role in cognition processes through an interaction between the cholinergic and/or histaminergic systems in the hippocampus or in the cortical areas ([11,34,35] for review). Stimulation of 5-HT$_4$ receptors increases extracellular histamine and acetylcholine levels in rodents in these brain regions involved in cognitive function. In bulbectomized mice, a mouse model of depression, chronic fluoxetine was able to restore normal exploratory behavior in the novel object test. In this model in which a 2.5 fold increase in corticosterone levels in serum was observed, chronic fluoxetine was also able to reverse the stress-induced increase in hippocampal acetylcholine esterase (AChE), but also to reduce AChE activity in the prefrontal cortex, corroborating the notion that this SSRI modulates cholinergic neurotransmission [36]. These cholinergic mechanisms may participate to chronic RS67333 and/or fluoxetine-reversed corticosterone induced behavioral deficit in the novelty in the novel object.

In the present study, differential fluoxetine and RS67333 behavioral cognitive effects also rely on different mechanisms of action characterizing these drugs. Whereas RS67333 selectively stimulates 5-HT$_4$ receptors [29], fluoxetine acts as an indirect 5-HT receptors' agonist, by selectively inhibiting serotonin re-uptake. Beneficial as well as detrimental cognitive effects of fluoxetine may thus involve other serotoninergic receptors, which play a crucial role in learning and memory processes [14,37,38]. For instance, current strategies blocking 5-HT$_3$, 5-HT$_6$ and 5-HT$_7$ receptors have been shown to improve cognition [13,39,40]. In regards to the detrimental effects of fluoxetine, a recent report using the novel object recognition, suggest that 5-HT$_{2A}$ receptors activation is involved. Indeed, fluoxetine (10 mg/kg i.p.) impaired mice performance test 24 h post-administration [41]. Multiple medication therapy, based on the modulation of these receptors, may represent a more efficient strategy than the use of each drug alone to alleviate cognition deficits in MDD.

Preclinical evidences for cognitive efficacy of SSRIs in animal models of depression are inconsistent (See [42] for review). Effects of chronic fluoxetine on stress-induced memory impairments vary according to the nature of the task and the chronic stress procedures. For example, stress-induced deficits in episodic-like memory was reversed [43] or unchanged in the recognition task [44] following a chronic fluoxetine treatment. Depending on the spatial navigation task, a chronic fluoxetine treatment produced either reversal in deficits [45,46], no effect in the Morris water maze or

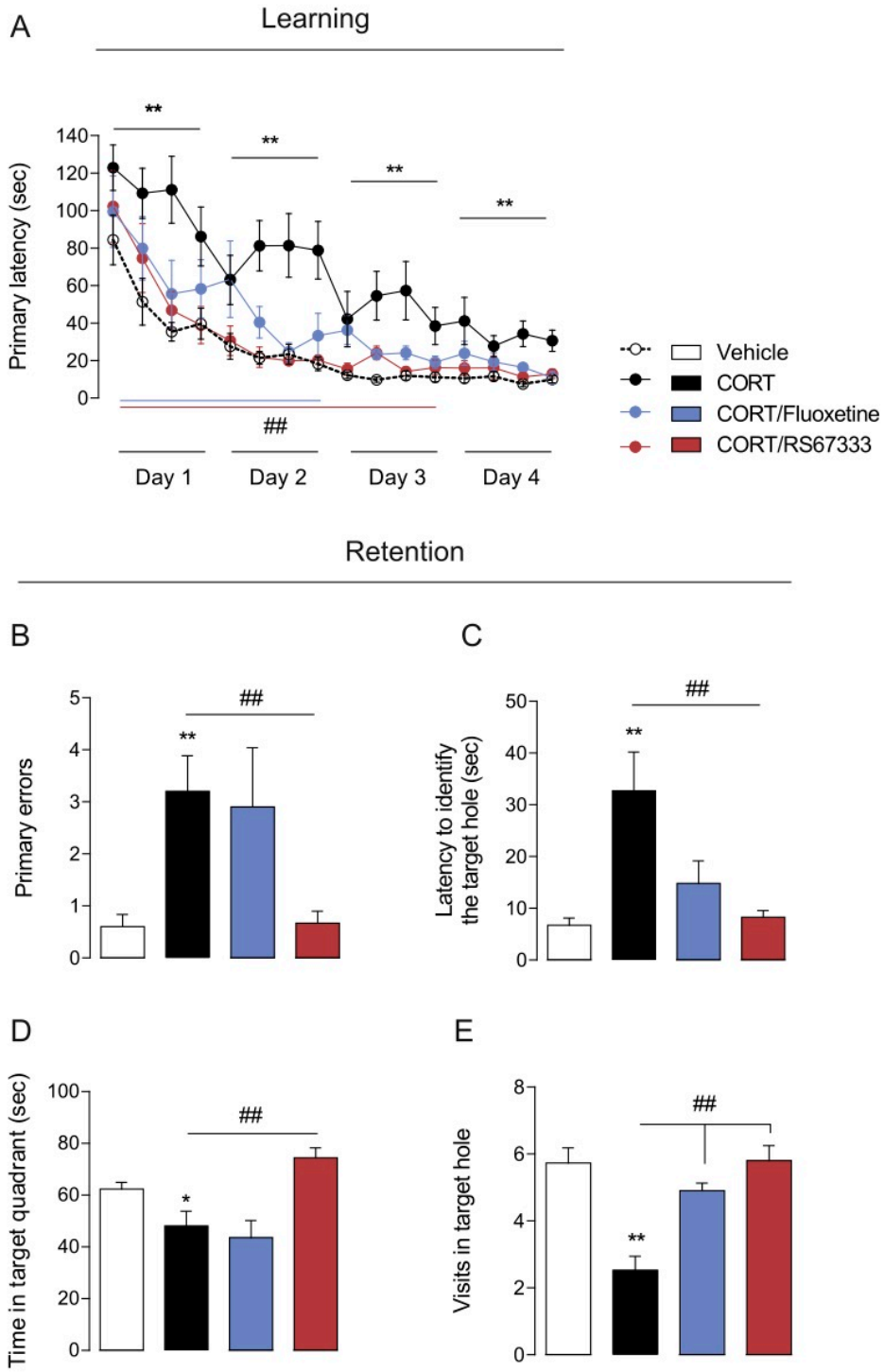


Fig. 2. Effects of chronic fluoxetine or 5-HT_4 receptor agonist treatments on CORT-induced spatial memory deficit. Animals were trained during 4 days to learn the location of the target box. A probe trial estimates reference memory (Day 5). During acquisition (Day 1–4), learning was monitored by recording primary latency during the training sessions (A). During retention trial (Day 5, B–E), the target box was removed and blocked. Reference memory retention was evaluated by measuring the latency to identify the target hole (B), primary errors (C), the time spent into the target quadrant (D) and the number of visits in the target hole (E). Values are mean ± SEM, n = 10–15 animals per group; *p < 0.05; **p < 0.01 *versus* vehicle group; ##p < 0.01 *versus* CORT-treated group.

impairment in the Radial arm water maze [47]. In our study using the Barnes Maze, opposing results regarding learning parameters (latency and errors) after a chronic fluoxetine treatment reopen the question whether mice actually used a spatial strategy to solve the task [48]. Here, fluoxetine-treated mice adopted a "serial" search strategy, by randomly choosing a hole and visiting adjacent holes until they found the target hole (see Fig. S4A and B). This suggests that these mice correctly learned the rule (since their latency to identify the hole decreased over time), but did not use, or partially, the spatial visual cues to locate the target hole.

To our knowledge, no study has focused on the effects of fluoxetine in the contextual fear-conditioning task in animal models of depression. Indeed, most of the studies investigating effects of SSRIs in conditioned fear response were conducted in naïve animals (see Ref. [49] for review). Following sub-chronic or chronic SSRI treatments in rats, freezing behavior was found to be decreased [50–52] or unchanged [53] compared to controls. Interestingly, Karpova et al. [54] showed that a 3-weeks fluoxetine treatment did not change fear-conditioning behavior, but had a positive synergistic effect when combined with extinction episodes to lose fearful memories. Additionally, chronic citalopram (another SSRI) treatment has been reported to impair the acquisition of contextual fear conditioning in naïve rats because of its anxiolytic-like activity [52]. A direct role of the NR2B subunit in the amygdala in mediating these effects, *i.e.*, a downregulation of the NR2B subunit, has been suggested [49]. Similar mechanism cannot be ruled out for fluoxetine since anxiolytic-like effects have been observed in our study and elsewhere in the CORT model [9,20]. Nevertheless, additional studies are needed to understand the link between SSRIs and fear conditioning in a pathological stressful context.

From a clinical point of view, several studies support the idea that monoaminergic antidepressant drugs may ameliorate the cognitive functions of depressed patients. Specifically, fluoxetine was found to improve memory and mental processing speed [55,56]. However, some studies did not observe any improvement in cognitive functions after antidepressant drug treatment in depressed patients [6,57], or that medication is ineffective without additional psychotherapeutic intervention [7].

By restoring all of the CORT-induced cognitive deficits as well as anxio/depressive-like symptoms, the present study highlighted 5-HT_4 receptor agonists as a promising antidepressive strategy that may also treat cognitive-associated symptoms in MDD. Since 5-HT_4 receptor density does not decrease with age, it may also represent a new strategy to treat cognitive deficits in elderly patients with or without MDD.

Disclosures

DJD serves as a consultant for Lundbeck, Roche, and Servier. All other authors report no biomedical financial interests or potential conflicts of interest.

Acknowledgements

This work was supported by the technical assistance of Valerie Dupont-Domergue and the staff of the animal care facility of the SFR-UMS Institut Paris Saclay Innovation Thérapeutique. This work was supported by the Brain & Behavior Research Foundation (formerly NARSAD) and the Pierre Deniker Foundation (DJD), by the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche (MENESR, Paris, France) Fellowship (FD). We thank Pr Rene Hen (Columbia University) for insightful comments on this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2016.01.055.

References

[1] R.S. Lee, D.F. Hermens, M.A. Porter, M.A. Redoblado-Hodge, A meta-analysis of cognitive deficits in first-episode major depressive disorder, J. Affect. Disord. 140 (2012) 113–124.
[2] M.J. Millan, Y. Agid, M. Brune, E.T. Bullmore, C.S. Carter, N.S. Clayton, R. Connor, S. Davis, B. Deakin, R.J. DeRubeis, B. Dubois, M.A. Geyer, G.M. Goodwin, P. Gorwood, T.M. Jay, M. Joels, I.M. Mansuy, A. Meyer-Lindenberg, D. Murphy, E. Rolls, B. Saletu, M. Spedding, J. Sweeney, M. Whittington, L.J. Young, Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy, Nat. Rev. Drug Discov. 11 (2012) 141–168.
[3] R.J. Porter, P. Gallagher, J.M. Thompson, A.H. Young, Neurocognitive impairment in drug-free patients with major depressive disorder, Br. J. Psychiatry 182 (2003) 214–220.
[4] S.L. Naismith, I.B. Hickie, K. Turner, C.L. Little, V. Winter, P.B. Ward, K. Wilhelm, P. Mitchell, G. Parker, Neuropsychological performance in patients with depression is associated with clinical, etiological and genetic risk factors, J. Clin. Exp. Neuropsychol. 25 (2003) 866–877.
[5] I.H. Gotlib, J. Joormann, Cognition and depression: current status and future directions, Annu. Rev. Clin. Psychol. 6 (2010) 285–312.
[6] M.E. Culang, J.R. Sneed, J.G. Keilp, B.R. Rutherford, G.H. Pelton, D.P. Devanand, S.P. Roose, Change in cognitive functioning following acute antidepressant treatment in late-life depression, Am. J. Geriatric Psychiatry 17 (2009) 881–888.
[7] A.G. Bastos, L.S. Guimaraes, C.M. Trentini, Neurocognitive changes in depressed patients in psychodynamic psychotherapy, therapy with fluoxetine and combination therapy, J. Affect. Disord. 151 (2013) 1066–1075.
[8] G.I. Papakostas, Antidepressants and their effect on cognition in major depressive disorder, J. Clin. Psychiatry 76 (2015) e1046.
[9] I. Mendez-David, D.J. David, F. Darcet, M.V. Wu, S. Kerdine-Romer, A.M. Gardier, R. Hen, Rapid anxiolytic effects of a 5-HT(4) receptor agonist are mediated by a neurogenesis-independent mechanism, Neuropsychopharmacology 39 (2014) 1366–1378.
[10] B.A. Samuels, I. Mendez-David, C. Faye, S.A. David, K.A. Pierz, A.M. Gardier, R. Hen, D.J. David, Serotonin 1A and serotonin 4 receptors: essential mediators of the neurogenic and behavioral actions of antidepressants, Neuroscientist (2014).
[11] V. Lelong, L. Lhonneur, F. Dauphin, M. Boulouard, BIMU 1 and RS 67333 two 5-HT4 receptor agonists, modulate spontaneous alternation deficits induced by scopolamine in the mouse, Naunyn Schmiedebergs Arch. Pharmacol. 367 (2003) 621–628.
[12] M. Orsetti, A. Dellarole, S. Ferri, P. Ghi, Acquisition, retention, and recall of memory after injection of RS67333, a 5-HT(4) receptor agonist, into the nucleus basalis magnocellularis of the rat, Learn. Memory 10 (2003) 420–426.
[13] A. Quiedeville, M. Boulouard, K. Hamidouche, V. Da Silva Costa-Aze, G. Nee, C. Rochais, P. Dallemagne, F. Fabis, T. Freret, V. Bouet, Chronic activation of 5-HT4 receptors or blockade of 5-HT6 receptors improve memory performances, Behav. Brain Res. 293 (2015) 10–17.
[14] M.V. King, C.A. Marsden, K.C. Fone, A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory, Trends Pharmacol. Sci. 29 (2008) 482–492.
[15] A.C. Lo, J.H. De Maeyer, B. Vermaercke, Z. Callaerts-Vegh, J.A. Schuurkes, R. D'Hooge, SSP-002392 a new 5-HT4 receptor agonist, dose-dependently reverses scopolamine-induced learning and memory impairments in C57Bl/6 mice, Neuropharmacology 85 (2014) 178–189.
[16] M. Cachard-Chastel, S. Devers, S. Sicsic, M. Langlois, F. Lezoualc'h, A.M. Gardier, C. Belzung, Prucalopride and donepezil act synergistically to reverse scopolamine-induced memory deficit in C57Bl/6j mice, Behav. Brain Res. 187 (2008) 455–461.
[17] G. Patki, N. Solanki, F. Atrooz, F. Allam, S. Salim, Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress, Brain Res. 1539 (2013) 73–86.
[18] S.H. Richter, B. Zeuch, K. Lankisch, P. Gass, D. Durstewitz, B. Vollmayr, Where have I been? Where should I go? Spatial working memory on a radial arm maze in a rat model of depression, PLoS One 8 (2013) e62458.
[19] J.A. Markham, A.R. Taylor, S.B. Taylor, D.B. Bell, J.I. Koenig, Characterization of the cognitive impairments induced by prenatal exposure to stress in the rat, Front. Behav. Neurosci. 4 (2010) 173.
[20] D.J. David, B.A. Samuels, Q. Rainer, J.W. Wang, D. Marsteller, I. Mendez, M. Drew, D.A. Craig, B.P. Guiard, J.P. Guilloux, R.P. Artymyshyn, A.M. Gardier, C. Gerald, I.A. Antonijevic, E.D. Leonardo, R. Hen, Neurogenesis-dependent and −independent effects of fluoxetine in an animal model of anxiety/depression, Neuron 62 (2009) 479–493.
[21] F. Darcet, I. Mendez-David, L. Tritschler, A.M. Gardier, J.P. Guilloux, D.J. David, Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression, Front. Behav. Neurosci. 8 (2014) 136.
[22] J. Pascual-Brazo, E. Castro, A. Diaz, E.M. Valdizan, F. Pilar-Cuellar, R. Vidal, B. Treceno, A. Pazos, Modulation of neuroplasticity pathways and antidepressant-like behavioural responses following the short-term (3 and 7 days) administration of the 5-HT(4) receptor agonist RS67333, Int. J. Neuropsychopharmacol. 15 (2012) 631–643.
[23] A. Sahay, K.N. Scobie, A.S. Hill, C.M. O'Carroll, M.A. Kheirbek, N.S. Burghardt, A.A. Fenton, A. Dranovsky, R. Hen, Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation, Nature 472 (2011) 466–470.
[24] M.R. Drew, C.A. Denny, R. Hen, Arrest of adult hippocampal neurogenesis in mice impairs single- but not multiple-trial contextual fear conditioning, Behav. Neurosci. 124 (2010) 446–454.
[25] D.J. Fontana, S.E. Daniels, E.H. Wong, R.D. Clark, R.M. Eglen, The effects of novel, selective 5-hydroxytryptamine (5-HT) 4 receptor ligands in rat spatial navigation, Neuropharmacology 36 (1997) 689–696.
[26] G. Lucas, V. Compan, Y. Charnay, R.L. Neve, E.J. Nestler, J. Bockaert, M. Barrot, G. Debonnel, Frontocortical 5-HT4 receptors exert positive feedback on

serotonergic activity: viral transfections, subacute and chronic treatments with 5-HT4 agonists, Biol. Psychiatry 57 (2005) 918–925.
[27] E. Gomez-Lazaro, L. Garmendia, G. Beitia, J. Perez-Tejada, A. Azpiroz, A. Arregi, Effects of a putative antidepressant with a rapid onset of action in defeated mice with different coping strategies, Progress Neuro Psychopharmacol. Biol. Psychiatry 38 (2012) 317–327.
[28] J.H. De Maeyer, R.A. Lefebvre, J.A. Schuurkes, 5-HT4 receptor agonists: similar but not the same, Neurogastroenterol. Motil. 20 (2008) 99–112.
[29] J. Bockaert, S. Claeysen, V. Compan, A. Dumuis, 5-HT4 receptors, Current drug targets, CNS Neurol. Disord. 3 (2004) 39–51.
[30] R.M. Eglen, D.W. Bonhaus, L.G. Johnson, E. Leung, R.D. Clark, Pharmacological characterization of two novel and potent 5-HT4 receptor agonists, RS 67333 and RS 67506, in vitro and in vivo, Br. J. Pharmacol. 115 (1995) 1387–1392.
[31] http://pdsp.med.unc.edu/pdsp.php.
[32] T. Freret, V. Bouet, A. Quiedeville, G. Nee, P. Dallemagne, C. Rochais, M. Boulouard, Synergistic effect of acetylcholinesterase inhibition (donepezil) and 5-HT(4) receptor activation (RS67333) on object recognition in mice, Behav. Brain Res. 230 (2012) 304–308.
[33] L. Lamirault, H. Simon, Enhancement of place and object recognition memory in young adult and old rats by RS 67333, a partial agonist of 5-HT4 receptors, Neuropharmacology 41 (2001) 844–853.
[34] D.E. Johnson, E. Drummond, S. Grimwood, A. Sawant-Basak, E. Miller, E. Tseng, L.L. McDowell, M.A. Vanase-Frawley, K.E. Fisher, D.M. Rubitski, K.J. Stutzman-Engwall, R.T. Nelson, W.E. Horner, R.R. Gorczyca, M. Hajos, C.J. Siok, The 5-hydroxytryptamine4 receptor agonists prucalopride and PRX-03140 increase acetylcholine and histamine levels in the rat prefrontal cortex and the power of stimulated hippocampal theta oscillations, J. Pharmacol. Exp. Ther. 341 (2012) 681–691.
[35] R. Penas-Cazorla, M.T. Vilaro, Serotonin 5-HT4 receptors and forebrain cholinergic system: receptor expression in identified cell populations, Brain Struct. Funct. 220 (2015) 3413–3434.
[36] D.G. Machado, M.P. Cunha, V.B. Neis, G.O. Balen, A. Colla, J. Grando, P.S. Brocardo, L.E. Bettio, J.C. Capra, A.L. Rodrigues, Fluoxetine reverses depressive-like behaviors and increases hippocampal acetylcholinesterase activity induced by olfactory bulbectomy, Pharmacol. Biochem. Behav. 103 (2012) 220–229.
[37] A.V. Terry Jr., J.J. Buccafusco, W.J. Jackson, M.A. Prendergast, D.J. Fontana, E.H. Wong, D.W. Bonhaus, P. Weller, R.M. Eglen, Enhanced delayed matching performance in younger and older macaques administered the 5-HT4 receptor agonist, RS 17017, Psychopharmacology 135 (1998) 407–415.
[38] C. Lecoutey, D. Hedou, T. Freret, P. Giannoni, F. Gaven, M. Since, V. Bouet, C. Ballandonne, S. Corvaisier, A. Malzert Freon, S. Mignani, T. Cresteil, M. Boulouard, S. Claeysen, C. Rochais, P. Dallemagne, Design of donecopride, a dual serotonin subtype 4 receptor agonist/acetylcholinesterase inhibitor with potential interest for Alzheimer's disease treatment, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) E3825–3830.
[39] G. Beaudet, V. Bouet, C. Jozet-Alves, P. Schumann-Bard, F. Dauphin, E. Paizanis, M. Boulouard, T. Freret, Spatial memory deficit across aging: current insights of the role of 5-HT7 receptors, Front. Behav. Neurosci. 8 (2014) 448.
[40] D.J. Fontana, S.E. Daniels, C. Henderson, R.M. Eglen, E.H. Wong, Ondansetron improves cognitive performance in the Morris water maze spatial navigation task, Psychopharmacology 120 (1995) 409–417.
[41] A. Castane, L. Kargieman, P. Celada, A. Bortolozzi, F. Artigas, 5-HT2A receptors are involved in cognitive but not antidepressant effects of fluoxetine, Eur. Neuropsychopharmacol. 25 (2015) 1353–1361.
[42] A.L. Pehrson, S.C. Leiser, M. Gulinello, E. Dale, Y. Li, J.A. Waller, C. Sanchez, Treatment of cognitive dysfunction in major depressive disorder–a review of the preclinical evidence for efficacy of selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors and the multimodal-acting antidepressant vortioxetine, Eur. J. Pharmacol. 753 (2015) 19–31.
[43] W. El Hage, S. Peronny, G. Griebel, C. Belzung, Impaired memory following predatory stress in mice is improved by fluoxetine, Progress Neuro Psychopharmacol. Biol. Psychiatry 28 (2004) 123–128.
[44] M. Bianchi, K.C. Fone, A.J. Shah, A.R. Atkins, L.A. Dawson, C.A. Heidbreder, J.J. Hagan, C.A. Marsden, Chronic fluoxetine differentially modulates the hippocampal microtubular and serotonergic system in grouped and isolation reared rats, Eur. Neuropsychopharmacol. 19 (2009) 778–790.
[45] L. Song, W. Che, W. Min-Wei, Y. Murakami, K. Matsumoto, Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress, Pharmacol. Biochem. behavior 83 (2006) 186–193.
[46] D. Ibi, K. Takuma, H. Koike, H. Mizoguchi, K. Tsuritani, Y. Kuwahara, H. Kamei, T. Nagai, Y. Yoneda, T. Nabeshima, K. Yamada, Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice, J. Neurochem. 105 (2008) 921–932.
[47] E. Gumuslu, O. Mutlu, D. Sunnetci, G. Ulak, I.K. Celikyurt, N. Cine, F. Akar, The effects of tianeptine, olanzapine and fluoxetine on the cognitive behaviors of unpredictable chronic mild stress-exposed mice, Drug Res. 63 (2013) 532–539.
[48] F.E. Harrison, R.S. Reiserer, A.J. Tomarken, M.P. McDonald, Spatial and nonspatial escape strategies in the Barnes maze, Learn. Memory 13 (2006) 809–819.
[49] N.S. Burghardt, E.P. Bauer, Acute and chronic effects of selective serotonin reuptake inhibitor treatment on fear conditioning: implications for underlying fear circuits, Neuroscience 247 (2013) 253–272.
[50] S. Hashimoto, T. Inoue, I. Muraki, T. Koyama, Effects of acute citalopram on the expression of conditioned freezing in naive versus chronic citalopram-treated rats, Progress Neuro Psychopharmacol. Biol. Psychiatry 33 (2009) 113–117.
[51] J.M. Santos, R.C. Martinez, M.L. Brandao, Effects of acute and subchronic treatments with fluoxetine and desipramine on the memory of fear in moderate and high-intensity contextual conditioning, Eur. J. Pharmacol. 542 (2006) 121–128.
[52] N.S. Burghardt, G.M. Sullivan, B.S. McEwen, J.M. Gorman, J.E. LeDoux, The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: a comparison with tianeptine, Biol. Psychiatry 55 (2004) 1171–1178.
[53] G. Spennato, C. Zerbib, C. Mondadori, R. Garcia, Fluoxetine protects hippocampal plasticity during conditioned fear stress and prevents fear learning potentiation, Psychopharmacology 196 (2008) 583–589.
[54] N.N. Karpova, A. Pickenhagen, J. Lindholm, E. Tiraboschi, N. Kulesskaya, A. Agustsdottir, H. Antila, D. Popova, Y. Akamine, A. Bahi, R. Sullivan, R. Hen, L.J. Drew, E. Castren, Fear erasure in mice requires synergy between antidepressant drugs and extinction training, Science 334 (2011) 1731–1734.
[55] Y. Levkovitz, R. Caftori, A. Avital, G. Richter-Levin, The SSRIs drug Fluoxetine but not the noradrenergic tricyclic drug Desipramine, improves memory performance during acute major depression, Brain Res. Bull. 58 (2002) 345–350.
[56] R. Gallassi, R. Di Sarro, A. Morreale, M. Amore, Memory impairment in patients with late-onset major depression: the effect of antidepressant therapy, J. Affect. Disord. 91 (2006) 243–250.
[57] J.M. Ferguson, K.A. Wesnes, G.E. Schwartz, Reboxetine versus paroxetine versus placebo: effects on cognitive functioning in depressed patients, Int. Clin. Psychopharmacol. 18 (2003) 9–14.

Chronic 5-HT_4 receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression

Flavie Darcet, Alain M Gardier, Denis J. David, Jean-Philippe Guilloux

INSERM UMR-S 1178, Univ. Paris-Sud, Université Paris-Saclay, Châtenay-Malabry, 92290, France

Supplementary Elements

1 Supplementary Methods

1.1 The mouse CORT model

The model of elevated glucocorticoids (also named "CORT model") is able to blunt the response of the hypothalamic-pituitary-adrenal axis (HPA) as shown by the markedly attenuated stress-induced corticosterone levels observed in these mice [1]. This model displays hallmark characteristics of anxiety and depression. The dose and duration of corticosterone treatment was selected based on previous studies [1, 2].

1.2 Anxiety and depression behavior paradigms

1.2.1 Mouse body weight

Mouse body weight for each animal was followed once a week during all the procedure.

1.2.2 Fur Coat State

The score corresponding to the state of the coat resulted from the sum of the score of five different body parts: head, neck, dorsal/ventral coat, tail and fore-/hind paws. For each body area, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat [1, 3]. Fur coat state and mouse body weight was followed weekly during the whole treatment period.

1.2.3 Open Field paradigm (OF)

Motor activity was quantified in Plexiglas OF boxes 43x43 cm (MED associates, Georgia, VT, USA) during a 10-min session [4]. Two sets of 16 pulse-modulated infrared photobeams were placed on opposite walls 2.5 cm apart to record x-y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100 ms resolution. The center was defined as a 32x32-cm central arena. Dependent measures were: total time spent in the center, the numbers

of entries into the center and distance travelled in the center divided by total distance travelled. Overall motor activity was quantified as the total distance travelled (cm).

1.2.4 Splash Test (ST)

This test consisted in squirting a 10% sucrose solution on the mouse's snout. The grooming duration was then recorded over a 5 min period in the home cage of the animal [5].

1.3 Cognition behavioral paradigms

1.3.1 Episodic memory: novel object recognition test (NORT)

The NORT was divided into 4 training sessions and one test session as described previously [6]. Each exposure lasted 5 minutes with a 3-min inter-trial interval. During training sessions, two identical objects were present in the box and the mouse was allowed to freely explore the apparatus and the objects. During the test session, one of the familiar objects was removed from the cage and replaced by a novel object. Object exploration was defined as the orientation of the nose to the object at a distance ≤ 2 cm. Ambulatory activity was controlled during the entire experiment and objects exploration was hand-scored by an experimenter. Objects were cleaned with 70 % ethanol between trials to avoid olfactory cues. Results for this test were expressed as: 1) exploration (in percent) of each object during the test session and 2) a discrimination index (DI) between objects during the test session, calculated as the difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects (DI=(N-F)/(N+F)).

1.3.2 Associative/Contextual memory: one-trial contextual fear conditioning (FC)

The experimental design ran over two consecutive days. On Day 1, mice were placed in the conditioning chamber and, 3 min later, received one shock (2 sec., 0.75 mA). Mice were removed 15 sec after the shock. On Day 2, animals returned to the conditioning chamber for a 4-min period in the exact same conditions of Day 1, but without electrical shock, for a test of context-elicited freezing. Training chambers were cleaned with 70% ethanol solution before and after each trial. Scoring was measured using Freezing software version 2.0.04 (Packwin, Harvard apparatus, Bioseb, France).

1.3.3 Spatial reference learning and memory: Barnes maze paradigm (BM)

Spatial acquisition was organized in 4 training sessions (Day 1 to Day 4). Each training session consisted in four 3-min trials, with 15-20 min intertrial intervals during which animals were returned to their home cage. Primary latency and primary errors to identify the target hole were manually scored during all learning trials. A hole was considered visited when mice tilted their head over it (nose poke) or introduced their paws into the hole. On Day 5, reference memory was evaluated by a retention probe trial (90 sec) during which the target box was removed and the target hole was closed. Mice were allowed to explore the maze and to visit the target and the adjacent holes. Latency to reach the target hole for the first time, number of errors before reaching the target hole, number of visits in the target hole and time spent in the target quadrant were recorded (ANY-maze Software)

1.4 Statistics

Results from data analyses were expressed as mean ± SEM. Statistical analyses were processed with Statview 5.0® Software (SAS Institute, Cary, NC). For all experiments, comparisons between CORT-treated and control animals were performed by using *t*-tests or one-way ANOVA with

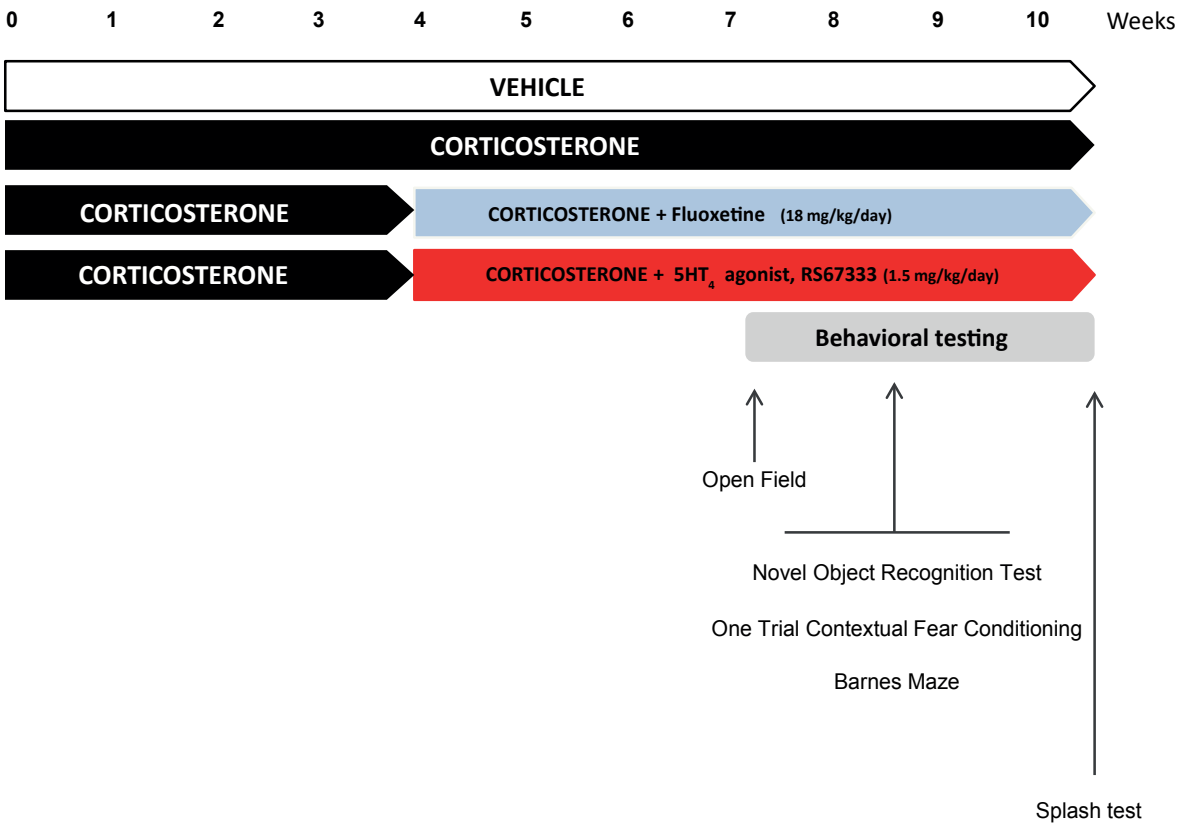
repeated-measures when appropriate. One-way ANOVA alone or with repeated-measures were applied to the data as appropriate. Significant main effects and/or interactions were followed by Fisher's PLSD *post hoc* analysis. One sample *t*-tests were used to compare the percent of time exploring the novel object *versus* the chance level (50%) in the novel object recognition test and the time spent in the target quadrant *versus* the chance level (22.5 sec) in the BM test. Statistical significance was set at $p<0.05$. A summary of statistical measures is included in **Supplementary Tables 1 and 2.**

2 Supplementary Figures

2.1 Supplementary Figure 1: Experimental design.

C57BL/6JRj mice received a 4-weeks corticosterone treatment in drinking water to induce the anxio-depressed phenotype. Mice were then given a 4-week chronic fluoxetine treatment in drinking water or the 5-HT_4 receptor agonist (RS67333) treatment via osmotic mini-pumps. Mice were then subjected to a complete characterization of cognitive functions in episodic-like, spatial and associative domains. Each animal was successively tested in the open field paradigm, the novel object recognition test, the Barnes maze, the one-trial contextual fear conditioning and the Splash test.

Figure S1


0
1
2
3
4
5
6
7
8
9
10
Weeks
VEHICLE
CORTICOSTERONE
CORTICOSTERONE
CORTICOSTERONE + Fluoxetine (18 mg/kg/day)
CORTICOSTERONE
CORTICOSTERONE + 5HT4 agonist, RS67333 (1.5 mg/kg/day)
Behavioral testing
Open Field
Novel Object Recognition Test
One Trial Contextual Fear Conditioning
Barnes Maze
Splash test

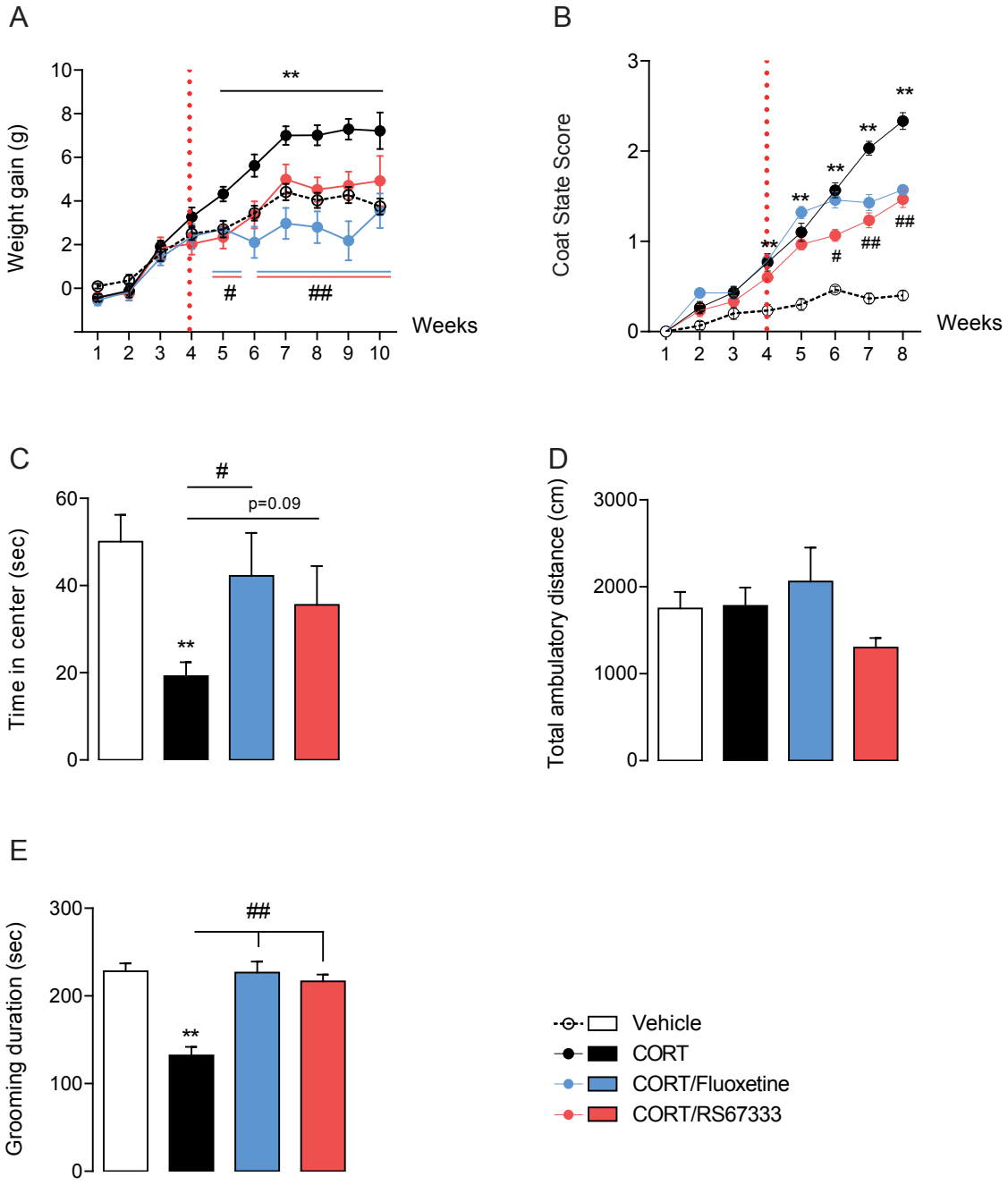
2.2 Supplementary Figure 2: 4 weeks of fluoxetine (18 mg/kg/day) or RS67333 (1.5 mg/kg/day) reversed increase in mouse body weight, deterioration of the coat state and anxio-depressed phenotype induced by chronic corticosterone.

The effects of 4 weeks of RS67333 (1.5 mg/kg/day) and fluoxetine (18 mg/kg/day) treatments started after 4 weeks of corticosterone regimen (35 µg/ml) on mouse body weight (in g) (A), on coat state (B), on anxiety (C) and depression-like (D) phenotypes were followed during all the procedure.

Chronic RS67333 and fluoxetine treatments reversed chronic corticosterone induced increase in mouse body weight (A) and deterioration of the coat state (B).

Anxiety, measured for various parameters in the center of OF paradigm, is expressed as mean total of the time-spent (in seconds) for the entire 10-min session (C) and also for the total ambulatory distance (D). A 4-week corticosterone regimen inducing anxiety-like phenotype was abolished with fluoxetine treatment. A trend for an increase in time spent in the center was observed for RS67333 treatment. Both drugs did not affect locomotor activity (D). Depressive-like phenotype assessed in the ST is expressed as mean duration of grooming (in seconds) after receiving a 10 % sucrose solution on the snout. Decrease in grooming duration induced with chronic corticosterone was abolished with a 4 week RS67333 and fluoxetine treatment (E) Values are mean ± SEM (n=10-15 animals per group). PLSD post hoc test: **p<0.01 *versus* vehicle group; # p<0.05, ## p<0.01 *versus* CORT-treated group.

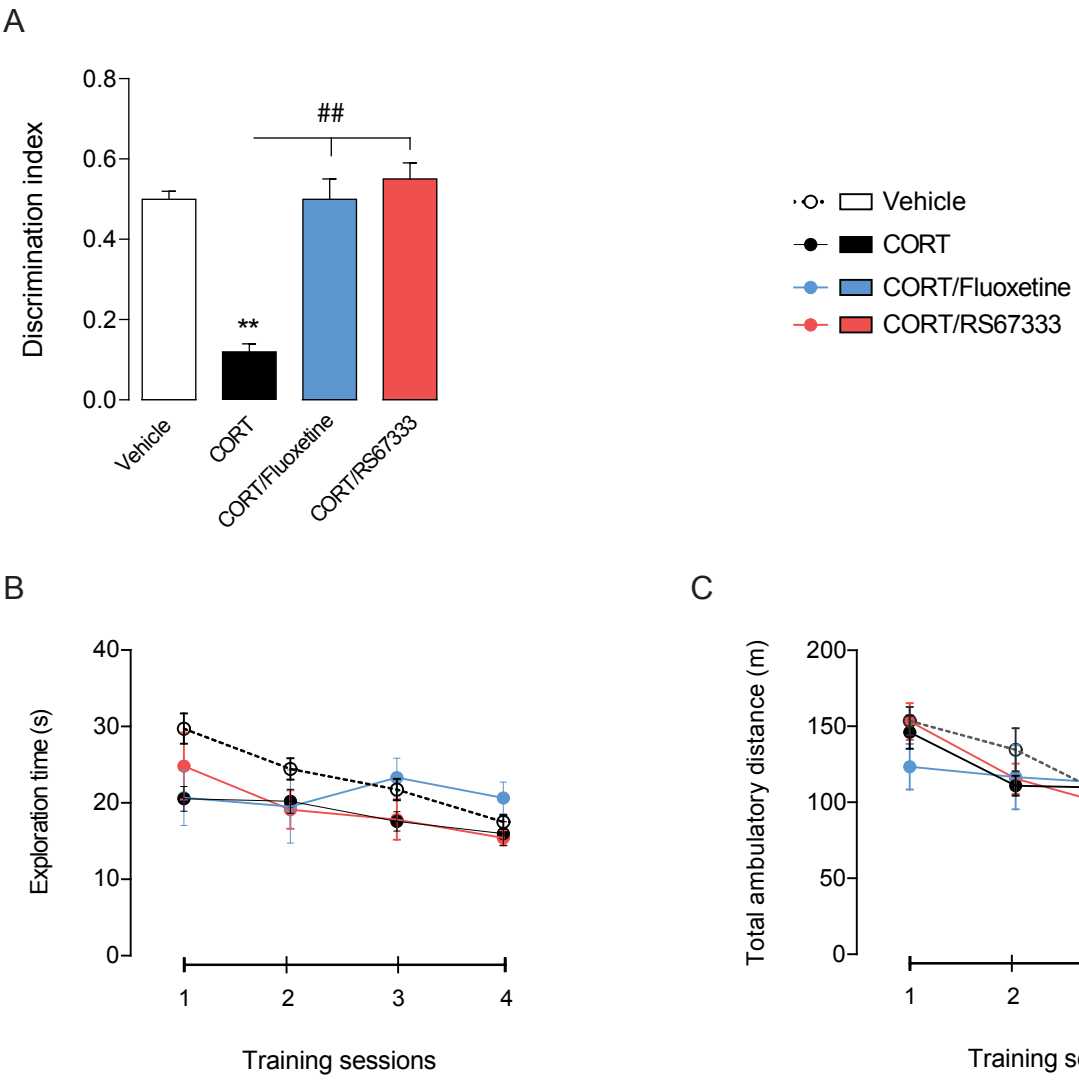
Figure S2


A
Weight gain (g)
Weeks
B
Coat State Score
Weeks
C
Time in center (sec)
p=0.09
D
Total ambulatory distance (cm)
E
Grooming duration (sec)
Vehicle
CORT
CORT/Fluoxetine
CORT/RS67333

2.3 Supplementary Figure 3: 4 weeks of fluoxetine (18 mg/kg/day) or RS67333 (1.5 mg/kg/day) did not affect exploration behavior or motor activity in the novel object recognition test.

The effects of 4 weeks of RS67333 (1.5 mg/kg/day) and fluoxetine (18 mg/kg/day) treatments started after 4 weeks of corticosterone regimen (35 µg/ml) on both objects exploration time along learning sessions (A) and ambulatory distance during training (B) and test (C) sessions were followed during the NORT. None of these parameters was affected by either chronic corticosterone or drugs (RS67333, fluoxetine) treatments. Values are mean ± SEM (n=10-15 animals per group).

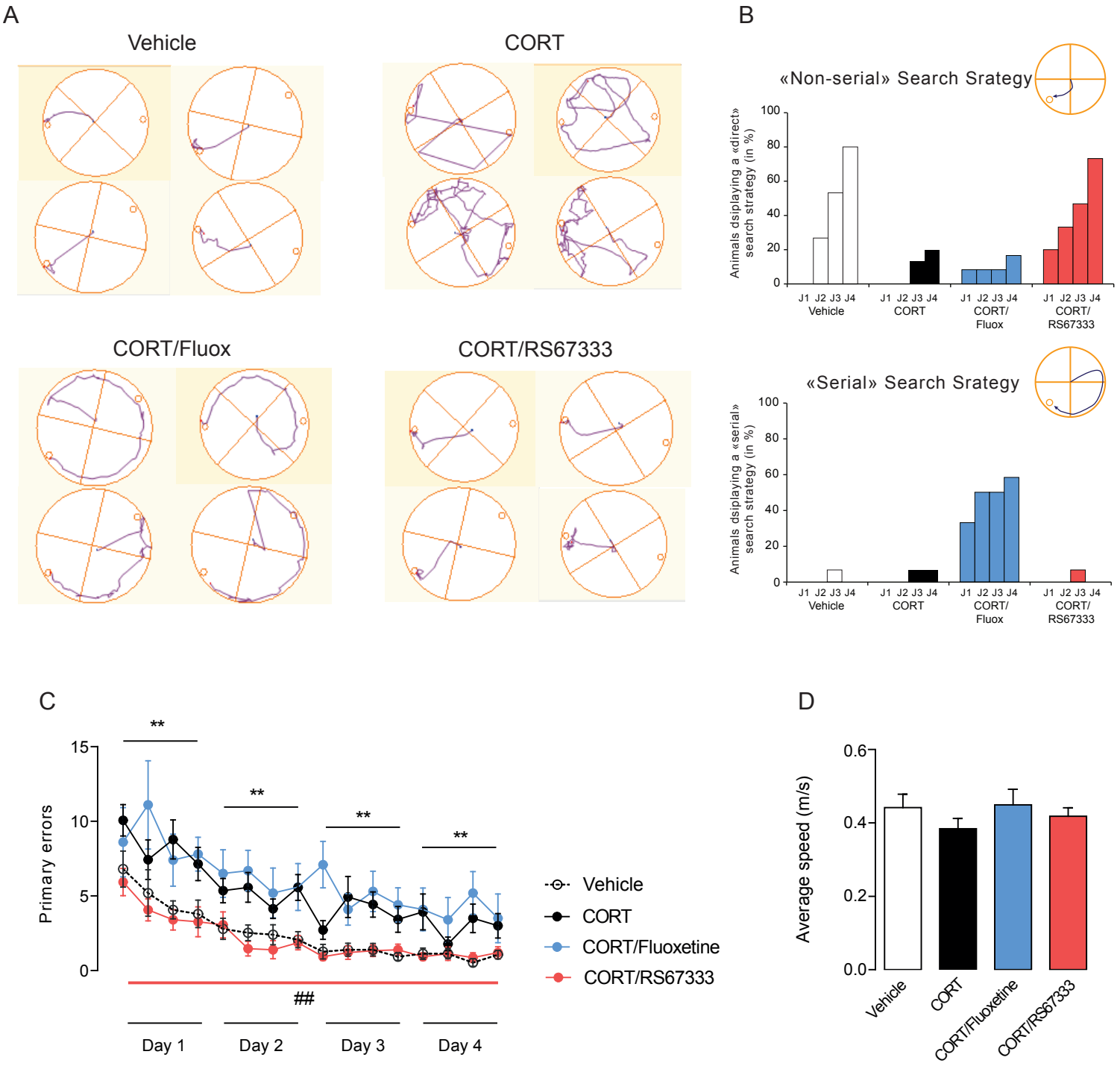
Figure S3


A
Discrimination index
0.8
0.6
0.4
0.2
0.0
##
**
Vehicle
CORT
CORT/Fluoxetine
CORT/RS67333
Vehicle
CORT
CORT/Fluoxetine
CORT/RS67333
B
Exploration time (s)
40
30
20
10
0
1
2
3
4
Training sessions
C
Total ambulatory distance (m)
200
150
100
50
0
1
2
3
4
Training sessions

2.4 Supplementary Figure 4: Distinct pattern searching strategies observed between fluoxetine (18 mg/kg/day) and RS67333 (1.5 mg/kg/day) –treated animals in Barnes maze paradigm.

Contradictory learning parameters revealed distinct pattern of searching strategies between CORT/Fluoxetine and CORT/RS67333 groups as shown with representative cartoons of 4 different animals per group (A). Percentage of animals displaying either a “non-serial” or “serial” search strategy during at least 2 out of 4 trials during the learning period (B). Number of errors before visiting the target hole (primary errors) scored along training sessions (C). Average speed during the first four trials of Day 1 training was measured to detect motivational variations between experimental groups (D).

Figure S4


A
Vehicle
CORT
CORT/Fluox
CORT/RS67333
B
«Non-serial» Search Sratégy
Animals dsiplaying a «direct» search strategy (in %)
100
80
60
40
20
0
J1 J2 J3 J4
Vehicle
CORT
CORT/ Fluox
CORT/ RS67333
«Serial» Search Sratégy
Animals dsiplaying a «serial» search strategy (in %)
C
Primary errors
15
10
5
0
**
##
Day 1
Day 2
Day 3
Day 4
Vehicle
CORT
CORT/Fluoxetine
CORT/RS67333
D
Average speed (m/s)
0.6
0.4
0.2
0.0
Vehicle
CORT
CORT/Fluoxetine
CORT/RS67333

2.5 Supplementary Table 1: complete statistical summary analysis for behavioral data after chronic corticosterone treatment

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Novel object recognition test	Exploration time (%)	*t*-test	CORT *vs* Veh	F=76.179	27	p<0.01**	1A
		One sample *t*-test	CORT *vs* 50%	t=17.967	13	p<0.01δ	
			Veh *vs* 50%	t=3.835	14	p<0.01δ	
	Discrimination Index	*t*-test	CORT *vs* Veh	F=76.179	27	p<0.01**	1B
	Exploration time across sessions	One-way ANOVA with repeated measures	Factor 1 Treatment	F=9.814	1, 39	p<0.01**	S3A
			Factor 2 Time	F=15.376	3, 39	p<0.01**	
			Interaction (F1 X F2)	F=3.056	3, 39	p>0.1	
		PLSD Post-hoc test	CORT *vs* Veh (Session 1, 3)			p<0.05*	
	Distance travelled across sessions	One-way ANOVA with repeated measures	Factor 1 Treatment	F=0.670	1, 81	p>0.05	S3B
			Factor 2 Time	F=24.289	3, 81	p<0.01**	
			Interaction (F1 X F2)	F=1.492	3, 81	p>0.1	
	Distance travelled (Probe test)	*t*-test	CORT *vs* Veh	F=0.559	27	p>0.5	S3C
One-trial contextual fear conditioning	Freezing duration (%)	*t*-test	CORT *vs* Veh	F=12.669	27	p<0.05*	1C
	Freezing episodes	*t*-test	CORT *vs* Veh	F=10.694	27	p<0.05*	1D
Barnes maze	Primary latency (acquisition)	One-way ANOVA with repeated measures	Factor 1 Treatment	F=136.7	1, 416	p<0.01**	2A
			Factor 2 Time	F=11.69	15, 416	p<0.01**	
			Interaction (F1 X F2)	F=1.366	15, 416	p>0.1	
		PLSD Post-hoc test	CORT *vs* Veh (Day 1 to Day 4)			p<0.01**	
	Primary errors (retention)	*t*-test	CORT *vs* Veh	F=13.210	27	p<0.01**	2B

	Primary latency (retention)	*t*-test	CORT *vs* Veh	F=10.985	27	p<0.01**	2C
	Time in target quadrant (retention)	*t*-test	CORT *vs* Veh	F=7.423	27	p<0.05*	2D
	Visits of the target hole (retention)	*t*-test	CORT *vs* Veh	F=24.568	27	p<0.01**	2E
	Primary errors (Acquisition)	One-way ANOVA with repeated measures	Factor 1 Treatment	F=82.26	1,432	p<0.01**	S4B
			Factor 2 Time	F=10.95	3,432	p<0.01**	
			Interaction (F1 X F2)	F=0.6566	3,432	p<0.05*	
		PLSD Post-hoc test	CORT *vs* Veh (Day 1 to Day 4)			p<0.01**	
	Average speed on Day 1	*t*-test	CORT *vs* Veh	F=1.842	27	p>0.5	S4C
Weight	Weight gain	One-way ANOVA with repeated measures	Factor 1 Treatment	F=45.50	2, 390	p<0.01**	S2A
			Factor 2 Time	F=41.42	9, 390	p<0.01**	
			Interaction (F1 X F2)	F=3.108	18, 390	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (from week 6 to 10)			p<0.01**	
Coat state	Score	One-way ANOVA with repeated measures	Factor 1 Treatment	F=580.6	1, 224	p<0.01**	S2B
			Factor 2 Time	F=110.6	7, 224	p<0.01**	
			Interaction (F1 X F2)	F=55.80	7, 224	p<0.01**	
		PLSD Post-hoc test	CORT *vs* Veh (Week 2 and 3)			p<0.05*	
			CORT *vs* Veh (Week 4 to week 7)			p<0.01**	
Open Field	Time in center (10')	*t*-test	CORT *vs* Veh	F=10.362	27	p<0.01**	S2C
	Ambulatory distance	*t*-test	CORT *vs* Veh	F=2.274	27	p>0.1	S2D
Splash test	Grooming duration	*t*-test	CORT *vs* Veh	F=53.04	27	p<0.01**	S2E

Legend: CORT: corticosterone; Veh: Vehicle

2.6 Supplementary Table 2: complete statistical summary analysis for behavioral data after chronic fluoxetine and RS67333 treatments

Behavioral paradigm	Measurement	Statistical Test	Comparison	Statistics	Degrees of freedom	p	Fig.
Novel object recognition test	Exploration time	One-way ANOVA	Factor Treatment	F=32.28	2.37	p<0.01##	1A
		PLSD Post-hoc test	CORT/Fluox *versus* CORT			p<0.01##	
			CORT/RS *versus* CORT			p<0.01##	
		One sample *t*-test	CORT/Fluox *vs* 50%	t=8.161	11	p<0.01 δ	
			CORT/RS *vs* 50%	t=17.069	14	p<0.01δ	
	Discrimination Index	One-way ANOVA	Factor Treatment	F=32.28	2,37	p<0.01##	1B
		PLSD Post-hoc test	CORT/Fluox versus CORT			p<0.01##	
			CORT/RS *versus* CORT			p<0.01##	
	Exploration time across sessions	One-way ANOVA with repeated measures	Factor 1 Treatment	F=0.8210	2, 56	p>0.1	S3A
			Factor 2 Time	F=1.384	3, 56	p>0.1	
			Interaction (F1 X F2)	F=0.7885	6, 56	p>0.1	
	Distance travelled across sessions	One-way ANOVA with repeated measures	Factor 1 Treatment	F=0.4758	2, 160	p>0.05	S3B
			Factor 2 Time	F=9.027	3, 160	p<0.01##	
			Interaction (F1 X F2)	F=1.840	6, 160	p>0.1	
	Distance travelled (Probe test)	One-way ANOVA	Factor Treatment	F=1.184	2, 39	p>0.5	S3C
One-trial contextual fear conditioning	Freezing duration (%)	One-way ANOVA	Factor Treatment	F=8.131	2, 39	p<0.05#	1C
		PLSD Post-hoc test	CORT/RS *versus* CORT			p<0.05#	
	Freezing episodes	One-way ANOVA	Factor Treatment	F=8.826	2, 39	p<0.01##	1D

		PLSD Post-hoc test	CORT/Fluox *versus* CORT			p<0.05#	
			CORT/RS *versus* CORT			p<0.05#	
Barnes maze	Primary latency	One-way repeated measures ANOVA	Factor 1 Treatment	F=44.37	2, 576	p<0.01##	2A
			Factor 2 Time	F=15.74	15, 576	p<0.01##	
			Interaction (F1 X F2)	F=0.8778	30, 576	p>0.6	
		PLSD Post-hoc test	CORT/Fluox *versus* CORT(Day1, Day 2)			p<0.01##	
			CORT/RS *versus* CORT (Day1, Day 2, Day 3)			p<0.01##	
	Primary errors (retention)	One-way ANOVA	Factor Treatment	F=6.248	2,37	p<0.01##	2B
		PLSD Post-hoc test	CORT/Fluox *versus* CORT			p<0.05#	
			CORT/RS *versus* CORT			p<0.01##	
	Primary latency (retention)	One-way ANOVA	Factor Treatment	F=4.497	2,37	p<0.05#	2C
		PLSD Post-hoc test	CORT/RS *versus* CORT			p<0.01##	
	Time in target quadrant (retention)	One-way ANOVA	Factor Treatment	F=9.801	2, 37	p<0.01##	2D
		PLSD Post-hoc test	CORT/RS *versus* CORT			p<0.01##	
	Visits of the target hole (retention)	One-way ANOVA	Factor Treatment	F=18.73	2, 37	p<0.01##	2E
		PLSD Post-hoc test	CORT/Fluox *versus* CORT			p<0.01##	

			CORT/RS *versus* CORT			p<0.01##	
	Primary errors (Acquisition)	One-way ANOVA with repeated measures	Factor 1 Treatment	F=61.51	2,576	p<0.01##	S4B
			Factor 2 Time	F=8.904	15, 576	p<0.01##	
			Interaction (F1 X F2)	F=0.8484	30, 576	p<0.05#	
			CORT/RS *versus* CORT (Day1 to Day 4)			p<0.01##	
	Mean speed on Day 1	One-way ANOVA	Factor Treatment	F=1.073	2, 37	p>0.1	S4C
Weight	Weight gain	One-way ANOVA with repeated measures	Factor 1 Treatment	F=81.74	1, 280	p<0.01##	S2A
			Factor 2 Time	F=67.46	9, 280	p<0.01##	
			Interaction (F1 X F2)	F=7.043	9, 280	p<0.01##	
		PLSD Post-hoc test	CORT/Fluox *versus* CORT (Week 5)			p<0.05#	
			CORT/RS *versus* CORT (Week 6)			p<0.05#	
			CORT/Fluox and CORT/RS *versus* CORT (Week 7-8)			p<0.01##	
Coat state	Score	One-way ANOVA with repeated measures	Factor 1 Treatment	F=42.74	2, 328	p<0.01##	S2B
			Factor 2 Time	F=252.9	7, 328	p<0.01##	
			Interaction (F1 X F2)	F=8.870	14, 328	p<0.01##	
		PLSD Post-hoc test	CORT/Fluox and CORT/RS *versus* CORT (Week 5)			p<0.05#	
			CORT/Fluox and			p<0.01##	

			CORT/RS *versus* CORT (Week 6-9)				
Open Field	Time in center (10')	One-way ANOVA	Factor Treatment	F=3.262	2, 39	p<0.05#	S2C
		PLSD Post-hoc test	CORT/Fluox *versus* CORT			p<0.05#	
			CORT/RS *versus* CORT			p=0.09	
	Ambulatory distance	One-way ANOVA	Factor Treatment	F=2.78	2, 39	p>0.1	S2D
Splash test	Grooming duration	One-way ANOVA	Factor Treatment	F=28.02	2, 39	p<0.01##	S2E
		PLSD Post-hoc test	CORT/Fluox versus CORT			p<0.01##	
			CORT/RS versus CORT			p<0.01##	

Legend: CORT: corticosterone; Fluox: fluoxetine; RS: RS67333

Additional References

[1] D.J. David, B.A. Samuels, Q. Rainer, J.W. Wang, D. Marsteller, I. Mendez, M. Drew, D.A. Craig, B.P. Guiard, J.P. Guilloux, R.P. Artymyshyn, A.M. Gardier, C. Gerald, I.A. Antonijevic, E.D. Leonardo, R. Hen, Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression, Neuron 62 (2009) 479-493.

[2] I. Mendez-David, D.J. David, F. Darcet, M.V. Wu, S. Kerdine-Romer, A.M. Gardier, R. Hen, Rapid anxiolytic effects of a 5-HT(4) receptor agonist are mediated by a neurogenesis-independent mechanism, Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 39 (2014) 1366-1378.

[3] A. Surget, M. Saxe, S. Leman, Y. Ibarguen-Vargas, S. Chalon, G. Griebel, R. Hen, C. Belzung, Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal, Biological psychiatry 64 (2008) 293-301.

[4] J.P. Guilloux, I. Mendez-David, A. Pehrson, B.P. Guiard, C. Reperant, S. Orvoen, A.M. Gardier, R. Hen, B. Ebert, S. Miller, C. Sanchez, D.J. David, Antidepressant and anxiolytic potential of the multimodal antidepressant vortioxetine (Lu AA21004) assessed by

behavioural and neurogenesis outcomes in mice, Neuropharmacology 73 (2013) 147-159.
[5] C. Ducottet, C. Belzung, Behaviour in the elevated plus-maze predicts coping after subchronic mild stress in mice, Physiology & behavior 81 (2004) 417-426.
[6] F. Darcet, I. Mendez-David, L. Tritschler, A.M. Gardier, J.P. Guilloux, D.J. David, Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression, Frontiers in behavioral neuroscience 8 (2014) 136.

Commentaires sur Article 3 :

Ce travail a étudié pour la première fois les activités cognitives de la fluoxetine et d'un agoniste du récepteur 5-HT_4 (RS67333) chez des souris présentant un phénotype anxio/dépressif. Si l'administration chronique de RS67333 a permis de corriger le déficit cognitif observé chez les souris dans tous les domaines cognitifs testés, il n'en n'a pas été de même avec la fluoxetine. Son efficacité concernant le versant cognitif reste modérée et test-dépendante.

En effet, seules les capacités de mémoire de type épisodique et d'apprentissage en mémoire spatiale sont restaurées après le traitement chronique par la fluoxetine. Ces résultats suggèrent que les effets pro-cognitifs de la fluoxetine sur ces types de mémoire pourraient être médiés, entre autre, par la stimulation des récepteurs 5-HT_4. Afin de confirmer cette hypothèse, une administration simultanée de fluoxetine avec un antagoniste spécifique des récepteurs 5-HT_4 (tel que le GR125487) permettrait de déterminer si les effets pro-cognitifs de la fluoxetine impliquent une activation du récepteur 5-HT_4 suite au blocage du transporteur sérotoninergique.

La correction complète du déficit cognitif suite à l'administration de RS67333 chez les animaux anxio/dépressifs comparés aux effets plus modérés de la fluoxetine soulève aussi des interrogations sur l'implication éventuelle d'autres récepteurs sérotoninergiques dans la réponse cognitive à la fluoxetine.

ARTICLE 4: Conditional β-arrestin 1 deletion in adult stem cells of the dentate gyrus alters emotional state and dampens antidepressant effects in adult mice

Flavie Darcet[1], Jean-Martin Beaulieu[2], René Hen[3], Fabienne Agasse[4], Caroline Benstaali[4], Indira Mendez-David[1], Alain M. Gardier[1], Jean-Philippe Guilloux[1], Denis J. David[1]

[1]CESP, INSERM, Univ. Paris-Sud, UVSQ, Université Paris-Saclay, Châtenay-Malabry, 92290, France

[2]Department of Psychiatry and Neurosciences, Université de Laval, Québec, QC

[3]Neuroscience and Psychiatry, Columbia Univ., New York City, NY

[4]Institut des Neurosciences, Grenoble, France

En préparation

Questions posées :

Quelles sont les modifications phénotypiques émotionnelles suite à la suppression spécifique génétique de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé ? Quelles sont les conséquences comportementales et neurogéniques d'un traitement chronique par de la fluoxetine chez ces animaux ?

Résumé de l'étude

De récentes études montrent que les protéines β-arrestines, qui régulent les récepteurs couplés aux protéines G, jouent un rôle important dans la physiopathologie des troubles de l'humeur et des mécanismes reliés à l'action des antidépresseurs (Avissar et al., 2004 ; Matuzany-Ruban et al., 2005). De récentes études précliniques ont confirmé l'utilisation de la protéine β-arrestine 1 comme un potentiel biomarqueur d'un état de type anxio/dépressif et de la réponse aux antidépresseurs (Mendez-David et al., 2013, Mendez-David et al., 2015). Ces études reportent une diminution de l'expression de la β-arrestine 1 dans l'hypothalamus dans un modèle d'anxiété/dépression basé sur l'administration chronique de corticosterone exogène qui a été restauré après un traitement chronique par de la fluoxetine (David et al., 2009). Ces résultats ouvrent la possibilité que l'expression de la β-arrestine 1 soit un substrat potentiel pour les effets comportementaux et neurogéniques des antidépresseurs monoaminergiques.

Puisque d'une part la neurogenèse hippocampique et notamment les jeunes neurones du GD âgés de 4-6 semaines semblent jouer un rôle prépondérant dans l'activité cognitive ou antidépressive (Sahay et al., 2011, Denny et al., 2014),nous avons souhaité étudier les conséquences de l'absence d'expression de la protéine β-arrestine 1 dans les jeunes neurones âgées de 4/7 semaines. Dans ce travail, nous avons caractérisé les conséquences comportementales d'une délétion sélective de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé de l'hippocampe dans plusieurs tests prédictifs d'une activité anxiolytique et/ou antidépressive en présence ou non d'un traitement chronique à la fluoxetine. Les conséquences neurogéniques de cette délétion sélective ont également été étudiées.

Contribution personnelle :

Au cours de ce travail :

- J'ai mis en place les cohortes d'animaux tissus-spécifiques et leurs contrôles avec la méthode de génotypage et induit la suppression génétique spécifique avec les injections de tamoxifène.

- J'ai réalisé le suivi des animaux incluant la préparation et l'administration des traitements pharmacologiques (traitement par la fluoxetine).

- J'ai mené l'ensemble des tests comportementaux émotionnels.

- J'ai réalisé l'ensemble des analyses immunohistochimiques des étapes de la neurogenèse hippocampique depuis la perfusion, le prélèvement et la coupe des cerveaux jusqu'au comptage des cellules.

- J'ai analysé les résultats et rédigé l'article sous la supervision du Pr. Denis David et du Dr. Jean-Philippe Guilloux.

Conditional β-arrestin 1 deletion in stem cells of the dentate gyrus alters emotional state and dampens antidepressant effects in adult mice

Flavie Darcet[1], Jean-Martin Beaulieu[2], René Hen[3], Fabienne Agasse[4], Caroline Benstaali[4], Indira Mendez-David[1]Alain M. Gardier[1], Jean-Philippe Guilloux[1], Denis J. David[1]

[1]CESP, INSERM, Univ. Paris-Sud, UVSQ, Université Paris-Saclay, Châtenay-Malabry, 92290, France

[2]Department of Psychiatry and Neurosciences,Université Laval, Québec, QC

[3]Neuroscience and Psychiatry, Columbia Univ., New York City, NY

[4]Institut des Neurosciences, Grenoble, France

En préparation

INTRODUCTION OF THE STUDY:

Elucidating the neurobiological basis of depression and anxiety are one of the foremost challenges for today's society. Severe forms of depression affect 2-5% of the U.S. population and mood disorders impact 7% of the world's population and rank among the top ten causes of disability. Selective Serotonin Reuptake Inhibitors (SSRIs) are the most commonly prescribed drugs for the treatment of depression and several anxiety disorders. However, key questions remain regarding the molecular and cellular mechanisms underlying the effects of SSRIs and other antidepressants (ADs). In accordance with the idea that ADs exert essential effects in multiple brain regions, we previously demonstrated that chronic corticosterone regimen induces anxiety/depression-like phenotype and subsequent fluoxetine treatment elicits effects on genes' expression not only in the hippocampus, but also in the hypothalamus in adult mice. Among the genes investigated, only 3, including β-arrestins 1 and 2, displayed a change in mRNA levels in the chronic corticosterone group that was reversed by fluoxetine treatment. Molecular functions of β-arrestins have placed them in pathways associated with mood and responsiveness to lithium, a drug currently used to treat bipolar disorders. This includes regulation of an Akt/GSK3 complex and classical functions such as desensitization of G-protein coupled receptors including serotonin and dopamine receptors. Finally, there is also evidence in humans implicating β-arrestins in depression and in response to stress, and that these changes are

reversible by various AD treatments. Thus, β-arrestins could be an important molecular determinant of the effects of fluoxetine and more generally to ADs. Recently, clinical data from Avissar's group (Avissar et al., 2004) suggest that β-arrestin-1 mRNA and protein levels are highest in peripheral blood leukocytes of MDD patients. Therefore, β-arrestin-1 may be a putative candidate biochemical marker in clinical practice for depressive pathophysiology and the response to antidepressants (for review see (Schreiber et al., 2009). β-arrestin mRNA levels and β-arrestin-1 protein levels in mononuclear leukocytes of untreated patients with MDD are lower than the levels found in healthy subjects. Furthermore, reduced levels of β- arrestin-1 protein and mRNA are significantly correlated with the severity of depressive symptoms (Avissar et al., 2004; Schreiber et al., 2009). However, the low β-arrestin-1 protein and mRNA levels are alleviated by antidepressant treatment. Therefore, these low levels may predict clinical improvement (Avissar et al., 2004; Golan et al., 2010). Finally, SSRIs are potent stimulators of adult hippocampal neurogenesis (David et al., 2009; Santarelli et al, 2003) and some of these effects are required for the anxiolytic/antidepressant-like effects of SSRIs (David et al., 2009). These results raise the possibility that β-arrestin 1 expression is a potential substrate for the behavioral antidepressant effects, including the neurogenic effects of antidepressant drugs. Here, we characterized the behavioral consequences of β-arrestin 1 (ARRB1) conditional deletion in stem cells in the dentate gyrus of the hippocampus in various behavioral paradigms predictive of anxiolytic/antidepressant therapy in presence or not of a chronic fluoxetine treatment.

MATERIALS AND METHODS:

Animals

Inducible tissue-specific male mice were created by interbreeding Nestin-CreER^{T2} (René Hen, Columbia University, USA) mice and ARRB1$^{flox/flox}$ mice (Jean-Martin Beaulieu, Université Laval, Canada). Nestin-CreER^{T2}; ARRB1$^{+/flox}$ and ARRB1$^{+/flox}$ mice from F1 were then crossed to generate NestinCreER$^{T2-/+}$; ARRB1$^{flox/flox}$ mice (ARRB1$^{-/-}$ mice). NestinCreER$^{T2-/+}$; ARRB1$^{+/+}$ mice (ARRB1$^{+/+}$) were used as controls. ARRB1 mice were maintained on a mixed (C57BL/6-129/Sv) genetic background. To induce CreER^{T2} mediated recombination in neural stem cells in the adult brain, ARRB1$^{-/-}$ mice were given 2 mg of tamoxifen (TAM) (Sigma, France), once a day, intraperitoneally for 5 consecutive days. TAM solution (10 mg/mL) was prepared in corn oil containing 10% ethanol. Control mice were injected with an identical volume of corn oil with 10% ethanol. Animals were maintained on a 12L:12D schedule (Lights ON at 7AM) and were housed 5 per cage. Food and water were provided *ad libitum.* All testing were conducted in compliance with the laboratory animal care guidelines, with protocols approved by the Institutional Animal Care and Use Committee (CEE26 authorization 2012-099) and with the European directive 2010/63/EU. Experiments were conducted on male transgenic mice between 12 and 14 weeks of age.

Two cohorts of male animals were generated to assess the behavioral and the neurogenic consequences of the conditional deletion of β-arrestin 1 in adult hippocampal stem cell in baseline and under chronic antidepressant treatment.

Control of selective deletion

In order to control the conditional and selective deletion of β-arrestin 1 protein in stem cells of the dentate gyrus, in situ hybridization and western blot from stem cells cultures were applied in ARRB1$^{-/-}$ mice and their littermates.

In situ hybridization

Coronal hippocampal sections (20 µm thickness) from ARRB1$^{+/+}$ and ARRB1$^{-/-}$ mice were collected fixed with paraformaldehyde and dehydrate in ethanol at room temperature. Slices were first treated with Pretreat solution for 10 minutes, rinsed and hybridize for 2h at 40°C with a complementary oligodeoxyribonucleotide probe of β-arrestine 1 protein in mice. Sections were then hybridized with different stabilizer reagents at 40°C from 15 to 30 minutes. To amplify the signal, sections were incubated in DAB during 10 minutes at room temperature then colored with 50% hematoxylin and a 0.02% ammoniac solution for 2 minutes. Finally, slices were dehydrated in successive baths: 70% ethanol, 100% ethanol and xylene. β-arrestin 1 mRNA was visualized with a microscope and scanned by ZEISS Axio Scan.Z1 apparatus. β-arrestin 1 expression was counted using Guide ZEN 2012 – Slidescan and FIJI softwares.

Stem cells culture

Neural stem cells were grown in monolayers according to the protocol of Babu et al, 2011 with modifications. Adult mice (2 to 4 month-old) were killed by cervical dislocation; dentate gyrus and subventricular zone were rapidly dissected and placed in sterile saline solution. The tissues were digested for 20 min at 37˚C in PBS containing papain (2,5 U/ml), DNase I250 U/ml (both from Sigma) and dispase (8 U/ml) (Roche) and viable cells were separated from myelin and cell debris by centrifugation on a percoll gradient (see Babu et al., 2011). Then, cells were diluted in serum-free culture medium (SFM) composed of Dulbecco's modified Eagle's medium/Ham's (DMEM) F-12 medium GlutaMAX-I supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 1% B27 supplement, 20 ng/ml epidermal growth factor (EGF) and 20 ng/ml basic fibroblast growth factor (FGF-2) (all from Life Technologies) and grown as monolayers for 7 days (SVZ stem cells) or 14 days (dentate gyrus stem cells) in petri dishes coated with 20 µg/ml poly-D-Lysine and 20 µg/ml laminin (both from Sigma) in a 95% air–5% CO_2 humidified atmosphere at 37°C.

Western Blot

For Western Blot analysis, protein concentration from stem cells cultures was quantified using BCA Protein Assay Kit (Pierce Biotechnology). Equal amount of proteins were separated by 10% sodium dodecyl sulfate–polyacrylamide electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (PVDF), (Amersham Biosciences, Les Ulis, France). After blocking in 10 Mm Tris-HCl, pH:7,6, 200 mM NaCl, 0,1% Tween-20 and 5% BSA, membranes were incubated with primary monoclonal antibody Mouse Anti- β-Arrestin 1 (BD Bioscience Pharmigen) and β-Actin (Santa Cruz Biotechnology, CA).Primary antibodies were detected with appropriate HRP-coupled secondary antibodies and ECL signals (SuperSignal West Pico Chemiluminescent Substrate (Pierce, Erembodegem, Belgium) were quantitated with Image lab software (Bio-Rad).

Drugs and administration

Fluoxetine hydrochloride (160 mg/mL, equivalent to 18mg/kg/day) was purchased from Anawa Trading, (Zurich, Switzerland) and administered in the drinking water in opaque bottles for 4 weeks. The dose of fluoxetine was based on previous data showing the ability of fluoxetine to produce antidepressant-like activity (David et al., 2009).

Behavioral testing

For each cohort, the same animal was tested in five different behavioral paradigms of anxiety and depression. Each animal, over a week, was successively tested in: Open Field (1), Elevated Plus maze (2), 4-plate test (3), Novelty Suppressed Feeding (4), Splash test (5) and Tail Suspension test (6). Z-score methodology was used to investigate the potential of combining results within and across the different behavior tests for depressive/anxious-like behaviors and investigate the anxiolytic/antidepressant-like response of fluoxetine treatment in ARRB1$^{-/-}$ mice and controls (Guilloux et al., 2011).

Open Field (OF)

This test was performed as described by David and colleagues (David *et al*, 2009). Motor activity was quantified in four 43 x 43 cm2 Plexiglas open field boxes (MED Associates, Georgia, VT). Two sets of 16 pulse-modulated infrared photobeams were placed on opposite walls 2.5-cm apart to record x–y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100-ms resolution. The computer defined grid lines that divided each open field into center and surround regions, with each of four lines being 11 cm from each wall. Dependent measures in the center were the total time and the number of entries over a 30-min test period. The activity in the center was quantified as distance traveled in the center divided by total distance traveled.

Elevated Plus Maze (EPM)

This test was performed as described by David and colleagues (David *et al*, 2009). The maze is a plus-cross-shaped apparatus, with two open arms and two arms closed by walls linked by a central platform 50 cm above the floor. Mice were individually put in the center of the maze facing an open arm and were allowed to explore the maze during 5 min. The time spent and the number of entries into the open arms were used as an anxiety index. All parameters were measured using a videotracker (EPM3C, Bioseb, Vitrolles, France).

4-plates test (4-PT)

The 4-plates test (Aron et al., 1971) is a fear-related anxiety paradigm in which the natural exploration of a novel environment is inhibited by the delivery of a footshock. The equipment consists in a small cage (25×18×16 cm) in which the floor is composed of 4 similar rectangular metal plates (11×8 cm) separated from each other with a 4-mm space (Bioseb, France). Each plate is connected to a foot shock generator (0.6 mA; 0.5 s). After a 15-sec habituation period, a foot shock is administrated to animals when they move from one plate to another. Anxiety parameter is measured by the number of punitions administered to animals over a 60-sec period.

Novelty-Suppressed-Feeding (NSF)

The NSF is a conflict test that elicits competing motivations: the drive to eat and the fear of venturing into the center of a brightly lit arena. The latency to begin eating is used as an index of anxiety/depression-like behavior, because classical anxiolytic drugs as well as chronic antidepressants decrease this measure. The NSF test was carried out during a 10-min period as previously described (David et al, 2009). Briefly, the testing apparatus consisted of a plastic box (50x40x20 cm), the floor of which was covered with approximately 2 cm of wooden bedding. Twenty-four hours prior to behavioral testing, all food was removed from the home cage. At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the center of the box. Each animal was placed in a corner of the box, and a stopwatch was immediately started. The latency to eat (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was timed. Immediately afterwards, the animal was transferred to its home cage, and the amount of food consumed by the mouse in the subsequent 5 min was measured, serving as a control for change in appetite as a possible confounding factor.

Tail Suspension Test (TST)

The TST is an antidepressant activity-screening test (Steru et al, 1985) used to test compounds that are expected to affect depression related behaviors. Mice are suspended by their tails with tape, in a position that they cannot escape or hold on to nearby surfaces. During this 6 minutes test, the resulting escape oriented behaviors are quantified using an automated tail suspension test apparatus (Bioseb, Vitrolles, France). A specific strain gauge linked to a computer quantifies the time spent by the animal trying to escape.

Splash Test

This test consisted of squirting 200 µl of a 10% sucrose solution on the mouse's snout. The grooming duration was assessed at the end of the corticosterone regimen in the presence or absence of 4-weeks of drug treatment according to a protocol previously described (David *et al*, 2009).

Saccharine test

This test relies on the natural preference of mice for a sweet beverage (saccharine or sucrose solution) instead of water. This test measures the anhedonia behavior, known to be one of the core symptoms in depression. Briefly, mice are housed singly the day before the start of the experiment and are habituated to drink into water from 2 plastic Falcon tubes with a hole at the bottom during 3 consecutive days. On the 4^{th} day, mice have a free access to one tube containing water and one tube containing 0.1% saccharin solution during a period of 48 hours. The amount of consumed liquid in each tube is measured every 12 hours at 8AM and 8PM each day. Furthermore, tubes positions are alternated every 12 h to avoid any place preference (left/right). Saccharin preference (in %) is calculated as followed: Preference= [saccharin consumption (mL)/ total consumption (mL) (water + saccharin)]*100.

Immunochemistry

The effects of chronic fluoxetine treatment on cell proliferation, cell survival and cell maturation of newborn neurons were assessed in $ARRB1^{+/+}$ and $ARRB1^{-/-}$ animals. Mice were perfused transcardially (cold saline for 2 min, followed by 4% cold paraformaldehyde at 4°C) after anesthesia with ketamine and xylazine (100 mg/ml ketamine; 20mg/ml xylazine). Brains were then removed and post-fixed in PFA during 24h. Brains were coronnaly sectionned (35 µm thickness) to collect hippocampus slices and free-floating sections were stored in PBS + 0.1% azide solution at 4°C until immunohistochemistry methods were performed.

Proliferation of newborn cells

We first looked at proliferation of newborn cells using Ki-67 immunohistochemistry as described previously (Xia *et al*, 2012). Sections were washed in PBS, blocked (PBS containing 0.3% Triton and 10% NDS) and incubated with primary antibody overnight at 4° C (Ki67 rabbit, 1:100, Vector,

Burlingame, CA). Following washes in PBS, sections were incubated with fluorescence coupled rabbit secondary antibody (Jackson ImmunoResearch, Beckman, France). Stereological quantification of Ki-67 labeling was performed using an Olympus BX51 microscope (Germany).

Survival of newborn cells

Mice were administered with 5-Bromo-2-Deoxyuridin (BrdU) (100 mg/kg, twice a day during 3 days, intraperitoneally, dissolved in saline) before the start of fluoxetine treatment. The identification of survival cells was performed as previously described (David et al., 2009). Sections were mounted on slides and boiled in citric acid (pH 6.0) for 5 min, rinsed with PBS and treated with 0.01% trypsin in Tris/CaCl2 for 10 minutes. Brain sections were incubated for 30 minutes with 2N HCl and blocked with 5% NGS. Sections were then incubated overnight at room temperature with anti-mouse BrdU (1:100) (Beckman-Dickson, France). After washing with PBS, sections were incubated for 1h with secondary antibody (1:200 biotinylated goat anti-mouse) followed by amplification with an avidin-biotin complex. The staining was visualized with DAB. Stereological quantification of Ki-67 labeling was performed using an Olympus BX51 microscope (Germany).

Maturation of newborn neurons

For doublecortin staining, the procedure consisted of the following steps: 1hr incubation in 0.1M TBS with 0.5% Triton X-100 and 10% normal donkey serum (NDS), followed by goat anti-doublecortin primary antibody (1:100) in TBS/Tx/NDS for 24 hrs at 4°C. The secondary antibody was biotinylated donkey anti-goat (1:500) in TBS/NDS for 1 hr at room temperature, followed by a 1hr amplification step using an avidin-biotin complex (Vector, USA). The immunohistochemistry protocol was adapted from David et al. (David et al, 2009). DCX-positive (DCX^+) cells were subcategorized according to their dendritic morphology: DCX^+ cells without and DCX^+ cells with tertiary (or higher order) dendrites. The maturation index was defined as the ratio of DCX^+ cells possessing tertiary dendrites to the total number of DCX^+ cells.

Data analysis:

Results were expressed as mean ± SEM for all experiments. T-tests, one-way or two-way ANOVA (genotype and treatment factors) were applied when appropriate. Significant main effects and/or interactions were followed by a Fisher's PLSD post hoc analysis. In the NSF test, we used the Kaplan-Meier survival analysis due to the lack of normal distribution of the data. Mantel-Cox log-rank test was used to evaluate differences between experimental groups. Statistical significance was set at $p<0.05$.

Figure 1:

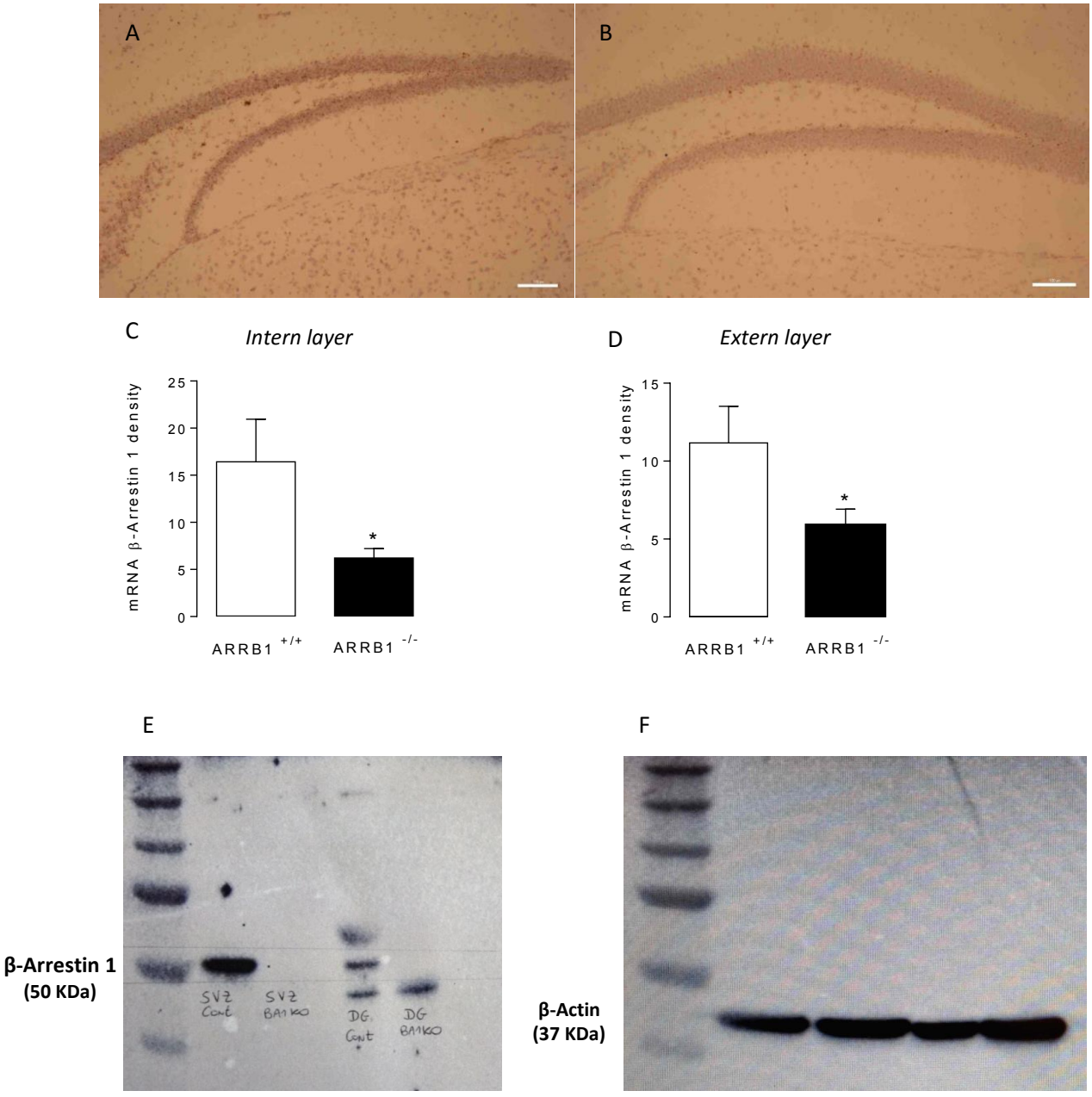


Figure 1: Conditional deletion of β-arrestin 1 protein in adult stem cells of the dentate gyrus. *In situ* hybridization slices visualized with ImageJ software in ARRB1$^{+/+}$ (A) and ARRB1$^{-/-}$ (B) animals. mRNA quantification in ARRB1$^{+/+}$ and ARRB1$^{-/-}$ animals in intern layer (1/3) of the dentate gyrus (C) and extern layer (2/3) of the dentate gyrus (D). Representative western blot of β-arrestin 1 (E) and β-actin (F) levels in stem cells of the dentate gyrus. β-actin was used as a control.

Figure 2 :

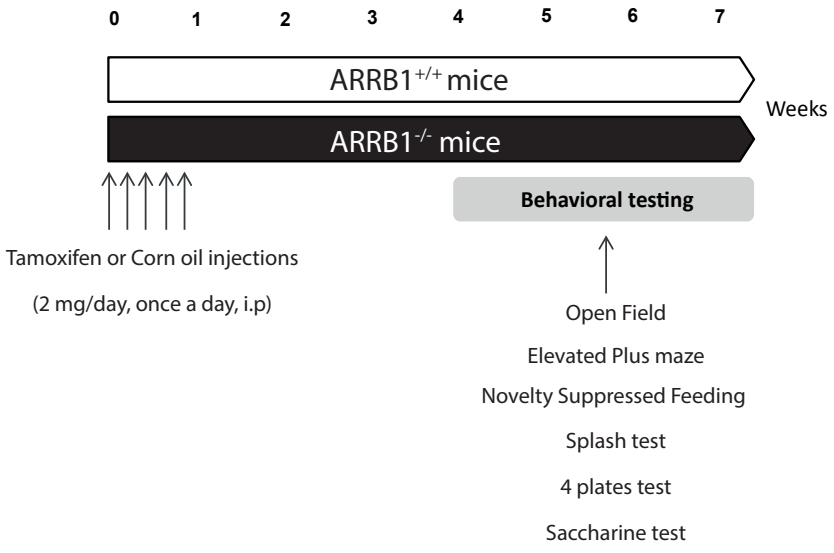


Figure 2: Experimental design of the phenotype study. Tamoxifen was injected intraperitoneally once a day (2 mg/day) on 5 consecutive days at the beginning of the study. In week 4, all animals were successively submitted to behavioral paradigms.

Figure 3 :

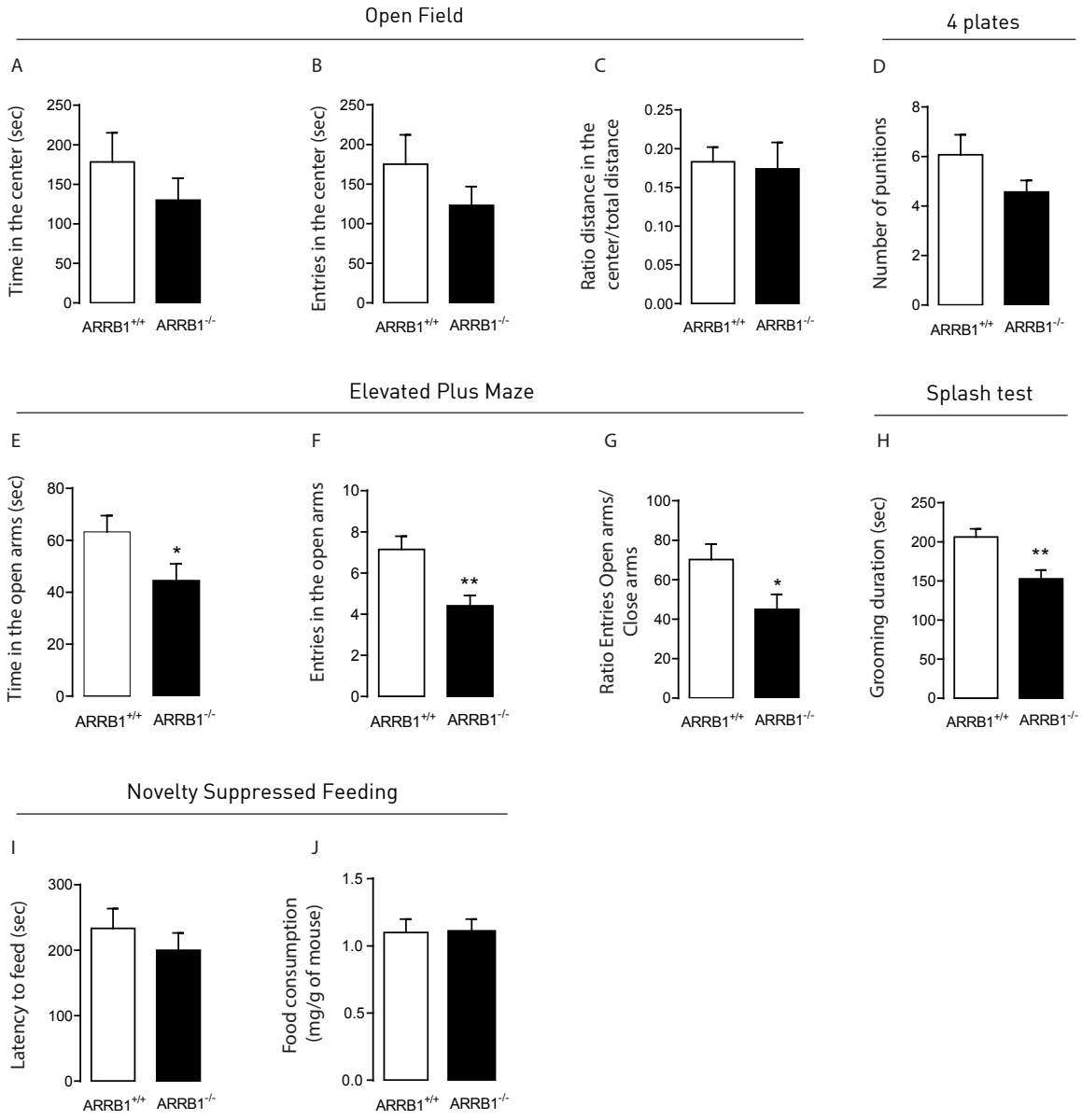


Figure 3: Behavioral consequences of conditional deletion of β-arrestin 1 protein in adult newborn granule cells of the dentate gyrus on anxiety or/and depressive phenotype.

Effects of selective deletion of β-arrestin 1 protein in adult newborn granule cells of the dentate gyrus on anxiety or/and depressive-like behaviors in the Open Field (OF) paradigm (A-C), the Four Plates test (FPT) paradigm (D), the Elevated Plus maze (EPM) paradigm (E-G), the Splash test (H) and the Novelty Suppressed Feeding paradigm (NSF) (I-J).

(n = 10–12 per group).

*p< 0.05, **p<0.01 versus Vehicle/ARRB1$^{+/+}$ mice.

Figure 4 :

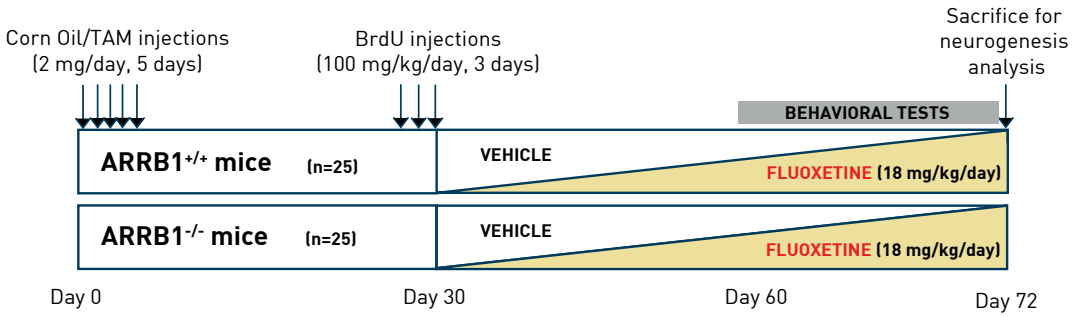


Figure 4: Experimental design of the pharmacological study. Tamoxifen was injected intraperitoneally once a day (2 mg/day) on 5 consecutive days at the beginning of the study. In Day 30, ARRB1$^{+/+}$ and ARRB1$^{-/-}$ animals were subdivided into two groups. In place of normal drinking water, one of the two groups was administered a 4-weeks fluoxetine treatment for each genotype. The same cohort of mice performed successively all of the behavioral tests from day 60 to day 72.

Figure 5 :

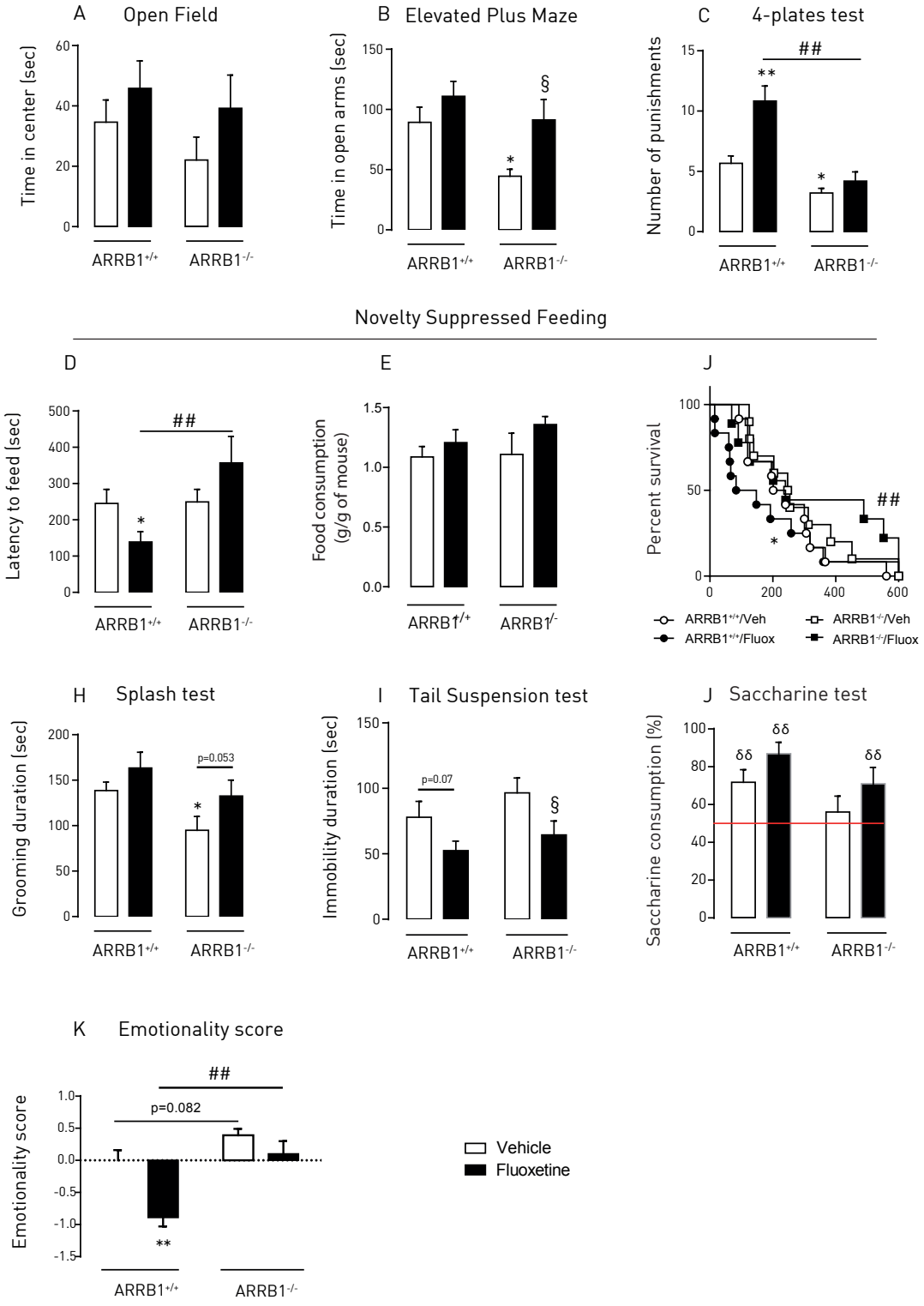

A Open Field
Time in center (sec)
ARRB1+/+
ARRB1-/-
B Elevated Plus Maze
Time in open arms (sec)
C 4-plates test
Number of punishments
Novelty Suppressed Feeding
D
Latency to feed (sec)
E
Food consumption (g/g of mouse)
J
Percent survival
ARRB1+/+/Veh
ARRB1+/+/Fluox
ARRB1-/-/Veh
ARRB1-/-/Fluox
H Splash test
Grooming duration (sec)
p=0.053
I Tail Suspension test
Immobility duration (sec)
p=0.07
J Saccharine test
Saccharine consumption (%)
K Emotionality score
Emotionality score
p=0.082
Vehicle
Fluoxetine

Figure 5 : Arresting β-Arrestin 1 expression in adult newborn granule cells of the dentate gyrus prevents fluoxetine induced anxiolytic/antidepressant-like activity in a neurogenesis-dependent task.

Effects of 4 weeks of fluoxetine treatment (18 mg/kg/day) in ARRB1$^{-/-}$ and ARRB1$^{+/+}$ mice on anxiety or/and depressive-like behaviors in the Open Field (OF) paradigm (A), the Elevated Plus Maze (EPM) paradigm (B), the Four Plates test (FPT) paradigm (C), the Novelty Suppressed Feeding paradigm (NSF) (D-F), the Splash test (G), the Tail Suspension test (TST) (H) and the saccharine preference test (I). Anxiety parameters are represented by time in the center in the OF, time in the open arms in the EPM and number of punishments in the FPT. The depressive-like behavior in the Splash test was expressed with grooming duration. In the TST, the immobility duration has been used as parameter to predict antidepressant-like activity. In the NSF, the anxiety/depressive-like parameter is represented with the latency to feed. In the saccharin preference test, the anhedonia is measured by the percent of saccharin consumption. Emotionality score was used to normalize emotionally-related data across tests. An increase of this score indicates a higher anxio-depressed phenotype of mice (K).
(n = 10–12 per group)

*p< 0.05, **p<0.01 versus Vehicle/ARRB1$^{+/+}$ mice;
**p<0.01 versus Vehicle/ARRB1$^{+/+}$ mice
##p<0.01 versus fluoxetine-treated/ARRB1$^{+/+}$ mice;
§p< 0.05 versus Vehicle/ARRB1$^{-/-}$ mice;
δδ p<0.01 versus 50% saccharine consumption.

Figure 6 :

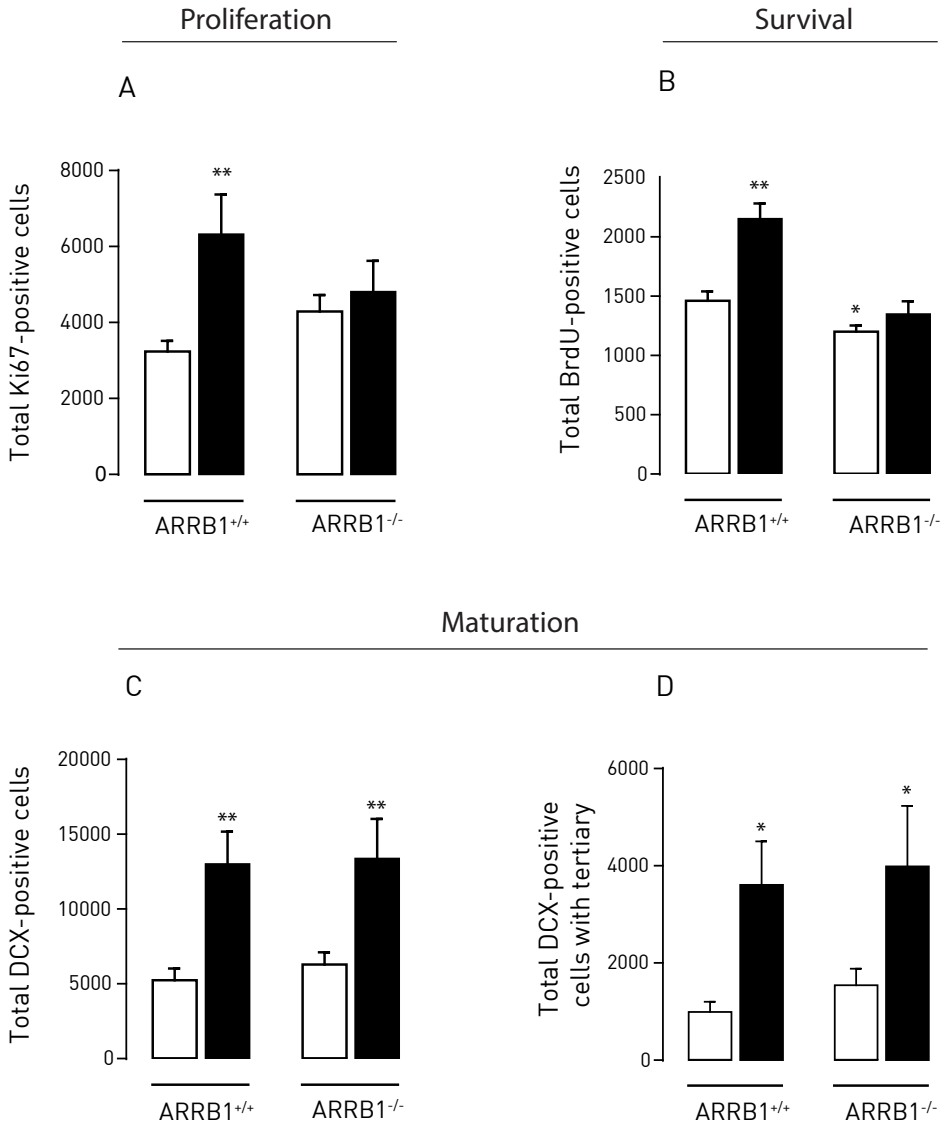


Figure 6: Arresting β-Arrestin 1 expression in adult newborn granule cells of the dentate gyrus prevents fluoxetine induced increase in proliferation of progenitor cells and survival of newborn neurons. (A) Proliferation is represented as the total number of Ki67-positive cells in the dentate gyrus of adult mouse hippocampus. (B) Survival was expressed as the total number of BrdU-positive cells in the dentate gyrus of adult mouse hippocampus. For the quantification of maturation cells, DCX-positive cells were categorized according to their dendritic morphology: total DCX-positive cells (C) and DCX-positive cells with tertiary dendrites (D).
(n= 7-9 per group). *p<0.05; **p<0.01 versus Vehicle/ARRB1+/+ mice.

DISCUSSION OF THE STUDY:

In this study, arresting β-arrestin 1 expression in adult stem cells of the dentate gyrus did not significantly change overall the emotionality in mice but altered behavior in specific tests such as Elevated Plus Maze, 4-plates test and Splash test. Interestingly arresting β-arrestin 1 expression dampened anxiolytic/antidepressant-like effects of chronic fluoxetine treatment, as revealed by an absence of decrease of the emotionality score analysis, and prevents fluoxetine induced anxiolytic/antidepressant-like activity in a neurogenesis-dependent task, the Novelty Suppressed Feeding.

On another hand, neurogenesis study revealed that β-arrestin 1 protein expression in stem cells of the dentate gyrus is necessary for survival of newborn cells. Interestingly, β-arrestin 1 protein expression in stem cells of the dentate gyrus necessary for fluoxetine-induced proliferation and survival of newborn cells. Finally, β-arrestin 1 protein expression in stem cells of the dentate gyrus is not involved in fluoxetine-induced maturation of newborn granule cells

Further experiments using death cells staining (caspase-3) or triple staining (BrdU, NeuN and DCX) could help to better determine neurogenesis modifications after the specific deletion of β-arrestin 1 protein in stem cells of the dentate gyrus.

Résultats complémentaires:

Conditional β-arrestin 1 deletion in adult stem cells of the dentate gyrus alters cognitive phenotype and prevents pro-cognitive effects of chronic fluoxetine and 5-HT$_4$ receptor agonist treatments in mice

Flavie Darcet[1], Jean-Martin Beaulieu[2], René Hen[3], Alain M. Gardier[1], Jean-Philippe Guilloux[1], Denis J. David[1]

[1]CESP, INSERM, Univ. Paris-Sud, UVSQ, Université Paris-Saclay, Châtenay-Malabry, 92290, France
[2]Department of Psychiatry and Neurosciences, Université de Laval, Québec, QC
[3]Neuroscience and Psychiatry, Columbia Univ., New York City, NY

Questions posées :

La délétion conditionelle de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé induit-elle des modifications dans les performances d'apprentissage et de mémoire ? Si oui, quelles sont les conséquences comportementales d'un traitement chronique par de la fluoxetine ou par le RS67333, un agoniste du récepteur 5-HT$_4$ chez ces animaux adultes ?

Résumé de l'étude

De récentes études montrent que les protéines β-arrestines, qui régulent l'activité des récepteurs couplés aux protéines G, jouent un rôle important dans la physiopathologie des troubles de l'humeur et des mécanismes reliés à l'action des antidépresseurs (Avissar et al., 2004 ; Matuzany-Ruban et al., 2005). En plus d'être impliqués dans les mécanismes émotionnels et de réponse aux antidépresseurs, l'hippocampe d'une manière générale est également étroitement lié aux mécanismes de cognition. De plus, la relation fonctionnelle entre la protéine β-arrestine 1 et le récepteur 5-HT$_4$ est bien décrite dans la littérature et pourrait constituer une nouvelle piste dans l'étude des effets des ligands du récepteur 5-HT$_4$, tel que le RS67333 (Marin et al., 2012).

Dans cette étude, nous avons dans un premier temps caractérisé les conséquences d'une délétion conditionnelle de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé de l'hippocampe de souris adulte dans plusieurs tests d'apprentissage et de mémoire. Dans un second temps, les conséquences d'un traitement chronique à la fluoxetine ou d'un traitement chronique par un agoniste des récepteurs 5-HT$_4$, le RS67333 sur les performances cognitives de ces animaux ont été examinées dans le test de reconnaissance d'objet, le Barnes maze et le test de conditionnement par la peur.

Les résultats montrent que la délétion conditionnelle de la protéine β-arrestine 1 dans les jeunes neurones issus de la neurogenèse provoque une altération globale des performances cognitives des animaux, surtout au niveau de la mémoire de type épisodique et de la mémoire associative. De plus, aucun des traitements pharmacologiques appliqués aux animaux génétiquement modifiés n'a permis de corriger ces déficits d'apprentissage et de mémoire. Ces données suggèrent donc que l'expression de la protéine β-arrestine 1 dans les neurones nouvellement formés est nécessaire aux effets pharmacologiques pro-cognitifs de la fluoxetine et du RS67333. Ce travail souligne le rôle clé de la protéine β-arrestine 1 dans le processus de neurogenèse pour la réponse aux différentes stratégies thérapeutiques.

Contribution personnelle :

Au cours de ce travail :

- J'ai mis en place les cohortes d'animaux tissus-spécifiques et leurs contrôles avec la méthode de génotypage et induit la suppression génétique spécifique avec les injections de tamoxifène.

- J'ai réalisé le suivi des animaux incluant la préparation et l'administration des traitements pharmacologiques (traitement par la fluoxetine).

- J'ai mené l'ensemble des tests comportementaux d'apprentissage et de mémoire

- J'ai analysé les résultats et rédigé l'article sous la supervision du Pr. Denis David et du Dr. Jean-Philippe Guilloux

Conditional β-arrestin 1 deletion in stem cells of the dentate gyrus alters cognitive phenotype and prevents pro-cognitive effects of chronic fluoxetine and 5-HT_4 receptor agonist treatments in adult mice

Flavie Darcet[1], Jean-Martin Beaulieu[2], René Hen[3], Indira Mendez-David[1], Alain M. Gardier[1], Jean-Philippe Guilloux[1], Denis J. David[1]

[1]CESP, INSERM, Univ. Paris-Sud, UVSQ, Université Paris-Saclay, Châtenay-Malabry, 92290, France

[2]Department of Psychiatry and Neurosciences, Université de Laval, Québec, QC

[3]Neuroscience and Psychiatry, Columbia Univ., New York City, NY

MATERIALS AND METHODS:

Animals

Inducible tissue-specific male mice were created by interbreeding Nestin-CreER^{T2} (René Hen, Columbia University, USA) mice and ARRB1$^{flox/flox}$ mice (Jean-Martin Beaulieu, Université Laval, Canada). Nestin-CreER$^{T2-/+}$; ARRB1$^{+/flox}$ and ARRB1$^{+/flox}$ mice from F1 were then crossed to generate NestinCreER$^{T2-/+}$; ARRB1$^{flox/flox}$ mice (ARRB1$^{-/-}$ mice). NestinCreER$^{T2-/+}$; ARRB1$^{+/+}$ mice (ARRB1$^{+/+}$) were used as controls. To induce CreER^{T2} mediated recombination in neural stem cells in the adult brain, ARRB1$^{-/-}$ mice were given 2 mg of tamoxifen (TAM) (Sigma, France), once a day, intraperitoneally for 5 consecutive days. TAM solution (10 mg/mL) was prepared in corn oil containing 10% ethanol. Control mice were injected with an identical volume of corn oil with 10% ethanol. Animals were maintained on a 12L:12D schedule (Lights ON at 7AM) and were housed 5 per cage. Food and water were provided *ad libitum.* All testing were conducted in compliance with the laboratory animal care guidelines, with protocols approved by the Institutional Animal Care and Use Committee (CEE26 authorization 2012-099) and with the European directive 2010/63/EU. Experiments were conducted on male transgenic mice between 12 and 14 weeks of age.

Drugs and administration

Fluoxetine hydrochloride (160 mg/mL, equivalent to 18mg/kg/day) was purchased from Anawa Trading, (Zurich, Switzerland) and administered in the drinking water in opaque bottles for 4 weeks. The dose of fluoxetine was based on previous data showing the ability of fluoxetine to produce antidepressant-like activity (David et al., 2009). Hydrochloride (1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-butyl-4piperidinyl)-1-propanone hydrochloride) (RS67333), was purchased from Tocris Bioscience (Bristol, United Kingdom) and dissolved in 0.9% saline solution. RS67333 was chosen based on previous work (Lucas et al, 2007). RS67333 shows high binding affinity for the 5-HT$_4$ receptor with a pKi of 8.7 (Bockaert *et al*, 2004; Eglen *et al*, 1995). Except for the sigma receptors, which are bound at affinities comparable to 5-HT4 (sigma 1: pKi = 8.9; and sigma 2: pKi = 8.0),

RS67333 has a pKi of less than 6.7 for other neurotransmitter receptors including 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, dopamine D_1, D_2 and muscarinic M_{1-3} receptors. However little is known about the function of sigma receptors. However little is known about the function of sigma receptors.

Behavioral testing

In the cognitive phenotype study, different cohorts of mice were used to assess learning and memory performances in ARRB1$^{-/-}$ animals. To prevent any confounding effects between cognitive tasks, each animal was subjected to only one type of learning and memory test. Cognitive performances were evaluated using behavioral tests measuring episodic (novel object recognition test, NORT and novel object location test, NOLT), visuo- spatial (MWM; Barnes maze, BM) and associative (one-trial contextual fear conditioning, CFC) learning/memory. Animals were placed in the experimental room 30 min before the start of the behavioral experiments.

In the pharmacological study, the same cohort of animals was tested in three different learning and memory behavioral paradigms. Each animal, over 2 weeks, was successively tested in: the novel object recognition test (NORT), the Barnes maze (BM) and the one-trial contextual fear conditioning (CFC). To determine the effects of a chronic treatment of fluoxetine and RS67333 in episodic-like memory, the novel object recognition test with a 2h-ITI was chosen because the cognitive alteration in ARRB1$^{-/-}$ mice was the most pronounced with these conditions. The visuo-spatial Barnes maze was added in the list of behavioral tests in order to assess another type of memory.

Episodic-like memory

Novel object recognition test (NORT)

Because of a decrease in exploration behavior in this particular strain (C57BL6/Jx129Sv), the procedure was adapted from previous studies. The apparatus consisted in black plastic boxes (28x41x18 cm) slightly filled with sawdust (≈ 0.5-1 cm thickness) in a room with a low level of light. Locomotor activity was controlled during the entire experiment (parameter: ambulatory distance)

using a videotracking procedure (ANY-maze Software, Bioseb, France). Objects exploration was hand-scored by an experimenter.

The NORT was divided into one training session and one retention test session. Each exposure lasted 10 minutes separated with a 2 hours or 24 hours inter-trial interval. Between training and retention session, mice returned to their home cage, bedding of apparatus was changed and boxes were cleaned with 70 % ethanol solution. During the training session, two identical objects [cylindrical glassware (∅:3 cm, height: 8 cm) filled with white cotton) were present in the box. The mouse was placed in the middle of the box facing the wall and was allowed to freely explore the apparatus and the objects. During the test session, one of the familiar objects was removed from the cage and replaced by a novel object [Lego® rectangular structure (7x3x9 cm). The objects had been previously validated to ensure there was no inherent preference for either object (data not shown). The nature (Lego® *versus* glass) and the position of the novel object (left *versus* right) were chosen randomly.

Object exploration was defined as the orientation of the nose to the object at a distance ≤ 2 cm. Placing the forepaws on the objects was considered as exploratory behavior, but climbing on the objects was not. Objects were cleaned with 70 % ethanol between trials to avoid olfactory cues. Results for this test were expressed as: 1) exploration of each object (in seconds) during training and test sessions 2) exploration (in percent) of each object during the test session, calculated as time spent exploring familiar or novel object divided by total time spent exploring both objects and 3) a discrimination index (DI) between objects during the test session, calculated as the difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects (DI=(N-F)/(N+F)).

Novel object location test (NOLT)

The place recognition task examines the animal's ability to discriminate between an object that was moved during the delay between the acquisition and the retention phase and an object that remained stationary, adding a spatial component to the task. Briefly, the procedure was divided in one training session in which 2 identical objects are placed at the left side of the box, and one

retention session, in which one of the object was moved to the right side of the box. The initial position of objects (left/right) alternated between animals to avoid any side preference. Similarly as the NORT, training and retention session lasted 10 min with a 2 hours delay. Parameters to analyse were the same as in the NORT.

Spatial learning and memory: Barnes maze (BM)

The BM procedure was modified from a previous work (Sunyer et al., 2007). The apparatus consisted in a clear gray circular platform (Ø: 92cm, height: 100 cm; Bioseb, France) with 20 equally spaced holes (Ø: 5cm) located 2 cm from the border. In this open environment, mice naturally seek a dark enclosed surrounding place, provided by a black goal box (20x9x9 cm) located beneath one of the holes. During training sessions, the 19 other holes are closed. From the surface of the maze, the open escape hole looks identical to the closed holes so that mice can locate the target box only with the spatial extra cues surrounding the maze. The circular platform was virtually divided in 4 zones (including the target quadrant with the escape hole). To reduce anxiety behavior, mice were habituated to the platform and the target box on the day before the beginning of the experiment.

Each trial began by placing the animal in a black starting cylinder (Ø: 8 cm, height: 12,5 cm) at the center of the platform that was removed after 10s, allowing mice to freely explore the apparatus. Spatial acquisition was organized in 4 training session (Day 1-4). Each training session consisted in three 3-min trials, with 20 min inter-trials interval during which animals returned to their home cage. Mice that failed to find the target box within 3 min were gently guided to its location. For those mice, 180 sec were recorded as the escape latency. All animals remained in the target box for 60 sec after entering.

All trials were recorded by a camera and analyzed by ANY-maze Software version 4.99 (Stoelting, Bioseb, France). The following parameters were scored during all training trials : primary latency, latency to escape (total latency), primary errors and total errors. Primary latency was defined as the time required for mice to make initial contact with the target hole. Latency to escape was defined as

the time it took animals to completely enter in the target box (all 4 paws out of the platform). Primary errors were defined as the number of holes visited before the first contact with the target hole and total errors were defined as the total number of holes visited during the trial that did not lead to the target box. A hole was considered visited when mice tilted their head over it (nose poke) or introduced their paws into the hole.

On Day 5, reference memory was evaluated by a retention probe trial (90 sec) during which the target box was removed and the target hole was closed. Mice were allowed to explore the maze and to visit the target hole and the adjacent holes. Latency to reach the target hole for the first time, number of errors before reaching the target hole and time spent in target quadrant were recorded.

Associative memory: one-trial contextual fear conditioning (CFC)

Fear conditioning was conducted in Start Fear Combined system from Harvard apparatus (Bioseb, France) in chambers with internal dimensions of 25 × 25× 25 cm. The chambers had metal walls on each side, clear plastic front and back walls and ceilings, and stainless steel bars on the floor. A house light mounted directly on the side of the chamber provided illumination. Each chamber was located inside a larger insulated plastic cabinet (67 × 53× 55 cm) that provided protection from outside light and noise. Each cabinet contained a ventilation fan that was operated during the sessions. Mice were held outside the experimental room in their home cages prior to testing and transported to the conditioning apparatus individually in standard mouse cages. Training chambers were cleaned with 70% ethanol solution before and after each trial to avoid any olfactive cues. The experimental design was adapted from previous studies (Drew et al., 2010) and ran over two consecutive days. On Day 1, mice were placed in the conditioning chamber and 3 min later received one shock (2 s, 0.75 mA). Mice were removed from the chamber 15 s after the shock. On Day 2, animals returned to the conditioning chamber for a 4-min period in the exact same conditions of Day 1, but without electrical shock, for a test of context-elicited freezing. Scoring was measured using Freezing software version 2.0.04 (Packwin, Harvard apparatus, Bioseb, France). The StartFear system allows recording and

analyzing the signal generated by the animal movement through a high sensitivity weight transducer system. The behavior of mice was also recorded with digital video cameras mounted above the conditioning chamber.

Data analysis:

Results were expressed as mean ± SEM for all experiments. T-tests, one-way or two-way ANOVA (genotype and treatment factors) were applied when appropriate. Significant main effects and/or interactions were followed by a Fisher's PLSD post hoc analysis. In the NSF test, we used the Kaplan-Meier survival analysis due to the lack of normal distribution of the data. Mantel-Cox log-rank test was used to evaluate differences between experimental groups. Statistical significance was set at $p<0.05$.

Figure 1:

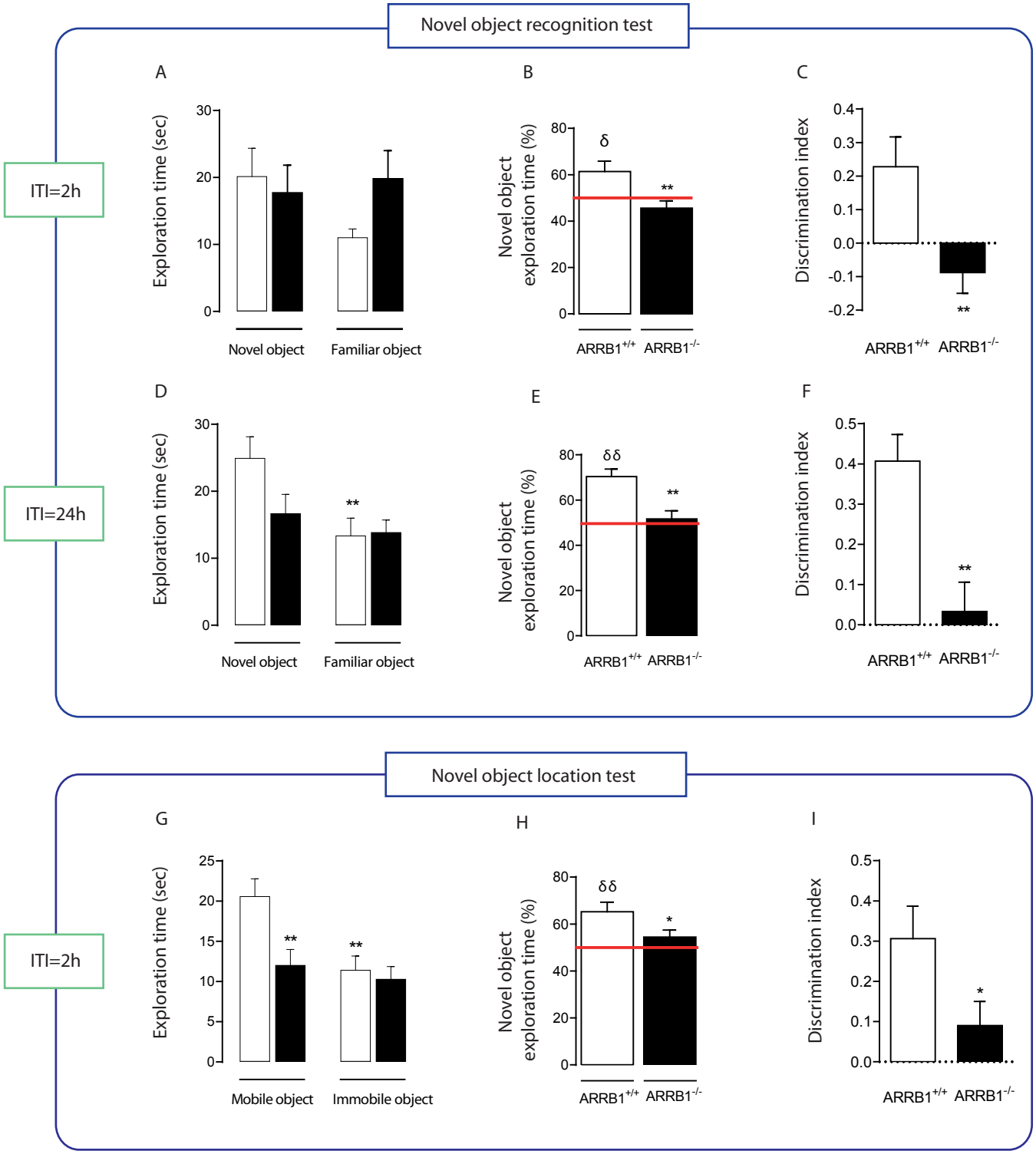


Figure 1: Behavioral consequences of conditional β-arrestin 1 deletion in adult newborn granule cells of the dentate gyrus on episodic-like memory. (A-D-G) Exploration time of the novel (or moved) object and the familiar (or stationary) objects were measured during the test session, with different intertrial intervals. (B-E-H) The exploration time was also expressed in percent of novel object exploration. The red line indicates the chance level (50%). (C-F-I) Discrimination index for each protocol was calculated. Values are mean ± SEM (n=7-12 per group)

*p< 0.05, **p<0.01 versus ARRB1$^{+/+}$ mice; δp< 0.05, δδp< 0.01 versus the chance level (50%)

Figure 2:

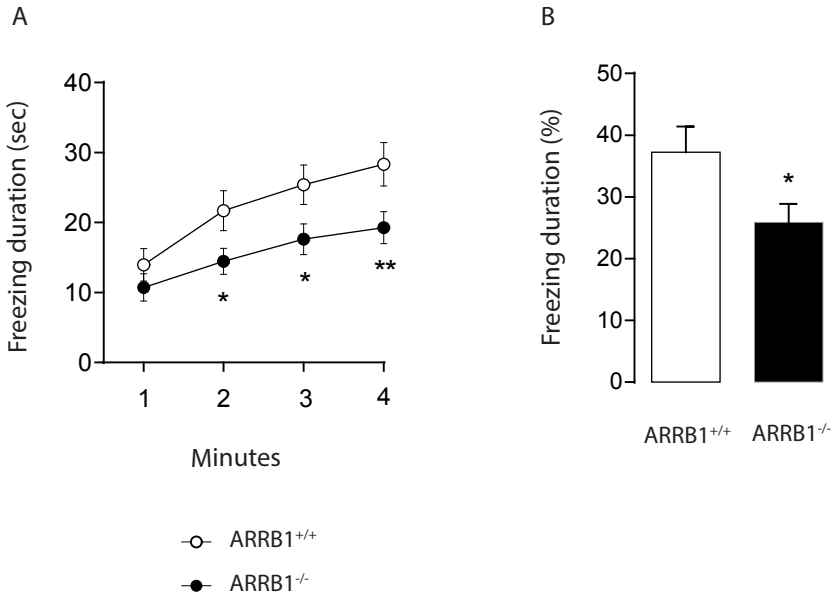


Figure 2: Behavioral consequences of conditional β-arrestin 1 deletionin adult newborn granule cells of the dentate gyrus on associative memory. Contextual fear conditioning was produced by placing a mouse into the conditioning chamber and delivering one footshock 180 sec later. Mice were returned to the conditioning chamber 24h later to assess for context-elicited freezing. During Day 2, context-elicited freezing was analyzed for each minute of the test (A) and during the whole session (B). Values are mean ± SEM (n=-10-15 per group)

*p< 0.05, **p<0.01 versus ARRB1$^{+/+}$ mice

Figure 3:

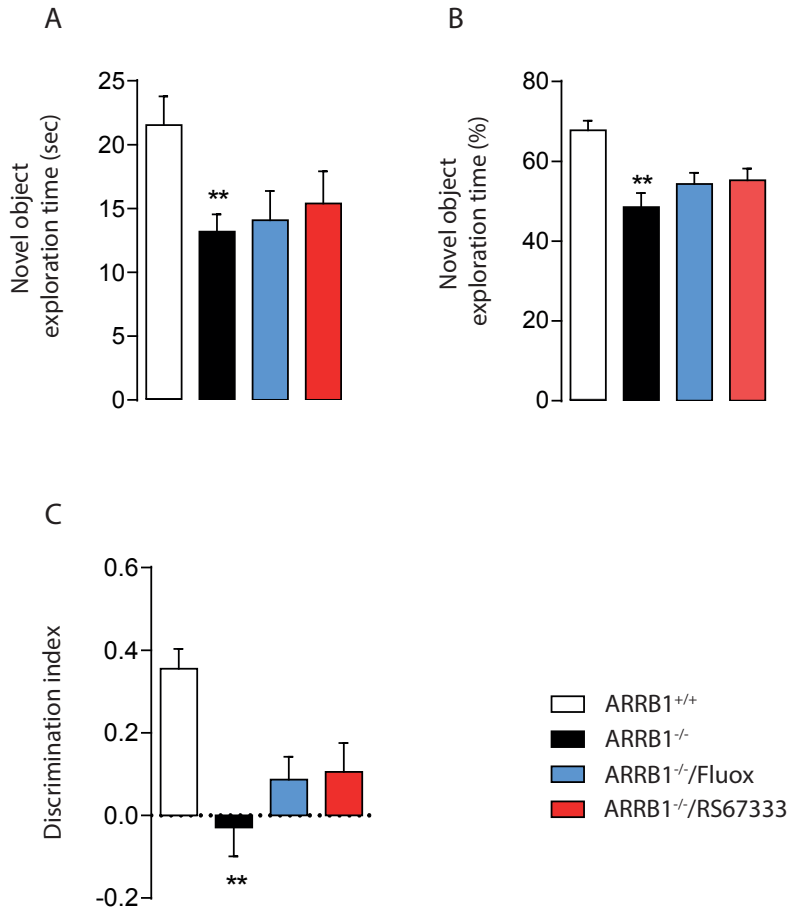


Figure 3: Chronic fluoxetine or RS67333 treatments are not able to reverse episodic-like memory deficits in ARRB1$^{-/-}$ mice. Novel object exploration time was measured in second (A) and in percent (B). Discrimination index was calculated in each group (C). Values are mean ± SEM (n=-12-15 per group)

**p<0.01 versus ARRB1$^{+/+}$ mice.

Figure 4:

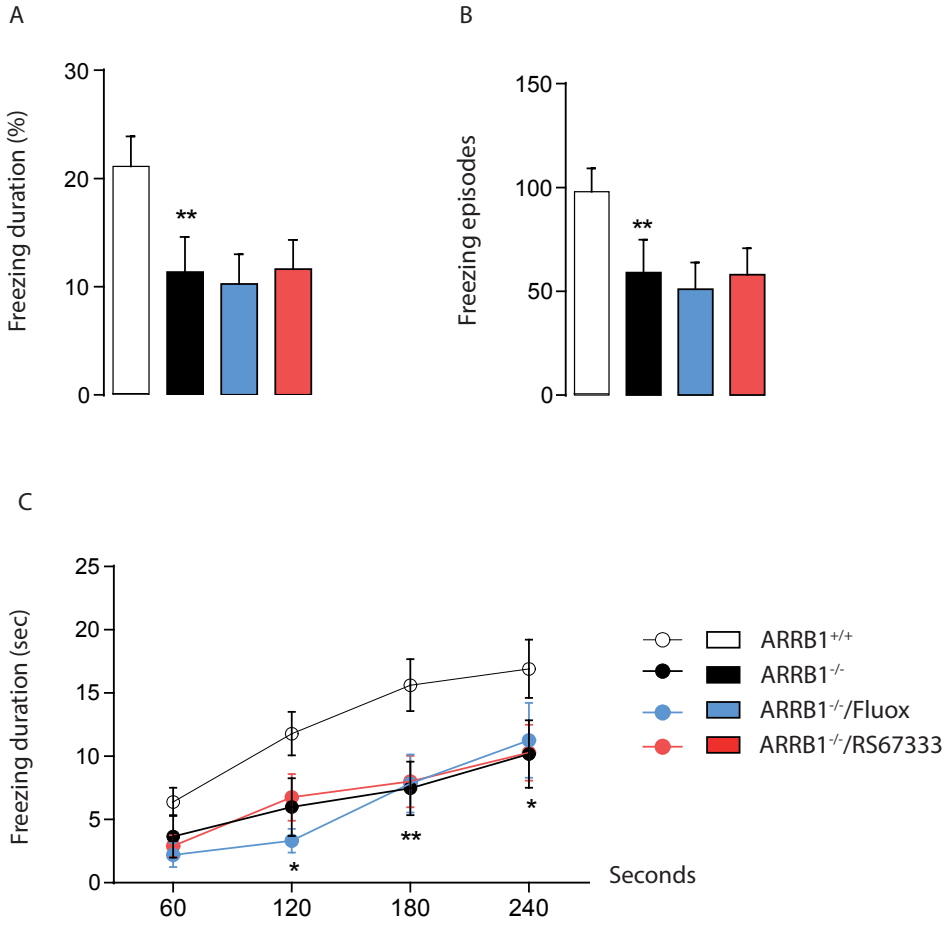


Figure 4: Chronic fluoxetine or RS67333 treatments are not able to reverse associative memory impairment in ARRB1$^{-/-}$ mice in the one-trial contextual fear conditioning. Context-elicited freezing was analyzed by measuring freezing duration (%) (A) and the number of freezing episodes (B). Freezing behavior was also monitored for each minute of the test (C). Values are mean ± SEM (n=-12-15 per group)

*p< 0.05, **p<0.01 versus ARRB1+/+ mice.

Figure 5:

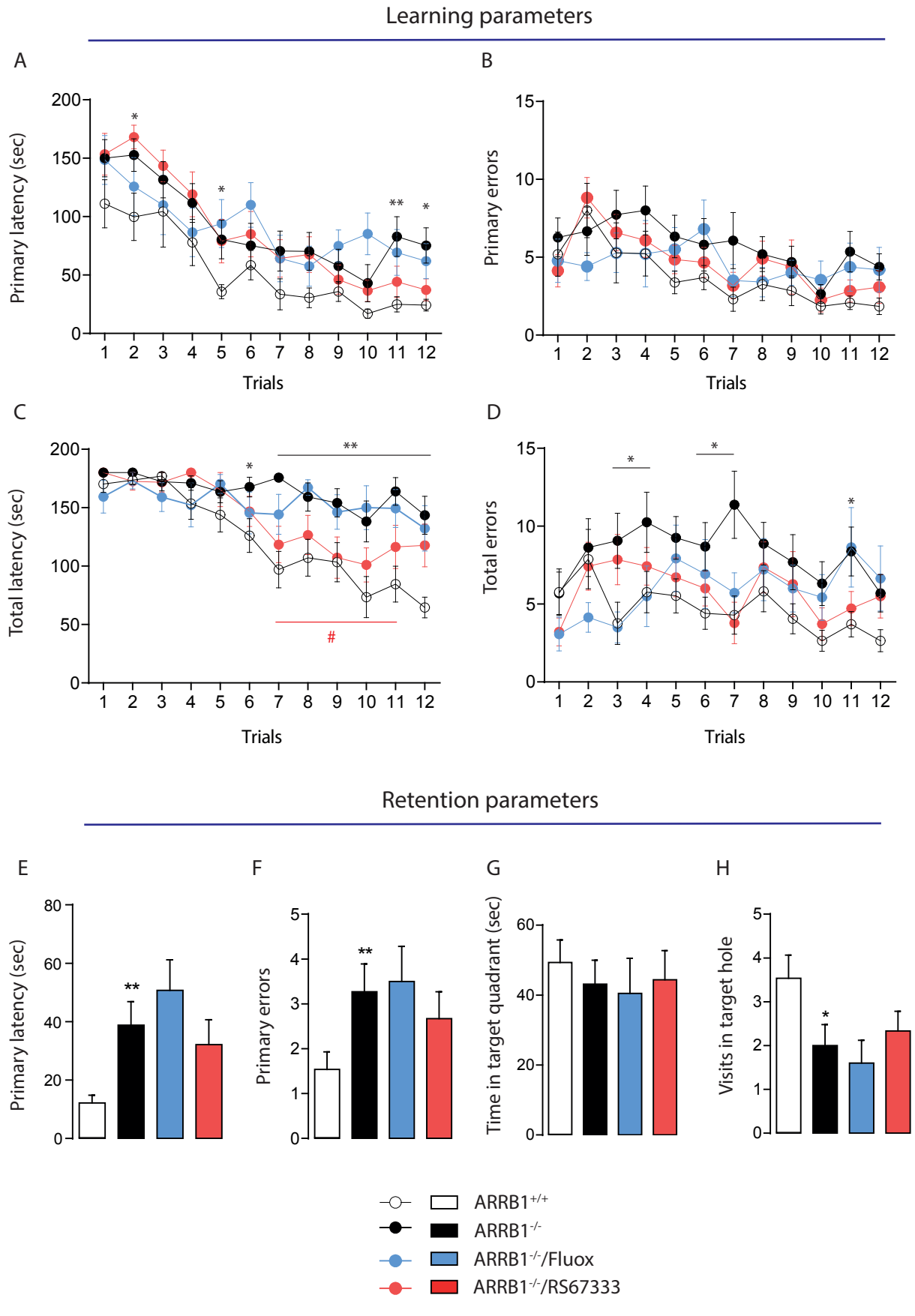
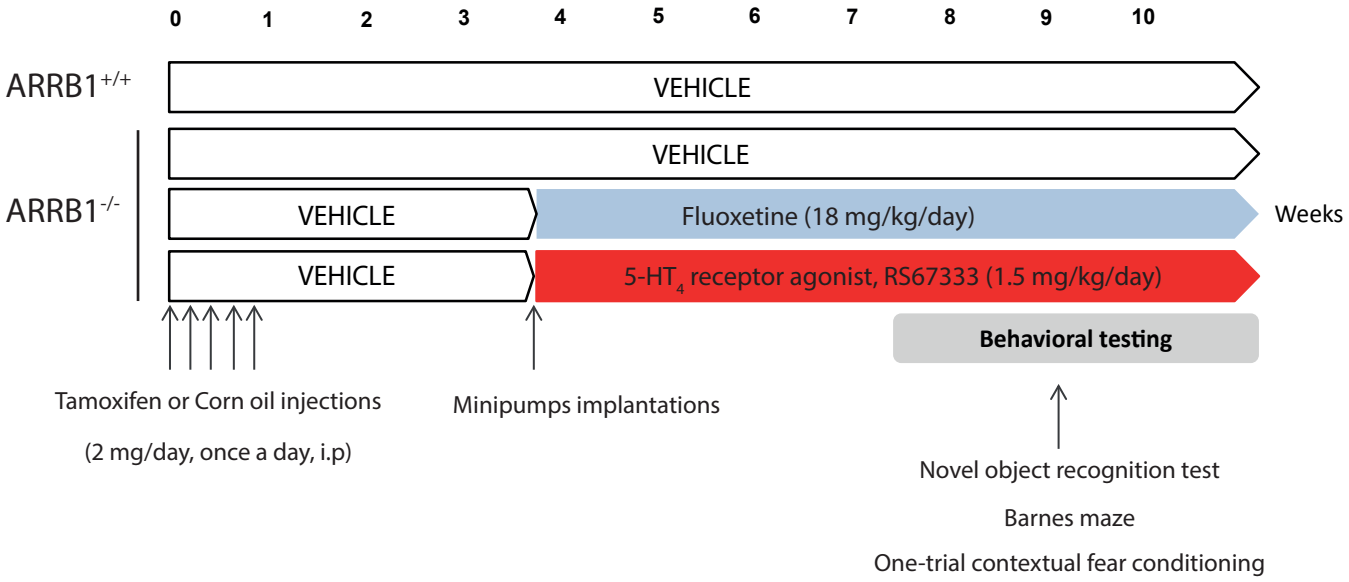

Learning parameters
A
Primary latency (sec)
Trials
B
Primary errors
Trials
C
Total latency (sec)
Trials
D
Total errors
Trials
Retention parameters
E
Primary latency (sec)
F
Primary errors
G
Time in target quadrant (sec)
H
Visits in target hole
ARRB1+/+
ARRB1-/-
ARRB1-/-/Fluox
ARRB1-/-/RS67333

Figure 5: Chronic fluoxetine or RS67333 treatments are not able to reverse spatial memory deficits in ARRB1$^{-/-}$ mice.

Animals were trained during 4 days to learn the location of the target box. During this acquisition period, learning was monitored by recording primary latency (A), total latency (B), primary errors (C) and total errors (D). At Day 5, a retention probe trial estimates reference spatial memory, by removing the target box and closing the target hole. Spatial retention was evaluated by measuring primary latency (E), primary errors (F), time in target quadrant (G) and the number of visits in the target hole (H). Values are mean ± SEM (n=-10-15 per group)

*p< 0.05, **p<0.01 versus ARRB1$^{+/+}$ mice;
#p<0.01 versus ARRB1$^{-/-}$ mice.

Supplemental Figure 1:



Supplemental Figure 1: Experimental design of the pharmacological study. Tamoxifen was injected intraperitoneally once a day (2 mg/day) on 5 consecutive days at the beginning of the study. From Week 4, in place of normal drinking water, 2 groups of ARRB1$^{-/-}$ mice were administered a 4-weeks fluoxetine or RS67333 treatment. The same cohort of mice performed successively all of the cognitive tests from week 8 to week 10: 1) novel object recognition test; 2) Barnes maze; 3) one-trial contextual fear conditioning.

DISCUSSION OF THE STUDY:

- *Conditional deletion of β-arrestin 1 in stem cells of the dentate gyrus alters cognitive performances in adult mice*

This study shows that conditional deletion of β-arrestin 1 in stem cells of the dentate gyrus alters cognitive performances in adult mice, especially episodic, associative and spatial memory. Indeed, discrimination index was significantly lower in ARRB1$^{-/-}$ mice in the novel object recognition test for both intertrial interval (2h and 24h) compared to ARRB1$^{+/+}$ animals. Episodic-like memory was also impaired in the novel objet recognition location with a decrease in time exploring the object that moved in ARRB1$^{-/-}$ mice compared to controls. Performances in the one-trial contextual fear conditioning revealed a decrease in freezing duration in ARRB1$^{-/-}$ mice, suggesting a deficit in associative memory in these mice. Finally, ARRB1$^{-/-}$ mice showed difficulties in learning and memory parameters in the spatial task, the Barnes maze. During learning trials, ARRB1$^{-/-}$ mice were longer to find the target hole and committed more errors before finding the target hole compared to ARRB1$^{+/+}$ animals. During the probe session in Day 5, primary latency and primary errors were increased and the number of visits in the target hole was decreased in ARRB1$^{-/-}$ mice compared to controls.

- *β-arrestin 1 expression in stem cells of the dentate gyrus is required to chronic fluoxetine and 5-HT$_4$ receptor agonist RS67333-induced pro-cognitive effects in adult mice*

Four weeks after tamoxifen injection, 2 groups of ARRB1$^{-/-}$ mice were administered during 28 days either fluoxetine or RS67333 in the drinking water or using Alzet® mini-pump respectively. The same cohort of mice performed successively all of the cognitive tests from week 8 to week 10: 1) novel object recognition test; 2) Barnes maze; 3) one-trial contextual fear conditioning. Based on discrimination index results (Figure 1), the novel object recognition test with 2h intertrial interval was chosen for the pharmacological study. Interestingly, chronic fluoxetine or RS67333 treatments were not able to reverse learning and memory deficits induced by the depletion of β-arrestin 1 in stem cells of the dentate gyrus, in all the cognitive tasks tested.

The conditional deletion of β-arrestin 1 prevents fluoxetine and RS67333-induce improvement of episodic-like, associative and spatial memory. In the novel object recognition test, chronic fluoxetine and RS67333 treatments were not able to correct the discrimination deficit observed in $ARRB1^{-/-}$ mice. In the one-trial contextual fear conditioning, freezing duration was still decreased in $ARRB1^{-/-}$/Fluox and $ARRB1^{-/-}$/RS67333-treated mice compared to $ARRB1^{+/+}$ animals, indicating that the alteration of associative memory is maintained in $ARRB1^{-/-}$ animals despite pharmacological treatments. Finally, $ARRB1^{-/-}$/Fluox and $ARRB1^{-/-}$/RS67333-treated mice failed to restore spatial performances, in both learning and memory parameters.

These data suggest that β-arrestin 1 expression in stem cells of the dentate gyrus is required to pro-cognitive effects of chronic fluoxetine and 5-HT_4 receptor agonist in adult mice and confirm that β-arrestin 1 protein is a key element in cognitive mechanisms and pharmacological cognitive responses.

Discussion générale

A ce jour, les déficits cognitifs pourtant présents chez plus de 94% des patients souffrant de dépression majeure, sont très peu pris en charge. Identifier des molécules thérapeutiques traitant les symptômes dépressifs au même titre que les symptômes cognitifs reste un problème majeur de santé publique. D'autre part, des données cliniques montrent que le déficit cognitif persiste après la rémission des symptômes dépressifs et pendant les épisodes récurrents de dépression (Hasselbalch, 2015; Hasselbalch et al., 2011) (Figure 29). Jusqu'à aujourd'hui, les études cliniques mettant en évidence l'efficacité des antidépresseurs et notamment des ISRS quant à la correction du déficit cognitif sont contradictoires (Bastos et al., 2013; Ferguson et al., 2003; Gallassi et al., 2006; Herrera-Guzman et al., 2010).

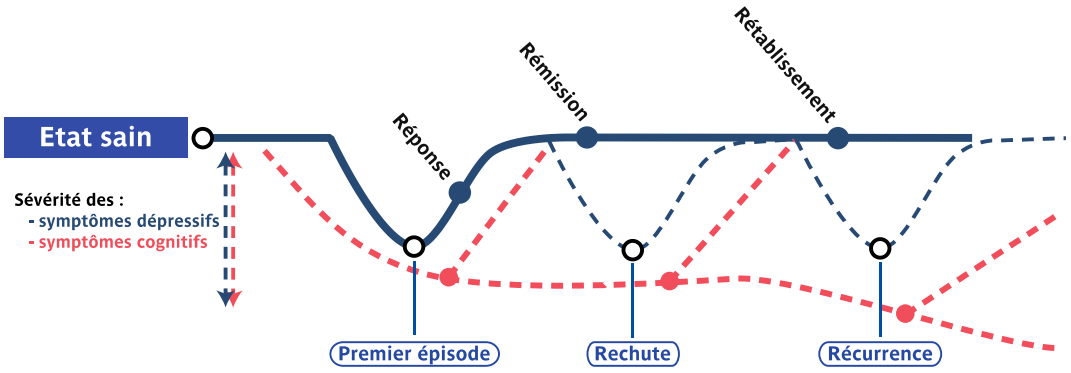


Figure 29: Représentation schématique de la trajectoire possible d'un patient dépressif.
Les lignes bleues pleines et pointillées représentent les formes variées de progression des symptômes dépressifs au cours de la dépression. La ligne rouge pointillée représente l'ampleur des symptômes cognitifs associés à la pathologie de la dépression selon le stade de l'épisode dépressif.

Une réelle évaluation d'abord préclinique, puis clinique des co-morbidités associées aux symptômes dépressifs est nécessaire afin de déterminer ensuite quelle approche thérapeutique pourrait le mieux les corriger.

Ce travail de recherche préclinique, décliné en 3 objectifs a permis d'approfondir la compréhension du rôle du récepteur 5-HT_4 et de la protéine β-arrestine 1 dans la modulation des processus émotionnels et cognitifs dans un modèle d'anxiété/dépression chez la Souris. Pour cela, nous avons caractérisé les troubles de la mémoire et de l'apprentissage dans un modèle animal murin d'anxiété/dépression : le modèle CORT. Nous avons ensuite évalué l'efficacité pro-cognitive de 2 stratégies thérapeutiques à l'aide d'un antidépresseur ISRS de référence (la fluoxetine) et d'une molécule à visée antidépressive possédant des propriétés agonistes du récepteur 5-HT_4 (le RS67333). Enfin, nous avons mis en évidence le rôle joué par la protéine β-arrestine 1 au niveau des jeunes neurones du GD, dans la physiopathologie des troubles de l'humeur et de la mémoire et dans la réponse aux traitements et notamment celle de l'agoniste du récepteur 5-HT_4.

1.1 Le phénotype anxio/dépressif induit par un traitement chronique de corticostérone chez la Souris est associé à des déficits cognitifs.

Pour mieux comprendre les mécanismes responsables des déficits cognitifs dans les épisodes dépressifs majeurs, la modélisation chez l'animal est essentielle. De nombreuses études se sont d'ailleurs intéressées aux troubles d'apprentissage et de mémoire dans différents modèles d'anxiété/dépression chez le rongeur (cf. **Revue**). Malgré des résultats très hétérogènes, il semble néanmoins que les troubles des fonctions exécutives et de l'attention soient constants, quelle que soit la procédure de stress chronique utilisée. D'autre part, les modèles de stress chronique induits pendant l'âge adulte (comme le UCMS et le modèle CORT) présentent de plus fortes similarités avec les symptômes cognitifs observés chez les patients déprimés, par rapport aux procédures de stress pré- ou périnatal.

Dans ce travail, le phénotype anxio/dépressif a été induit suite à l'administration chronique de corticostérone à faible dose chez la souris C57BL/6J (le modèle CORT) (David et al., 2009). Une revue de la littérature, reprenant différents travaux ayant étudiés l'impact d'une exposition chronique de corticostérone chez le Rongeur dans différentes tâches cognitives montre que la majorité des différents types de mémoires est affectée par ce protocole de stress chronique. Seule la mémoire de travail (alternation ou spatiale) semble insensible au stress généré par la CORT (Tableau 16).

Tableau 15: Effets d'une administration chronique de corticostérone sur les performances cognitives chez le Rongeur - Partie 1

Type de mémoire	Test comportemental	Espèce/Souche	Sexe	Corticostérone (dose, voie d'administration, durée)	Effets observés	Références
Episodique	NORT	Souris C57BL/6J	♂	100 µg/mL, v.o., 28 jours	↓ indice de discrimination	Solas et al 2013
		Rat Wi	♂	5 mg/kg, s.c., 21 jours		Walesiuk et al 2005
	MWM	Rat SD	♂	40 mg/kg/j, s.c., 21 jours	↑temps de latence et ↓ rétention	Lee et l 2014
		Rat Wi	♂	5 mg/kg/day, s.c., 21 jours	↑temps de latence	Trofimiuk et al 2015
	BM				↑nombre erreurs	
		Rat F-BN	♂	150 mg/ Implant (s.c.), 21 jours	↑ nombre d'erreurs et latence	MacLay et al 1998
		Rat SD	♂	26.8 mg/kg, 10 mg/day, s.c., 21 jours	↑ nombre d'erreurs	Coburn-Litvak et al 2003
Associative	Evitement passif	Rat SD	♂	100 mg, Implant (s.c.), 22 days	↓ temps pour entrer dans la chambre sombre	Bisagno et al 2000
			♂	40 mg/kg/j, s.c., 21 jours		Lee et l 2014
	CFC	Rat LE	♂	5 mg/kg or 40 mg/kg, s.c., 21 jours	Effet dose-dépendant	Marks et al 2015
		Rat SD	♂	50 µg/mL 14 jours, 25 µg/mL 3 jours, 12.5 µg/mL 3 jours	↑freezing, ↓ de l'extinction	Gourley et al 2009

Tableau 16: Effets d'une administration chronique de corticostérone sur les performances cognitives chez le Rongeur - Partie 2

Type de mémoire	Test comportemental	Espèce/Souche	Sexe	Corticostérone (dose, voie d'administration, durée)	Effets observés	Références
Travail	Y-maze	Rat SD	♂	26,8 mg/kg, 10 mg/day, s.c., 21 jours ou 56 jours	Ø après 21 jours de traitement ↓ alternation après 56 jours de traitement	Coburn-Litvak et al 2003
				200 mg/L, v.o., 21 jours	Ø	Gururajan et al 2015
	Spatiale	Rat SD	♂	40 mg/kg/j, s.c., 18 jours	Ø	Workman et al 2015
		Rat Wi	♂	25 mg/kg, s.c., 28 jours	Ø	Cerquiera et al 2005
Fonctions exécutives	5-CSTT	Rat SD	♂	50 µg/mL 14 jours, 25 µg/mL 3 jours, 12.5 µg/mL 3 jours	↓ action impulsive et ↑choix impulsifs	Terregrossa et al. 2012
	ASST	Rat Lister	♂	50 µg/mL, v.o., 15 jours	↑ nombre essais pour atteindre le critère (REV1, ID, REV2, REV3)	Wallace et al 2014
	Flexibilité mentale (MWM)	Rat Wi	♂	25 mg/kg, s.c., 28 jours	↓ apprentissage inversé	Cerquiera et al 2005

Abréviations : NORT : test de reconnaissance d'objet ; MWM : test de la piscine de Morris ; BM : labyrinthe Barnes; CFC : test de conditionnement par la peur ; 5-CSTT : test d'attention s.c : sous-cutanée ; v.o : voie orale (eau de boisson) ; LE : Long-Evans ; SD : Sprague-Dawley ; Wi : Wistar ; F-BN : Fischer 344 x Brown Norway

Au cours d'un premier travail expérimental effectué avec le modèle CORT (**Article 1**), nous avons caractérisé les performances d'apprentissage et de mémoire dans plusieurs domaines de cognition. Le Tableau 17 résume les effets observés au cours de ces expériences.

Tableau 17 : Récapitulatif des effets d'une administration chronique de corticostérone sur les différents types de mémoires. (= : capacité de mémoire intacte ; ↓ : mémoire altérée)

Type de mémoire	Test cognitif	Paramètre testé	Véhicule	CORT
Mémoire de type épisodique	Reconnaissance d'objet	Rétention à 5 min	=	↓
Mémoire spatiale	Piscine de Morris	Apprentissage (acquisition)	=	↓
		Rétention à 24h	=	↓
		Apprentissage (flexibilité)	=	↓
		Rétention à 24h	=	↓
	Labyrinthe de Barnes	Apprentissage	=	↓
		Rétention à 24h	=	↓
		Rétention à 7 jours	=	↓
Mémoire à composante associative	Conditionnement par la peur	Rétention à 24h	=	↓

Mécanismes neurobiologiques expliquant les troubles cognitifs dans le modèle CORT

Bien que nous n'ayons pas directement vérifié cette hypothèse, il est plausible qu'une diminution de la neurogenèse hippocampique puisse être responsable d'une partie des déficits cognitifs observés après traitement chronique de corticostérone, notamment dans le NORT et dans le CFC (**Article 1**). En effet, de précédentes études, utilisant une stratégie d'ablation génétique de la neurogenèse hippocampique adulte, ont montré que les jeunes neurones de 4 à 6 semaines participaient aux processus cognitifs (Sahay et al., 2011 ; Denny et al., 2014). Par ailleurs, l'activité des cellules granulaires adultes issues de la neurogenèse hippocampique participe grandement à l'encodage d'une nouvelle information et donc à l'apprentissage (Danielson et al., 2016). A ce jour, l'implication ou non de la neurogenèse hippocampique adulte dans les processus d'apprentissage et de mémoire reste un débat (cf **Revue**).

Même si la survie des jeunes neurones n'est pas affectée, il a été observé dans le modèle CORT une diminution d'environ 30% de la prolifération cellulaire (David et al., 2009). Par ailleurs, de récentes données obtenues au laboratoire avec une analyse de Sholl de l'arborisation dendritique des jeunes neurones, montre une diminution de la longueur de l'arborisation dendritique et du nombre d'intersections suite à une administration chronique de corticosterone. Cependant, il serait insuffisant de résumer les déficits d'apprentissage ou de mémoire observés dans ce modèle à un effet exclusivement neurogenèse-dépendant. D'autres modifications, par exemple des systèmes glutamatergiques, gabaergiques ou cholinergiques centraux peuvent expliquer en partie l'apparition de ces troubles cognitifs.

Des structures cérébrales autres que l'hippocampe peuvent voir leur fonctionnement altéré suite à une exposition chronique à la CORT. Ainsi, la mémoire associative est en étroite relation avec l'amygdale (Roozendaal et al., 2009), et la mémoire épisodique comme la discrimination d'objets est aussi reliée au cortex entorhinal (Reagh and Yassa, 2014; Wilson et al., 2013). Des modifications moléculaires et structurelles au sein de ces régions cérébrales peuvent également être à l'origine des difficultés cognitives induites par un stress prolongé.

Récemment, il a été démontré que les glucocorticoïdes altéraient la mémoire spatiale et la consolidation en diminuant l'excitabilité des synapses dans le subiculum (temporo-ammonique)-TA-CA1 (Kvarta et al., 2015). Ces auteurs ont montré qu'un traitement chronique de corticostérone, en mimant les effets d'un stress chronique, réduirait l'excitation induite par l'activation des récepteurs AMPA du glutamate. Ces effets, dus à l'activation des récepteurs aux glucocorticoïdes, diminuent la plasticité synaptique et affectent notamment la consolidation de la mémoire.

D'autre part, la neurotransmission glutamatergique joue un rôle majeur dans les processus de PLT, de DLP et de mémorisation via l'activation des récepteurs NMDA du glutamate, suivie de la cascade d'évènements provoqués par l'influx calcique (McEwen and Sapolsky, 1995; Riedel et al., 2003). Or, si un stress aigu semble avoir des effets bénéfiques sur la transmission glutamatergique et sur les performances cognitives, un stress chronique provoquerait à l'inverse des changements structurels délétères tels qu'une perte et une simplification dendritique ou encore une réduction de la densité d'épines dendritiques dans le cortex et dans l'hippocampe (Popoli et al., 2012; Sandi, 2011). Ces modifications quantitatives et qualitatives affectent la transmission synaptique et sont alors responsables des déficits cognitifs comportementaux. Dans le cadre du travail expérimental de l'**Article 1**, et comme viennent de le démontrer Kvarta et collaborateurs (2015), nous pouvons émettre l'hypothèse que l'administration chronique de CORT freine la plasticité synaptique notamment dans le circuit TA-CA1.

Le système cholinergique central est lui aussi un système clé dans la modulation des fonctions cognitives dans un contexte de stress (Gold, 2003; Hesen and Joels, 1996; Mizoguchi et al., 2008; Mizoguchi et al., 2001; Picciotto et al., 2015). Il a d'ailleurs récemment été montré qu'un traitement chronique par la CORT réduit l'expression des récepteurs nicotiniques de type α4 et α7 dans des neurones cholinergiques (Baier et al., 2014). Une autre étude montre que la nicotine serait capable de restaurer les déficits cognitifs induits par un stress psychosocial dans le test hippocampe-dépendant de la piscine de Morris (Alzoubi et al., 2013), confirmant le rôle essentiel de la transmission cholinergique centrale dans les altérations cognitives en environnement stressant.

1.2 L'activation du récepteur 5-HT4, une cible privilégiée du traitement des épisodes dépressifs associés à des troubles cognitifs.

En utilisant le modèle CORT, nous avons montré qu'un traitement chronique avec un agoniste du récepteur 5-HT_4 (RS67333, 1,5 mg/kg/jour) induit, tout comme un ISRS (fluoxetine, 18 mg/kg/jour), des effets de type anxiolytiques et antidépresseurs, mais aussi une augmentation de la neurogenèse dans le gyrus dentelé (**Article 2**). Nous avons également mis en évidence, que l'antagonisme du récepteur 5-HT_4 par le GR125487, est suffisant pour bloquer les effets comportementaux et neurogéniques de la fluoxetine. La sérotonine a une forte affinité pour les récepteurs 5-HT_4 humains et de rats (pKd = 8,4 et 7,7, respectivement) (Adham et al., 1996; Bender et al., 2000). L'activation des récepteurs 5-HT_4 suite à l'augmentation de la sérotonine synaptique induite par le blocage du SERT participe en partie aux effets de type anxiolytiques/antidépresseurs de la fluoxetine. Dans un second temps, nous avons voulu déterminer si des traitements chroniques par de la fluoxetine ou par le RS67333, parvenaient à restaurer les fonctions cognitives chez les animaux traités par la corticostérone. Le Tableau 18 rassemble les principaux résultats de cette étude expérimentale (**Article 3**). L'activation du récepteur 5-HT_4 corrige le déficit cognitif chez des animaux présentant un phénotype anxio/dépressif.

Tableau 18: Récapitulatif des effets d'un traitement chronique de fluoxetine ou d'agoniste du récepteur 5-HT_4 (RS67333) sur les performances cognitives chez les animaux anxio-déprimés (Ø: capacité de mémoire non corrigée ; ↑: capacité de mémoire restaurée).

Type de mémoire	Test cognitif	Paramètre testé	Fluoxetine	RS67333
Mémoire de type épisodique	Reconnaissance d'objet	Rétention à 5 min	↑	↑
Mémoire spatiale	Labyrinthe de Barnes	Apprentissage	↑	↑
		Rétention à 24h	Ø	↑
Mémoire à composante associative	Conditionnement par la peur	Rétention à 24h	Ø	↑

Comment expliquer une efficacité supérieure du RS67333 comparée à celle de la fluoxetine?

Alors que le traitement chronique par le RS67333 corrige la totalité des déficits induits par la corticostérone, les effets du traitement chronique par la fluoxetine sont moins nombreux puisque seuls les déficits de mémoire épisodique et d'apprentissage spatial sont corrigés. L'avantage principal du RS67333 est d'être un composé sélectif qui active uniquement le récepteur 5-HT_4. La fluoxetine, quant à elle, inhibe la recapture de la sérotonine, provoquant alors une augmentation des taux de sérotonine disponible dans la fente synaptique. Cette grande quantité de 5-HT se fixe alors de façon non sélective sur les récepteurs sérotoninergiques pour exercer ses effets comportementaux. Il est donc probable qu'il existe des effets inhibiteurs induits par la stimulation de récepteurs 5-HT qui limitent ainsi l'efficacité de la fluoxetine dans certains tests de cognition. De façon à mieux comprendre les mécanismes mis en jeu dans la réponse cognitive à la fluoxetine, il serait intéressant d'identifier le ou les récepteur(s) sérotoninergique(s) postsynaptique(s) responsable(s) de l'absence d'effets pro-cognitifs. Une fois ce(s) récepteur(s) déterminé(s), il faudrait alors identifier un antagoniste de ce récepteur 5-HT qui, associé à la fluoxetine permettrait d'atteindre l'efficacité du RS67333 dans les tests de cognition. Parmi les 14 sous-types de récepteurs sérotoninergiques existants, l'activation des récepteurs 5-HT_3, 5-HT_6 et 5-HT_7 semble être un frein à l'activité pro-cognitive bien qu'ils soient tous les trois associés à une dépolarisation/excitation membranaire. Ainsi des antagonistes de ces récepteurs pourraient être de bons candidats pour corriger les déficits cognitifs (pour revue, (Meneses, 2015)).

Le récepteur 5-HT_3, canal ionotropique, fait partie des récepteurs sérotoninergiques d'intérêts en raison de son influence excitatrice sur la libération d'acétylcholine et de GABA suite à sa stimulation par la sérotonine endogène (Giovannini et al., 1998). Dès les années 1990, des études ont montré que le blocage du récepteur 5-HT_3 par l'ondansetron améliorait certaines fonctions cognitives telle que la navigation spatiale dans la piscine de Morris (Fontana et al., 1996). Plus récemment, des travaux ont mis en exergue le rôle du tropisetron, antagoniste sélectif du récepteur 5-HT_3, non seulement dans le traitement des vomissements induits par la chimiothérapie mais aussi lors de déficits cognitifs, et notamment dans le contexte clinique de la maladie d'Alzheimer (Fakhfouri et al., 2015; Hashimoto, 2015).

Au même titre que le récepteur 5-HT_4, l'activation du récepteur 5-HT_6 mène à une activation de la voie de signalisation de l'AMPc via une augmentation de l'adénylate cyclase. Pourtant, la plupart des études rapportent un effet bénéfique du blocage du récepteur 5-HT_6 sur les fonctions cognitives, probablement dû à l'existence d'une voie alternative de type inhibitrice, activée par le

récepteur 5-HT_6. (Ramirez et al., 2014). Parmi ces travaux, la majorité est conduite dans un contexte pathologique bien précis. Ainsi, le blocage du récepteur 5-HT_6 par un antagoniste sélectif provoque globalement des effets de type anti-amnésiques voire pro-cognitifs chez les animaux âgés (Callaghan et al., 2012), chez les animaux présentant des déficits cognitifs induits par la scopolamine (Da Silva Costa-Aze et al., 2012). Cela a aussi été observé chez les patients atteints de la maladie d'Alzheimer (Claeysen et al., 2015; Wilkinson et al., 2014). Récemment, il a été démontré qu'une administration chronique chez la souris naïve d'un antagoniste du récepteur 5-HT_6, le SB271046, permettait d'améliorer la capacité de discrimination des animaux dans le test de la reconnaissance d'objet (Quiedeville et al., 2015). Cependant, à notre connaissance, aucune étude préclinique ou clinique n'a encore exploré les propriétés pro-cognitives des antagonistes des récepteurs 5-HT_6 dans le contexte de la dépression majeure. L'ensemble de ces données fait donc du récepteur 5-HT_6 une cible à privilégier dans les perspectives de ce travail de thèse.

Enfin, une revue récente suggère des perspectives encourageantes concernant le rôle du récepteur 5-HT_7 dans différentes pathologies du SNC (anxiété, épilepsie, schizophrénie) (Nikiforuk, 2015). Des études précliniques ont d'ailleurs montré que l'utilisation d'antagonistes des récepteurs 5-HT_7 entrainait des effets anti-amnésiques (Gasbarri and Pompili, 2014) voire pro-cognitifs (Gasbarri et al., 2008) si la tâche cognitive présente un degré élevé de difficulté. Cependant, d'autres travaux rapportent ces mêmes effets favorisant la mémoire suite à la stimulation des récepteurs 5-HT_7 par un agoniste (Di Pilato et al., 2014; Freret et al., 2014; Perez-Garcia and Meneses, 2005) (Figure 30). En raison de ces résultats hétérogènes, le récepteur 5-HT_7 ne constitue pas l'hypothèse prioritaire dans notre recherche du (des) récepteur(s) impliqué(s) dans la réponse cognitive à la fluoxetine.

Il est important de disséquer le mécanisme d'action impliqué dans les effets cognitifs de la fluoxetine induits par l'activation du récepteur 5-HT_4. Les arguments précédemment développés donnent quelques pistes à explorer pour de prochaines études. La Figure 30 ci-après récapitule les conséquences de la liaison de la fluoxetine sur le transporteur du 5-HT, puis l'activation des différents types de récepteurs sérotoninergiques par le 5-HT endogène sur le comportement cognitif.

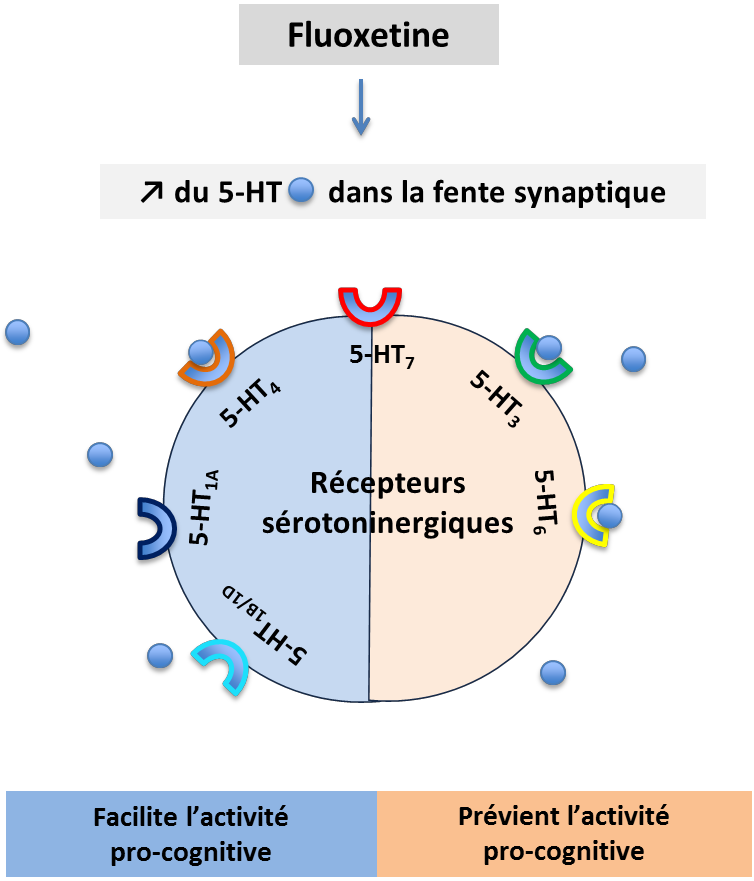


Figure 30: Représentation schématique du rôle facilitateur ou limitant de l'activation des récepteurs sérotoninergiques sur l'activité pro-cognitive. Les ISRS en bloquant le transporteur sérotoninergique et en permettant l'augmentation des concentrations extracellulaires de sérotonine après traitement chronique, sont aussi communément appelés agonistes « indirects » des récepteurs postsynaptiques. La 5-HT endogène en activant certains sous types de récepteurs sérotoninergiques (5-HT_{1A}, 5-$HT_{1B/1D}$, 5-HT_4) facilitera l'activité pro-cognitive tandis que l'activation des sous-types de récepteurs 5-HT_3, 5-HT_6 aura des effets opposés. Le sens de modulation du récepteur 5-HT_7 reste encore un point à identifier.

1.3 Implication de la protéine β-arrestine 1

1.3.1 Implication de la β-arrestine 1 dans le phénotype anxio-dépressif et dans la réponse à un traitement par la fluoxetine

Bien que l'expression des protéines β-arrestines soit ubiquitaire, de nombreuses études se sont donc intéressées à leur implication dans les pathologies du SNC en tant que nouvelle cible thérapeutique potentielle (Latapy and Beaulieu, 2013). A titre d'exemple, les rôles de la protéine β-arrestine 1 dans la physiopathologie de la maladie d'Alzheimer (Liu et al., 2013), de la maladie de Parkinson (Wu et al., 2013) ou de la dépendance à la nicotine chez les fumeurs (Sun et al., 2008) sont disséqués avec un intérêt grandissant. La diminution d'expression de β-arrestine 1 périphérique chez

les patients dépressifs a été, quant à elle, mise en évidence en 2004 (Avissar et al., 2004). Rapidement, l'implication de la β-arrestine 1 dans la réponse aux traitements antidépresseurs a été étudiée aussi bien en clinique (Avissar et al., 2004 ; Matuzany-Ruban et al., 2005) qu'en préclinique (Mendez-David et al., 2015; Golan et al 2009). Dans le domaine préclinique, l'étude de l'implication des protéines β-arrestines (1 et 2) peut faire appel à des lignées de souris génétiquement modifiées. De manière intéressante, la double mutation KO ARRB1/ARRB2 est létale chez la Souris, alors que les modèles de souris constitutives ARRB1$^{-/-}$ ou ARRB2$^{-/-}$ sont viables. Il semble donc que ces deux protéines aient des propriétés chevauchantes.

Puisque la neurogenèse hippocampique et notamment les jeunes neurones du GD âgés de 4-6 semaines semblent jouer un rôle prépondérant dans l'activité cognitive ou antidépressive (Denny et al., 2012; Sahay et al., 2011), nous avons souhaité étudier les conséquences de l'absence d'expression de la protéine β-arrestine 1 dans les jeunes neurones nouvellement formés. Pour cela, nous avons induit une délétion conditionnelle de la protéine β-arrestine 1 dans les cellules souches et attendu 4/7 semaines avant de caractériser leur phénotype. Les résultats obtenus dans l'**Article 4** sont regroupés dans le Tableau 19 et le Tableau 20. Au regard du score d'émotionalité, la délétion conditionnelle, à l'âge adulte, de l'expression de la protéine β-arrestine 1 dans les jeunes neurones issus de la neurogenèse du GD de l'hippocampe n'a pas modifié globalement le phénotype émotionnel. Pourtant des différences apparaissent quand on analyse les résultats de chaque test séparément. Un phénotype anxio/dépressif est apparu dans le labyrinthe en croix surélevée, le test des 4 plaques et le Splash test. Ceci confirme que l'expression de la β-arrestine 1 au niveau des jeunes neurones issus de la neurogenèse a des conséquences bien au-delà du GD (Bergami and Berninger, 2012). Il apparaît que le score d'émotionalité est beaucoup plus pertinent dans le cadre d'une étude de criblage que lors d'une étude mécanistique. De plus, l'absence d'expression de la β-arrestine 1 dans les jeunes neurones issus de la neurogenèse, a bloqué les effets de type anxiolytiques et antidépresseurs d'un traitement chronique par la fluoxetine, comme le révèle le score d'émotionalité, même si là encore des disparités entre les tests existent (Tableau 20).

Tableau 19: Conséquences comportementales et neurogéniques de la délétion conditionnelle de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé (Ø : absence d'effets ; ↑ : effets anxiolytiques et/ou antidépresseur ; ↓ : effets anxiogéniques et/ou dépressiogènes)

Tests		VEHICULE	
		ARRB1$^{+/+}$	**ARRB1$^{-/-}$**
Open Field		Ø	Ø
EPM		Ø	↓
NSF		Ø	Ø
Splash test		Ø	↓
TST		Ø	Ø
Test des 4-plaques		Ø	↓
Test à la saccharine		Ø	Ø
Neurogenèse hippocampique	Prolifération	Ø	↓
	Survie	Ø	Ø
	Maturation	Ø	Ø

Tableau 20: Conséquences comportementales et neurogéniques de la délétion conditionnelle de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé après un traitement chronique de fluoxetine (Ø : absence d'effets ; ↑ : effets anxiolytiques et/ou antidépresseur ; ↓ : effets anxiogéniques et/ou dépressiogènes)

Tests		FLUOXETINE	
		ARRB1$^{+/+}$	**ARRB1$^{-/-}$**
Open Field		Ø	Ø
EPM		Ø	↑
NSF		↑	Ø
Splash test		Ø	↑
TST		↑	↑
4-plates		↑	Ø
Saccharine test		↑	↑
Neurogenèse hippocampique	Prolifération	↑	Ø
	Survie	↑	Ø
	Maturation	↑	↑

Concernant l'analyse de la neurogenèse hippocampique, ces travaux ont montré que l'expression de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé de l'hippocampe participe à l'augmentation de la prolifération des progéniteurs ainsi qu'à la survie des jeunes neurones induit par l'administration chronique de fluoxetine. En revanche, cette expression ne semble pas indispensable aux effets de la fluoxetine sur l'étape de maturation des cellules granulaires. Ces effets passent donc par un mécanisme β-arrestine 1 indépendant.

Devant l'augmentation de l'étape de la maturation chez les animaux ARRB1$^{-/-}$ traités par la fluoxetine et l'absence d'effets de type anxiolytiques/antidépresseurs dans le test neurogenèse-dépendant du NSF, on peut se demander si les jeunes neurones dépourvus de protéine β-arrestine 1 s'intègrent pleinement au réseau neuronal préexistant. Pour répondre à cette question, il est primordial d'étudier l'intégration de ces jeunes neurones en regardant leur devenir en présence ou non de la protéine β-arrestine 1. Seul un triple marquage BrdU/DCX/NeuN permettrait de différencier les neurones en phase de maturation (BrdU+/DCX+/NeuN+ ou-) des neurones matures (BrdU+/NeuN+). Nous pouvons imaginer que l'absence d'expression de la protéine β-arrestine 1 dans les cellules souches du GD induise une augmentation de cellules présentant le phénotype (BrdU+/DCX+/NeuN+ ou-) sans aboutir au stade de neurone mature (BrdU+/NeuN+). D'autre part, un marquage à la caspase 3 permettrait de savoir si la diminution de la prolifération à l'état basal et l'absence d'augmentation des étapes de prolifération et de survie neuronale en présence de fluoxetine chez les animaux ARRB1$^{-/-}$est associée à une augmentation du nombre de cellules en apoptose.

1.3.2 Implication de la β-arrestine 1 dans les performances cognitives et dans la réponse à un traitement par la fluoxetine ou un agoniste du récepteur 5-HT4

Les données générées dans les résultats complémentaires sont récapitulées dans le Tableau 21 et dans le Tableau 22 ci-après.

Tableau 21: Conséquences de la délétion conditionnelle de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé sur les performances cognitives (Ø : absence d'effets ; ↓ : capacité de mémoire altérée)

Tests	Paramètres testés	VEHICULE	
		ARRB1$^{+/+}$	ARRB1$^{-/-}$
Reconnaissance d'objet	Rétention à 2H	Ø	↓
	Rétention à 24H	Ø	↓
Reconnaissance de place	Rétention à 2H	Ø	↓
Labyrinthe de Barnes	Apprentissage	Ø	↓
	Rétention à 24h	Ø	↓
Conditionnement par la peur	Rétention à 24h	Ø	↓

Tableau 22: Conséquences de la délétion conditionnelle de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé après un traitement chronique de fluoxetine ou de RS67333 sur les performances cognitives (Ø : absence d'effets ; ↓ : capacité de mémoire altérée)

Tests	Paramètres testés	FLUOXETINE		RS67333	
		ARRB1$^{+/+}$	ARRB1$^{-/-}$	ARRB1$^{+/+}$	ARRB1$^{-/-}$
Reconnaissance d'objet	Rétention à 2H		Ø		Ø
	Rétention à 24H				
Reconnaissance de place	Rétention à 2H				
Labyrinthe de Barnes	Apprentissage		Ø		Ø
	Rétention à 24h		Ø		Ø
Conditionnement par la peur	Rétention à 24h		Ø		Ø

Comment expliquer l'altération différentielle des comportements émotionnels et cognitifs suite à la délétion conditionnelle de la β-arrestine 1 dans les cellules souches du GD de l'hippocampe ?

Contrairement au phénotype émotionnel faiblement impacté par cette délétion, l'altération du phénotype cognitif est beaucoup plus prononcée. Tous les types de mémoire que j'ai étudiés sont altérés suite à l'absence d'expression de la β-arrestine 1 dans les jeunes neurones du DG issus de la neurogenèse. En effet, ces résultats révèlent un déficit de la mémoire de type épisodique, de la mémoire spatiale ainsi que de la mémoire associative (***Résultats complémentaires***).

Dans une récente revue de la littérature, Miller et Hen concluent que si l'ablation de la neurogenèse hippocampique induit un déficit cognitif relatif aux fonctions émotionnelles, une minorité d'étude montre que cette ablation induit un phénotype anxio/dépressif (Miller and Hen, 2015). Nos résultats suggèrent que la délétion de la β-arrestine 1 dans les jeunes neurones nouvellement formés n'est pas suffisante pour affecter le comportement émotionnel basal. Ce constat est au final en accord avec une étude clinique montrant que l'altération de la neurogenèse adulte n'est pas à l'origine de l'apparition des symptômes de la dépression (Cameron and McKay, 2001) et plus généralement avec la littérature. La protéine β-arrestine 1 serait donc impliquée de façon plus importante dans les processus mnésiques plutôt que dans la physiopathologie des troubles de l'humeur. Cependant, aucune étude clinique visant à préciser le rôle de la protéine β-arrestine 1 dans les troubles cognitifs n'a été menée à ce jour. Finalement, très peu d'études précliniques et cliniques se concentrent sur l'implication de la β-arrestine 1 dans les troubles émotionnels ou cognitifs. Ces thématiques gagneraient à être plus largement examinées afin de mieux comprendre les mécanismes mis en jeu dans ces pathologies mentales.

Considérations et limites méthodologiques

Impact de la souche de souris

Il est aujourd'hui bien reconnu que le fond génétique des animaux, et notamment chez la Souris, influence les phénotypes comportementaux (Clement et al., 2002; Crawley et al., 1997). Dans le cadre de ce travail conduit chez des animaux dans un contexte d'anxiété et de dépression, il est impératif de faire la distinction entre des troubles du comportement d'un apprentissage réel *versus* des altérations sensorielles, inhérentes aux différentes souches, pouvant mener à une performance cognitive amoindrie. Par exemple, le paramètre d'acuité visuelle pour les tâches spatiales, la capacité auditive dans les tests de conditionnement incluant un son, et le seuil de sensibilité au choc électrique peuvent varier d'une souche à une autre. Les différences de motivation des animaux, devant un stimulus appétant ou aversif, doivent être rigoureusement dissociées des troubles réels de l'apprentissage et de la mémoire. D'autre part, les capacités d'apprentissage diffèrent selon les souches de souris. Dans la piscine de Morris, les souris de souche C57BL/6J possèdent de meilleures capacités d'apprentissage que la souche 129Sv/J (Crawley et al., 1997) tandis que les deux souches semblent apprendre de la même manière dans le test de conditionnement par la peur (Crawley et al., 1997). Une étude comparant les performances cognitives spatiales de 13 souches de souris différentes dans le « Barnes maze », a montré que les souris 129S1/SvImJ mettent significativement plus de temps à localiser le trou cible, à entrer dedans et commettent plus d'erreurs que les autres souches. Ceci serait probablement plus dû à une hypoactivité plutôt qu'à une altération de

l'apprentissage (O'Leary et al., 2011). Ce niveau plus faible d'exploration des souris 129S1/SvImJ semble pourtant spécifique aux tests « aériens » puisque ces souris mettent autant de temps que les autres souches à rejoindre la plateforme dans le test de la piscine de Morris (Brown and Wong, 2007). A l'inverse, les souris C57BL/6J sont plus rapides à localiser et entrer dans le trou cible et commettent moins d'erreurs que les autres souches (O'Leary et al., 2011).

Durant cette thèse, il m'a fallu adapter certains protocoles de tests cognitifs en fonction de la souche utilisée. La mise au point des tests de cognition a été réalisée chez des souris C57BL/6 tandis que les souris ayant servies à l'étude du rôle de la β-arrestine 1 sont de fond mixte C57BL/6 - 129Sv/J. De façon surprenante, le protocole dont nous nous sommes inspirés pour la caractérisation du phénotype cognitif dans le modèle CORT (fond C57BL/6, **Article 1**) était basé sur un protocole validé pour des animaux de fond génétique mixte C57BL/6 - 129Sv/J (Sahay et al., 2011). Pourtant, ce même protocole s'est avéré inopérant lors des essais chez nos souris de fond génétique mixte C57BL/6 - 129Sv/J (**Article 4**). Ceci est bien la preuve qu'un même protocole, utilisé dans des laboratoires différents, par des expérimentateurs différents, avec du matériel différent est susceptible d'induire des comportements différents même si le fond génétique est similaire et souligne toute la difficulté et la rigueur à tenir dans les études comportementales.

Devant le manque d'exploration, la peur de la nouveauté, différentes stratégies peuvent être développées (Leger et al., 2013) (Tableau 23):
- diminuer le nombre de sessions d'apprentissage,
- instaurer des sessions d'habituation,
- augmenter la durée des sessions

Tableau 23: Modifications des protocoles de tests de cognition en fonction de la souche de souris utilisée.

	Test de reconnaissance d'objet			Barnes maze		
	Nombre sessions	Durée des sessions	Session habituation	Nombre sessions	Durée des sessions	Session habituation
C57BL/6	5	5 min	NON	4, 4 jours	3 min	OUI
Mixte C57BL/6 x 129Sv/J	1	10 min	NON	3, 4 jours	3 min	OUI

D'autre part, la réponse aux traitements peut varier d'une souche à une autre. C'est pourquoi il n'est pas possible aujourd'hui d'affirmer avec certitude que des traitements chroniques par le RS67333 ou par la fluoxetine ont un effet pro-cognitif chez les souris ARRB1 contrôles, de fond génétique mixte C57BL/6 - 129Sv/J.

Développement d'un score de cognition

Lors de l'analyse des résultats des tests cognitifs dans le modèle CORT, nous nous sommes interrogés sur la pertinence et la possibilité de développer un outil statistique capable de fournir un score cognitif global en regroupant les différentes mesures des tests de cognition. Un tel outil pourrait permettre de considérer un état cognitif global en réponse aux différents traitements subis, de façon similaire au score d'émotionalité ou « Z-score » développé au laboratoire pour les symptômes anxio/dépressifs (Guilloux et al., 2013). Plus précisément, le z-score permet de regrouper les résultats de plusieurs tests comportementaux prédictifs d'une activité anxiolytique, antidépressive ou anhédonique et de livrer à travers une valeur chiffrée un indice global de l'état émotionnel de l'animal. La principale limite à l'application de ce concept dans le domaine cognitif concernerait la différence entre les types de mémoire étudiée et l'implication de structures cérébrales distinctes dans ces différents paradigmes (sachant que la réponse à un seul test à une portée limitée). Cependant, cette méthodologie n'a pas pour but de s'affranchir de la spécificité de chaque test, mais d'obtenir une vision globale de la cognition de la souris qui aura été soumise à plusieurs tests. De plus, le comportement émotionnel d'une souris peut fluctuer en fonction de différents facteurs externes (Ramos, 2008), ce qui peut affecter la réponse cognitive (Paul et al., 2005). A ce jour, nous ne connaissons aucune analyse statistique capable de générer une telle mesure mais la pertinence d'un tel outil reste un débat.

Nous avons néanmoins tenté de calculer ce « z-score cognitif » en suivant la même méthodologie d'analyse que pour le « z-score émotionnel » mais en remplaçant les paramètres anxio/dépressifs par les paramètres cognitifs. Ainsi, lorsque toutes les données sont normalisées par rapport au groupe Véhicule, nous obtenons les scores de cognition indiqués dans la Figure 31A. Plus ce z-score est élevé, plus le déficit est cognitif est fort. La Figure 31B permet de visualiser quantitativement la participation de chaque type de mémoire selon les traitements à l'intérieur du z-score de cognition global.

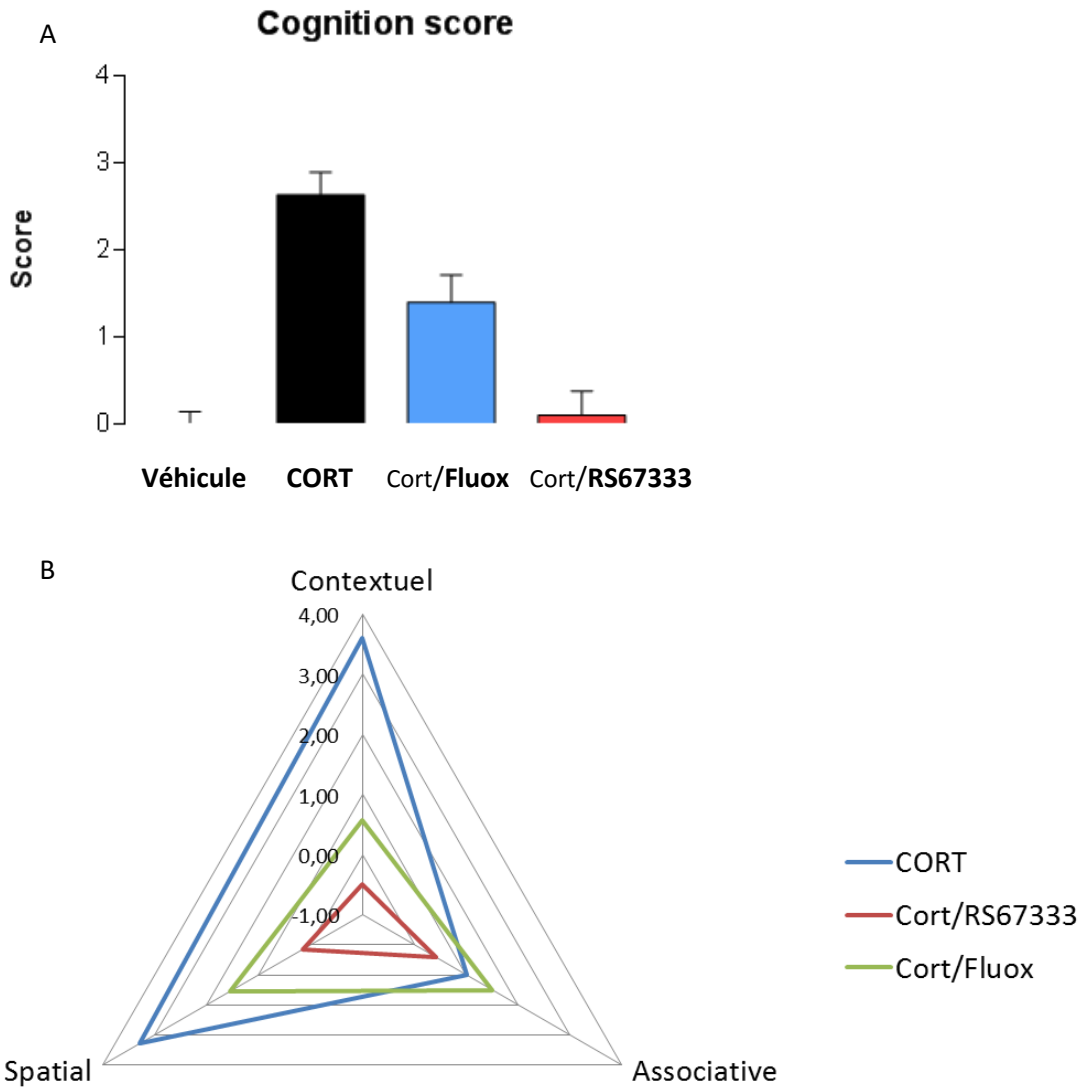


Figure 31: Proposition de score de cognition global (A) et répartition des capacités de mémoire selon les types de mémoire (B).

En combinaison avec les effets observés sur le comportement émotionnel (anxiété, dépression, anhédonie), sur les paramètres physiologiques (poids, état du pelage), les troubles du sommeil, nous pouvons proposer un graphique de type « radar », permettant d'apprécier les altérations induites par la corticostérone, et leur éventuelle correction par un traitement chronique avec de la fluoxetine et/ou du RS67333 (Figure 32). Ce graphique, encore incomplet, permet de mettre en évidence les bénéfices d'un traitement par un agoniste du 5-HT_4 sur les différents phénotypes mesurés dans notre modèle CORT.

Ces deux outils méthodologiques comportent néanmoins des limites. Qu'il s'agisse du « z-score cognitif » ou bien du radar, seuls les paramètres de rétention sont visibles, excluant alors les paramètres liés à la phase d'apprentissage. Or les processus mnésiques de d'apprentissage et de restitution de l'information sont étroitement liés, et l'absence de prise en compte de ce paramètre rend ces analyses incomplètes. Cette réflexion doit être poursuivie et débattue.

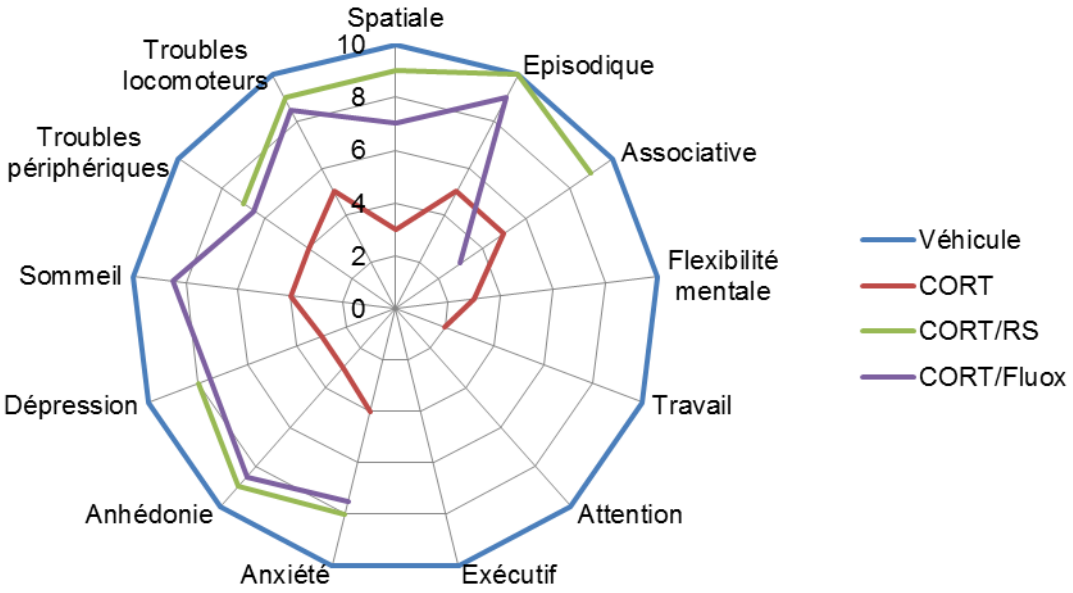


Figure 32: Profil des capacités cognitives et émotionnelles dans les différents groupes expérimentaux étudiés.

Conclusions et perspectives

Dans ce travail expérimental, nous avons mis en évidence l'efficacité d'un traitement chronique par le RS67333 aussi bien dans les troubles de l'humeur que dans les troubles cognitifs associés au phénotype anxio/dépressif. Ces données placent le RS67333 comme un excellent candidat dans le traitement de la dépression caractérisée, tant pour son action rapide, que pour son activité dans la correction des altérations cognitives. Cependant, malgré ces propriétés anxiolytiques, antidépressives et cognitives, la localisation variée du récepteur 5-HT_4 et de ses différentes fonctions peuvent être la source de possibles effets indésirables cérébraux et périphériques. Les études de tolérance de molécules innovantes possédant des propriétés agonistes pour le récepteur 5-HT_4 telle que le donecopride (RS67333 + Donepezil) permettront de mieux cerner les potentialités de cette nouvelle stratégie thérapeutique.

D'autre part, nos études ont permis d'identifier la protéine β-arrestine 1 comme un élément clé du phénotype cognitif et dans la réponse aux traitements (Figure 33).

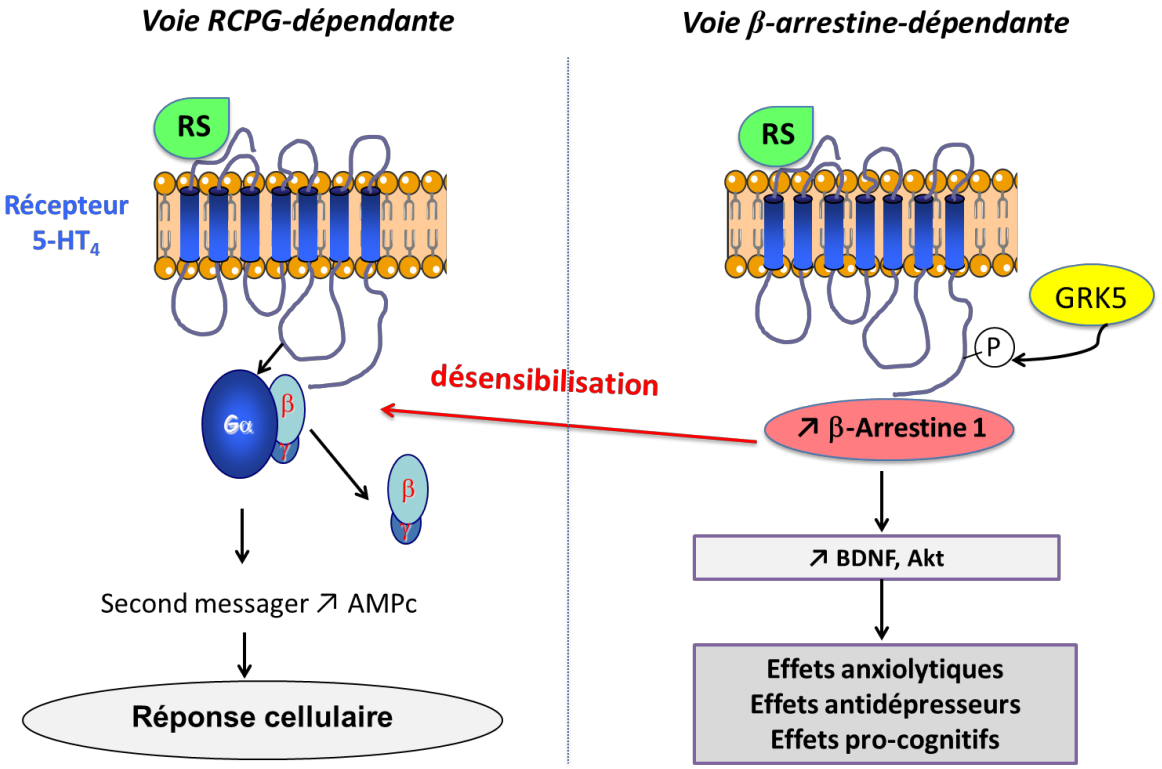


Figure 33: Différentes voies activées par le RS67333 et médiées par la protéine β-arrestine 1 dans les réponses comportementales anxiolytiques, antidépressives et cognitives.

Les suites de ce travail peuvent s'articuler selon plusieurs axes :

- Etudier les mécanismes moléculaires et cellulaires différentiellement mis en jeu par un traitement par la fluoxetine et le RS67333
- Vérifier si l'administration d'un antagoniste sélectif du récepteur 5-$HT_{4,}$ tel que le GR125487, bloque la réponse cognitive de la fluoxetine
- Etudier les effets cognitifs des antagonistes sélectifs des récepteurs 5-HT_3 et 5-HT_6 en association avec la fluoxetine
- Vérifier si la correction du déficit cognitif permet de prévenir la rechute des épisodes dépressifs

Enfin, plusieurs questions restent également en suspens et mériteraient la mise en place et la conception de nouvelles expérimentations :

- La correction des altérations cognitives est-elle préalable, associée ou consécutive à l'amélioration du phénotype anxio/dépressif ?
- La réponse rapide observée après administration aigue de RS67333 induit elle également une réponse cognitive ?
- Quels mécanismes expliquent les variations des différentes étapes de neurogenèse hippocampique adulte observées après administration de fluoxétine chez les souris ARRB1$^{-/-}$?

Références bibliographiques

A

Adham, N., Gerald, C., Schechter, L., Vaysse, P., Weinshank, R., Branchek, T., 1996. [3H]5-hydroxytryptamine labels the agonist high affinity state of the cloned rat 5-HT4 receptor. European journal of pharmacology 304, 231-235.

Ago, Y., Arikawa, S., Yata, M., Yano, K., Abe, M., Takuma, K., Matsuda, T., 2008. Antidepressant-like effects of the glucocorticoid receptor antagonist RU-43044 are associated with changes in prefrontal dopamine in mouse models of depression. Neuropharmacology 55, 1355-1363.

Ahmad, I., Nirogi, R., 2011. 5-HT4 Receptor Agonists for the Treatment of Alzheimer's Disease. Neuroscience & Medicine 2, 87-92.

Alam, F., Nayyar, S., Richie, W., Archibong, A., Nayyar, T., 2015. Beta-Arrestin1 Levels in Mononuclear Leukocytes Support Depression Scores for Women with Premenstrual Dysphoric Disorder. International journal of environmental research and public health 13.

Alzoubi, K.H., Srivareerat, M., Tran, T.T., Alkadhi, K.A., 2013. Role of alpha7- and alpha4beta2-nAChRs in the neuroprotective effect of nicotine in stress-induced impairment of hippocampus-dependent memory. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 16, 1105-1113.

American Psychiatric Association., American Psychiatric Association. DSM-5 Task Force., 2013. Diagnostic and statistical manual of mental disorders : DSM-5, 5th ed. American Psychiatric Association, Washington, D.C.
Ansanay, H., Dumuis, A., Sebben, M., Bockaert, J., Fagni, L., 1995. cAMP-dependent, long-lasting inhibition of a K+ current in mammalian neurons. Proceedings of the National Academy of Sciences of the United States of America 92, 6635-6639.

Ansanay, H., Sebben, M., Bockaert, J., Dumuis, A., 1992. Characterization of homologous 5-hydroxytryptamine4 receptor desensitization in colliculi neurons. Molecular pharmacology 42, 808-816.

Antonijevic, I., 2008. HPA axis and sleep: identifying subtypes of major depression. Stress 11, 15-27.

Aron, C., Simon, P., Larousse, C., Boissier, J.R., 1971. Evaluation of a rapid technique for detecting minor tranquilizers. Neuropharmacology 10, 459-469.

Avissar, S., Matuzany-Ruban, A., Tzukert, K., Schreiber, G., 2004. Beta-arrestin-1 levels: reduced in leukocytes of patients with depression and elevated by antidepressants in rat brain. The American journal of psychiatry 161, 2066-2072.

B

Babu, H., Claasen, J.H., Kannan, S., Runker, A.E., Palmer, T., Kempermann, G., 2011. A protocol for isolation and enriched monolayer cultivation of neural precursor cells from mouse dentate gyrus. Frontiers in neuroscience 5, 89.

Baier, C.J., Franco, D.L., Gallegos, C.E., Mongiat, L.A., Dionisio, L., Bouzat, C., Caviedes, P., Barrantes, F.J., 2014. Corticosterone affects the differentiation of a neuronal cerebral cortex-derived cell line through modulation of the nicotinic acetylcholine receptor. Neuroscience 274, 369-382.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083-1152.

Barthet, G., Carrat, G., Cassier, E., Barker, B., Gaven, F., Pillot, M., Framery, B., Pellissier, L.P., Augier, J., Kang, D.S., Claeysen, S., Reiter, E., Baneres, J.L., Benovic, J.L., Marin, P., Bockaert, J., Dumuis, A., 2009. Beta-arrestin1 phosphorylation by GRK5 regulates G protein-independent 5-HT4 receptor signalling. EMBO J 28, 2706-2718.

Barthet, G., Gaven, F., Framery, B., Shinjo, K., Nakamura, T., Claeysen, S., Bockaert, J., Dumuis, A., 2005. Uncoupling and endocytosis of 5-hydroxytryptamine 4 receptors. Distinct molecular events with different GRK2 requirements. The Journal of biological chemistry 280, 27924-27934
.
Bastos, A.G., Guimaraes, L.S., Trentini, C.M., 2013. Neurocognitive changes in depressed patients in psychodynamic psychotherapy, therapy with fluoxetine and combination therapy. Journal of affective disorders 151, 1066-1075.

Beaulieu, J.M., Marion, S., Rodriguiz, R.M., Medvedev, I.O., Sotnikova, T.D., Ghisi, V., Wetsel, W.C., Lefkowitz, R.J., Gainetdinov, R.R., Caron, M.G., 2008. A beta-arrestin 2 signaling complex mediates lithium action on behavior. Cell 132, 125-136.

Bender, E., Pindon, A., van Oers, I., Zhang, Y.B., Gommeren, W., Verhasselt, P., Jurzak, M., Leysen, J., Luyten, W., 2000. Structure of the human serotonin 5-HT4 receptor gene and cloning of a novel 5-HT4 splice variant. Journal of neurochemistry 74, 478-489.

Bergami, M., Berninger, B., 2012. A fight for survival: the challenges faced by a newborn neuron integrating in the adult hippocampus. Developmental neurobiology 72, 1016-1031.

Bockaert, J., Claeysen, S., Compan, V., Dumuis, A., 2004. 5-HT4 receptors. Curr Drug Targets CNS Neurol Disord 3, 39-51.

Bockaert, J., Claeysen, S., Compan, V., Dumuis, A., 2008. 5-HT(4) receptors: history, molecular pharmacology and brain functions. Neuropharmacology 55, 922-931.

Bohn, L.M., Schmid, C.L., 2010. Serotonin receptor signaling and regulation via beta-arrestins. Crit Rev Biochem Mol Biol 45, 555-566.

Bonaventure, P., Hall, H., Gommeren, W., Cras, P., Langlois, X., Jurzak, M., Leysen, J.E., 2000. Mapping of serotonin 5-HT(4) receptor mRNA and ligand binding sites in the post-mortem human brain. Synapse 36, 35-46.

Brodney, M.A., Johnson, D.E., Sawant-Basak, A., Coffman, K.J., Drummond, E.M., Hudson, E.L., Fisher, K.E., Noguchi, H., Waizumi, N., McDowell, L.L., Papanikolaou, A., Pettersen, B.A., Schmidt, A.W., Tseng, E., Stutzman-Engwall, K., Rubitski, D.M., Vanase-Frawley, M.A., Grimwood, S., 2012. Identification of multiple 5-HT(4) partial agonist clinical candidates for the treatment of Alzheimer's disease. Journal of medicinal chemistry 55, 9240-9254.

Brown, R.E., Wong, A.A., 2007. The influence of visual ability on learning and memory performance in 13 strains of mice. Learning & memory 14, 134-144.

Brunello, N., Mendlewicz, J., Kasper, S., Leonard, B., Montgomery, S., Nelson, J., Paykel, E., Versiani, M., Racagni, G., 2002. The role of noradrenaline and selective noradrenaline reuptake inhibition in depression. Eur Neuropsychopharmacol 12, 461-475.

Buwalda, B., Kole, M.H., Veenema, A.H., Huininga, M., de Boer, S.F., Korte, S.M., Koolhaas, J.M., 2005. Long-term effects of social stress on brain and behavior: a focus on hippocampal functioning. Neuroscience and biobehavioral reviews 29, 83-97.

C

Cai, X., Flores-Hernandez, J., Feng, J., Yan, Z., 2002. Activity-dependent bidirectional regulation of GABA(A) receptor channels by the 5-HT(4) receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. J Physiol 540, 743-759.

Caldieraro, M.A., Baeza, F.L., Pinheiro, D.O., Ribeiro, M.R., Parker, G., Fleck, M.P., 2013. Clinical differences between melancholic and nonmelancholic depression as defined by the CORE system. Compr Psychiatry 54, 11-15.

Callaghan, C.K., Hok, V., Della-Chiesa, A., Virley, D.J., Upton, N., O'Mara, S.M., 2012. Age-related declines in delayed non-match-to-sample performance (DNMS) are reversed by the novel 5HT6 receptor antagonist SB742457. Neuropharmacology 63, 890-897.

Cameron, H.A., McKay, R.D., 2001. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. The Journal of comparative neurology 435, 406-417.

Carroll, B.J., Cassidy, F., Naftolowitz, D., Tatham, N.E., Wilson, W.H., Iranmanesh, A., Liu, P.Y., Veldhuis, J.D., 2007. Pathophysiology of hypercortisolism in depression. Acta Psychiatr Scand Suppl, 90-103.

Cattoretti, G., Becker, M.H., Key, G., Duchrow, M., Schluter, C., Galle, J., Gerdes, J., 1992. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. The Journal of pathology 168, 357-363.

Chaudhury, D., Liu, H., Han, M.H., 2015. Neuronal correlates of depression. Cellular and molecular life sciences : CMLS 72, 4825-4848.

Cho, S., Hu, Y., 2007. Activation of 5-HT4 receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. Exp Neurol 203, 274-278.

Chourbaji, S., Zacher, C., Sanchis-Segura, C., Dormann, C., Vollmayr, B., Gass, P., 2005. Learned helplessness: validity and reliability of depressive-like states in mice. Brain Res Brain Res Protoc 16, 70-78.

Claeysen, S., Bockaert, J., Giannoni, P., 2015. Serotonin: A New Hope in Alzheimer's Disease? ACS chemical neuroscience 6, 940-943.

Claeysen, S., Sebben, M., Becamel, C., Bockaert, J., Dumuis, A., 1999. Novel brain-specific 5-HT4 receptor splice variants show marked constitutive activity: role of the C-terminal intracellular domain. Mol Pharmacol 55, 910-920.

Claeysen, S., Sebben, M., Journot, L., Bockaert, J., Dumuis, A., 1996. Cloning, expression and pharmacology of the mouse 5-HT(4L) receptor. FEBS Lett 398, 19-25.

Clement, Y., Calatayud, F., Belzung, C., 2002. Genetic basis of anxiety-like behaviour: a critical review. Brain research bulletin 57, 57-71.

Compan, V., Daszuta, A., Salin, P., Sebben, M., Bockaert, J., Dumuis, A., 1996. Lesion study of the distribution of serotonin 5-HT4 receptors in rat basal ganglia and hippocampus. The European journal of neuroscience 8, 2591-2598.

Conductier, G., Dusticier, N., Lucas, G., Cote, F., Debonnel, G., Daszuta, A., Dumuis, A., Nieoullon, A., Hen, R., Bockaert, J., Compan, V., 2006. Adaptive changes in serotonin neurons of the raphe nuclei in 5-HT(4) receptor knock-out mouse. The European journal of neuroscience 24, 1053-1062.

Consolo, S., Arnaboldi, S., Giorgi, S., Russi, G., Ladinsky, H., 1994. 5-HT4 receptor stimulation facilitates acetylcholine release in rat frontal cortex. Neuroreport 5, 1230-1232.

Coryell, W., 2007. The facets of melancholia. Acta Psychiatr Scand Suppl, 31-36.

Costall, B., Naylor, R.J., 1997. The influence of 5-HT2 and 5-HT4 receptor antagonists to modify drug induced disinhibitory effects in the mouse light/dark test. Br J Pharmacol 122, 1105-1118.

Couillard-Despres, S., Winner, B., Schaubeck, S., Aigner, R., Vroemen, M., Weidner, N., Bogdahn, U., Winkler, J., Kuhn, H.G., Aigner, L., 2005. Doublecortin expression levels in adult brain reflect neurogenesis. The European journal of neuroscience 21, 1-14.

Craft, C.M., Whitmore, D.H., Wiechmann, A.F., 1994. Cone arrestin identified by targeting expression of a functional family. The Journal of biological chemistry 269, 4613-4619.

Craig, D.A., Clarke, D.E., 1991. Peristalsis evoked by 5-HT and renzapride: evidence for putative 5-HT4 receptor activation. Br J Pharmacol 102, 563-564.

Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxson, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A., Paylor, R., 1997. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology 132, 107-124.

D

Da Silva Costa-Aze, V., Quiedeville, A., Boulouard, M., Dauphin, F., 2012. 5-HT6 receptor blockade differentially affects scopolamine-induced deficits of working memory, recognition memory and aversive learning in mice. Psychopharmacology 222, 99-115.

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A., Guiard, B.P., Guilloux, J.P., Artymyshyn, R.P., Gardier, A.M., Gerald, C., Antonijevic, I.A., Leonardo, E.D., Hen, R., 2009. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62, 479-493.

de Bodinat, C., Guardiola-Lemaitre, B., Mocaer, E., Renard, P., Munoz, C., Millan, M.J., 2010. Agomelatine, the first melatonergic antidepressant: discovery, characterization and development. Nat Rev Drug Discov 9, 628-642.

De Foubert, G., Carney, S.L., Robinson, C.S., Destexhe, E.J., Tomlinson, R., Hicks, C.A., Murray, T.K., Gaillard, J.P., Deville, C., Xhenseval, V., Thomas, C.E., O'Neill, M.J., Zetterstrom, T.S., 2004. Fluoxetine-induced change in rat brain expression of brain-derived neurotrophic factor varies depending on length of treatment. Neuroscience 128, 597-604.

Denny, C.A., Burghardt, N.S., Schachter, D.M., Hen, R., Drew, M.R., 2012. 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. Hippocampus 22, 1188-1201.

DeWire, S.M., Ahn, S., Lefkowitz, R.J., Shenoy, S.K., 2007. Beta-arrestins and cell signaling. Annual review of physiology 69, 483-510.

Di Pilato, P., Niso, M., Adriani, W., Romano, E., Travaglini, D., Berardi, F., Colabufo, N.A., Perrone, R., Laviola, G., Lacivita, E., Leopoldo, M., 2014. Selective agonists for serotonin 7 (5-HT7) receptor and their applications in preclinical models: an overview. Reviews in the neurosciences 25, 401-415.

Donati, R.J., Rasenick, M.M., 2003. G protein signaling and the molecular basis of antidepressant action. Life Sci 73, 1-17.

Dranovsky, A., Hen, R., 2006. Hippocampal neurogenesis: regulation by stress and antidepressants. Biological psychiatry 59, 1136-1143.

Ducottet, C., Griebel, G., Belzung, C., 2003. Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. Progress in neuro-psychopharmacology & biological psychiatry 27, 625-631.

Duman, R.S., Malberg, J., Nakagawa, S., 2001. Regulation of adult neurogenesis by psychotropic drugs and stress. The Journal of pharmacology and experimental therapeutics 299, 401-407.

Duman, R.S., Monteggia, L.M., 2006. A neurotrophic model for stress-related mood disorders. Biological psychiatry 59, 1116-1127.

Dumuis, A., Bouhelal, R., Sebben, M., Cory, R., Bockaert, J., 1988. A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. Mol Pharmacol 34, 880-887.

Dumuis, A., Sebben, M., Bockaert, J., 1989. The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT4) positively coupled to adenylate cyclase in neurons. Naunyn Schmiedebergs Arch Pharmacol 340, 403-410.

Dwivedi, Y., Rizavi, H.S., Conley, R.R., Roberts, R.C., Tamminga, C.A., Pandey, G.N., 2003. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. Archives of general psychiatry 60, 804-815.

E

Egeland, M., Warner-Schmidt, J., Greengard, P., Svenningsson, P., 2011. Co-expression of serotonin 5-HT(1B) and 5-HT(4) receptors in p11 containing cells in cerebral cortex, hippocampus, caudate-putamen and cerebellum. Neuropharmacology 61, 442-450.

Eglen, R.M., Cornett, C.M., Whiting, R.L., 1990. Interaction of p-F-HHSiD (p-Fluoro-hexahydrosila-difenidol) at muscarinic receptors in guinea-pig trachea. Naunyn Schmiedebergs Arch Pharmacol 342, 394-399.

Eglen, R.M., Wong, E.H., Dumuis, A., Bockaert, J., 1995. Central 5-HT4 receptors. Trends in pharmacological sciences 16, 391-398.

F

Fakhfouri, G., Mousavizadeh, K., Mehr, S.E., Dehpour, A.R., Zirak, M.R., Ghia, J.E., Rahimian, R., 2015. From Chemotherapy-Induced Emesis to Neuroprotection: Therapeutic Opportunities for 5-HT3 Receptor Antagonists. Molecular neurobiology 52, 1670-1679.

Fava, M., 2000. Management of nonresponse and intolerance: switching strategies. The Journal of clinical psychiatry 61 Suppl 2, 10-12.

Ferguson, J.M., Wesnes, K.A., Schwartz, G.E., 2003. Reboxetine versus paroxetine versus placebo: effects on cognitive functioning in depressed patients. International clinical psychopharmacology 18, 9-14.

Finamore, T.L., Port, R.L., 2000. Developmental stress disrupts habituation but spares prepulse inhibition in young rats. Physiol Behav 69, 527-530.

Fisher, P.M., Holst, K.K., Mc Mahon, B., Haahr, M.E., Madsen, K., Gillings, N., Baare, W.F., Jensen, P.S., Knudsen, G.M., 2012. 5-HTTLPR status predictive of neocortical 5-HT4 binding assessed with [(11)C]SB207145 PET in humans. NeuroImage 62, 130-136.

Fontana, D.J., Daniels, S.E., Eglen, R.M., Wong, E.H., 1996. Stereoselective effects of (R)- and (S)-zacopride on cognitive performance in a spatial navigation task in rats. Neuropharmacology 35, 321-327.

Freret, T., Bouet, V., Quiedeville, A., Nee, G., Dallemagne, P., Rochais, C., Boulouard, M., 2012. Synergistic effect of acetylcholinesterase inhibition (donepezil) and 5-HT(4) receptor activation (RS67333) on object recognition in mice. Behavioural brain research 230, 304-308.

Freret, T., Paizanis, E., Beaudet, G., Gusmao-Montaigne, A., Nee, G., Dauphin, F., Bouet, V., Boulouard, M., 2014. Modulation of 5-HT7 receptor: effect on object recognition performances in mice. Psychopharmacology 231, 393-400.

G

Gallassi, R., Di Sarro, R., Morreale, A., Amore, M., 2006. Memory impairment in patients with late-onset major depression: the effect of antidepressant therapy. Journal of affective disorders 91, 243-250.

Gasbarri, A., Cifariello, A., Pompili, A., Meneses, A., 2008. Effect of 5-HT(7) antagonist SB-269970 in the modulation of working and reference memory in the rat. Behavioural brain research 195, 164-170.

Gasbarri, A., Pompili, A., 2014. Serotonergic 5-HT7 receptors and cognition. Reviews in the neurosciences 25, 311-323.

Gerald, C., Adham, N., Kao, H.T., Olsen, M.A., Laz, T.M., Schechter, L.E., Bard, J.A., Vaysse, P.J., Hartig, P.R., Branchek, T.A., et al., 1995. The 5-HT4 receptor: molecular cloning and pharmacological characterization of two splice variants. EMBO J 14, 2806-2815.

Gillman, P.K., 2007. Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. Br J Pharmacol 151, 737-748.

Giovannini, M.G., Ceccarelli, I., Molinari, B., Cecchi, M., Goldfarb, J., Blandina, P., 1998. Serotonergic modulation of acetylcholine release from cortex of freely moving rats. The Journal of pharmacology and experimental therapeutics 285, 1219-1225.

Golan, M., Schreiber, G., Avissar, S., 2009. Antidepressants, beta-arrestins and GRKs: from regulation of signal desensitization to intracellular multifunctional adaptor functions. Current pharmaceutical design 15, 1699-1708.

Golan, M., Schreiber, G., Avissar, S., 2010. Antidepressants increase beta-arrestin 2 ubiquitinylation and degradation by the proteasomal pathway in C6 rat glioma cells. The Journal of pharmacology and experimental therapeutics 332, 970-976.

Gold, P.E., 2003. Acetylcholine modulation of neural systems involved in learning and memory. Neurobiology of learning and memory 80, 194-210.

Gorman, J.M., Kent, J.M., 1999. SSRIs and SNRIs: broad spectrum of efficacy beyond major depression. The Journal of clinical psychiatry 60 Suppl 4, 33-38; discussion 39.

Gornall, A.G., Bardawill, C.J., David, M.M., 1949. Determination of serum proteins by means of the biuret reaction. The Journal of biological chemistry 177, 751-766.

Gorzalka, B.B., Hanson, L.A., Hong, J.J., 2001. Ketanserin attenuates the behavioural effects of corticosterone: implications for 5-HT(2A) receptor regulation. European journal of pharmacology 428, 235-240.

Gourley, S.L., Kedves, A.T., Olausson, P., Taylor, J.R., 2009. A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF. Neuropsychopharmacology 34, 707-716.

Gourley, S.L., Kiraly, D.D., Howell, J.L., Olausson, P., Taylor, J.R., 2008. Acute hippocampal brain-derived neurotrophic factor restores motivational and forced swim performance after corticosterone. Biological psychiatry 64, 884-890.

Gourley, S.L., Taylor, J.R., 2009. Recapitulation and reversal of a persistent depression-like syndrome in rodents. Curr Protoc Neurosci Chapter 9, Unit 9 32.

Gregus, A., Wintink, A.J., Davis, A.C., Kalynchuk, L.E., 2005. Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behavioural brain research 156, 105-114.

Griebel, G., Simiand, J., Serradeil-Le Gal, C., Wagnon, J., Pascal, M., Scatton, B., Maffrand, J.P., Soubrie, P., 2002. Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. Proceedings of the National Academy of Sciences of the United States of America 99, 6370-6375.

Grippo, A.J., Beltz, T.G., Weiss, R.M., Johnson, A.K., 2006. The effects of chronic fluoxetine treatment on chronic mild stress-induced cardiovascular changes and anhedonia. Biological psychiatry 59, 309-316.

Grossman, C.J., Kilpatrick, G.J., Bunce, K.T., 1993. Development of a radioligand binding assay for 5-HT4 receptors in guinea-pig and rat brain. Br J Pharmacol 109, 618-624.

Guidi, J., Tomba, E., Fava, G.A., 2016. The Sequential Integration of Pharmacotherapy and Psychotherapy in the Treatment of Major Depressive Disorder: A Meta-Analysis of the Sequential Model and a Critical Review of the Literature. The American journal of psychiatry 173, 128-137.

Guilloux, J.P., Mendez-David, I., Pehrson, A., Guiard, B.P., Reperant, C., Orvoen, S., Gardier, A.M., Hen, R., Ebert, B., Miller, S., Sanchez, C., David, D.J., 2013. Antidepressant and anxiolytic potential of the multimodal antidepressant vortioxetine (Lu AA21004) assessed by behavioural and neurogenesis outcomes in mice. Neuropharmacology 73, 147-159.

Gunschmann, C., Chiticariu, E., Garg, B., Hiz, M.M., Mostmans, Y., Wehner, M., Scharfenberger, L., 2014. Transgenic mouse technology in skin biology: inducible gene knockout in mice. The Journal of investigative dermatology 134, e22.

Gurevich, E.V., Benovic, J.L., Gurevich, V.V., 2004. Arrestin2 expression selectively increases during neural differentiation. Journal of neurochemistry 91, 1404-1416.

H

Haahr, M.E., Fisher, P., Holst, K., Madsen, K., Jensen, C.G., Marner, L., Lehel, S., Baare, W., Knudsen, G., Hasselbalch, S., 2013. The 5-HT4 receptor levels in hippocampus correlates inversely with memory test performance in humans. Human brain mapping 34, 3066-3074.

Hajszan, T., Dow, A., Warner-Schmidt, J.L., Szigeti-Buck, K., Sallam, N.L., Parducz, A., Leranth, C., Duman, R.S., 2009. Remodeling of hippocampal spine synapses in the rat learned helplessness model of depression. Biological psychiatry 65, 392-400.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol 6, 278-296.

Hashimoto, K., 2015. Tropisetron and its targets in Alzheimer's disease. Expert opinion on therapeutic targets 19, 1-5.

Hasselbalch, B.J., 2015. Cognition and neuroplasticity in the remitted state of unipolar depressive disorder. Danish medical journal 62.

Hasselbalch, B.J., Knorr, U., Kessing, L.V., 2011. Cognitive impairment in the remitted state of unipolar depressive disorder: a systematic review. Journal of affective disorders 134, 20-31.

Hegde, S.S., Eglen, R.M., 1996. Peripheral 5-HT4 receptors. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 10, 1398-1407.

Herrera-Guzman, I., Herrera-Abarca, J.E., Gudayol-Ferre, E., Herrera-Guzman, D., Gomez-Carbajal, L., Pena-Olvira, M., Villuendas-Gonzalez, E., Joan, G.O., 2010. Effects of selective serotonin reuptake and dual serotonergic-noradrenergic reuptake treatments on attention and executive functions in patients with major depressive disorder. Psychiatry research 177, 323-329.

Hesen, W., Joels, M., 1996. Cholinergic Responsiveness of Rat CA1 Hippocampal Neurons In Vitro: Modulation by Corticosterone and Stress. Stress 1, 65-72.

J

Jacobsen, J.P., Mork, A., 2006. Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. Brain research 1110, 221-225.

K

Kalynchuk, L.E., Gregus, A., Boudreau, D., Perrot-Sinal, T.S., 2004. Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. Behav Neurosci 118, 1365-1377.

Kemp, A., Manahan-Vaughan, D., 2004. Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. Proceedings of the National Academy of Sciences of the United States of America 101, 8192-8197.

Kemp, A., Manahan-Vaughan, D., 2005. The 5-hydroxytryptamine4 receptor exhibits frequency-dependent properties in synaptic plasticity and behavioural metaplasticity in the hippocampal CA1 region in vivo. Cereb Cortex 15, 1037-1043.

Kennett, G.A., Bright, F., Trail, B., Blackburn, T.P., Sanger, G.J., 1997. Anxiolytic-like actions of the selective 5-HT4 receptor antagonists SB 204070A and SB 207266A in rats. Neuropharmacology 36, 707-712.

Kessler, R.C., Chiu, W.T., Demler, O., Merikangas, K.R., Walters, E.E., 2005. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Archives of general psychiatry 62, 617-627.

Kilbinger, H., Wolf, D., 1992. Effects of 5-HT4 receptor stimulation on basal and electrically evoked release of acetylcholine from guinea-pig myenteric plexus. Naunyn Schmiedebergs Arch Pharmacol 345, 270-275.

King, M.V., Marsden, C.A., Fone, K.C., 2008. A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory. Trends in pharmacological sciences 29, 482-492.

Kinney, J.L., 1985. Nomifensine maleate: a new second-generation antidepressant. Clin. Pharm. 4, 625-636.
Konarski, J.Z., McIntyre, R.S., Kennedy, S.H., Rafi-Tari, S., Soczynska, J.K., Ketter, T.A., 2008. Volumetric neuroimaging investigations in mood disorders: bipolar disorder versus major depressive disorder. Bipolar disorders 10, 1-37.

Krishnan, V., Han, M.H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., Laplant, Q., Graham, A., Lutter, M., Lagace, D.C., Ghose, S., Reister, R., Tannous, P., Green, T.A., Neve, R.L., Chakravarty, S., Kumar, A., Eisch, A.J., Self, D.W., Lee, F.S., Tamminga, C.A., Cooper, D.C., Gershenfeld, H.K., Nestler, E.J., 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391-404.

Kulla, A., Manahan-Vaughan, D., 2002. Modulation by serotonin 5-HT(4) receptors of long-term potentiation and depotentiation in the dentate gyrus of freely moving rats. Cereb Cortex 12, 150-162.

Kvarta, M.D., Bradbrook, K.E., Dantrassy, H.M., Bailey, A.M., Thompson, S.M., 2015. Corticosterone mediates the synaptic and behavioral effects of chronic stress at rat hippocampal temporoammonic synapses. Journal of neurophysiology 114, 1713-1724.

L

Larsen, M.H., Hay-Schmidt, A., Ronn, L.C., Mikkelsen, J.D., 2008. Temporal expression of brain-derived neurotrophic factor (BDNF) mRNA in the rat hippocampus after treatment with selective and mixed monoaminergic antidepressants. European journal of pharmacology 578, 114-122.

Latapy, C., Beaulieu, J.M., 2013. beta-Arrestins in the central nervous system. Progress in molecular biology and translational science 118, 267-295.

Le Strat, Y., Gorwood, P., 2008. Agomelatine, an innovative pharmacological response to unmet needs. J Psychopharmacol 22, 4-8.

Lecoutey, C., Hedou, D., Freret, T., Giannoni, P., Gaven, F., Since, M., Bouet, V., Ballandonne, C., Corvaisier, S., Malzert Freon, A., Mignani, S., Cresteil, T., Boulouard, M., Claeysen, S., Rochais, C., Dallemagne, P., 2014.

Design of donecopride, a dual serotonin subtype 4 receptor agonist/acetylcholinesterase inhibitor with potential interest for Alzheimer's disease treatment. Proceedings of the National Academy of Sciences of the United States of America 111, E3825-3830.

Lefebvre, H., Contesse, V., Delarue, C., Feuilloley, M., Hery, F., Grise, P., Raynaud, G., Verhofstad, A.A., Wolf, L.M., Vaudry, H., 1992. Serotonin-induced stimulation of cortisol secretion from human adrenocortical tissue is mediated through activation of a serotonin4 receptor subtype. Neuroscience 47, 999-1007.

Lefkowitz, R.J., Whalen, E.J., 2004. beta-arrestins: traffic cops of cell signaling. Curr Opin Cell Biol 16, 162-168.
Leger, M., Quiedeville, A., Bouet, V., Haelewyn, B., Boulouard, M., Schumann-Bard, P., Freret, T., 2013. Object recognition test in mice. Nature protocols 8, 2531-2537.

Letty, S., Child, R., Dumuis, A., Pantaloni, A., Bockaert, J., Rondouin, G., 1997. 5-HT4 receptors improve social olfactory memory in the rat. Neuropharmacology 36, 681-687.

Licht, C.L., Kirkegaard, L., Zueger, M., Chourbaji, S., Gass, P., Aznar, S., Knudsen, G.M., 2010. Changes in 5-HT4 receptor and 5-HT transporter binding in olfactory bulbectomized and glucocorticoid receptor heterozygous mice. Neurochemistry international 56, 603-610.

Liu, M.T., Kuan, Y.H., Wang, J., Hen, R., Gershon, M.D., 2009. 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 29, 9683-9699.

Liu, X., Zhao, X., Zeng, X., Bossers, K., Swaab, D.F., Zhao, J., Pei, G., 2013. beta-arrestin1 regulates gamma-secretase complex assembly and modulates amyloid-beta pathology. Cell research 23, 351-365.

Lohse, M.J., Benovic, J.L., Codina, J., Caron, M.G., Lefkowitz, R.J., 1990. beta-Arrestin: a protein that regulates beta-adrenergic receptor function. Science 248, 1547-1550.

Lucas, G., 2009. Serotonin receptors, type 4: a new hope? Curr Drug Targets 10, 1085-1095.

Lucas, G., Debonnel, G., 2002. 5-HT4 receptors exert a frequency-related facilitatory control on dorsal raphe nucleus 5-HT neuronal activity. The European journal of neuroscience 16, 817-822.

Lucas, G., Du, J., Romeas, T., Mnie-Filali, O., Haddjeri, N., Pineyro, G., Debonnel, G., 2010. Selective serotonin reuptake inhibitors potentiate the rapid antidepressant-like effects of serotonin4 receptor agonists in the rat. PLoS One 5, e9253.

Lucas, G., Rymar, V.V., Du, J., Mnie-Filali, O., Bisgaard, C., Manta, S., Lambas-Senas, L., Wiborg, O., Haddjeri, N., Pineyro, G., Sadikot, A.F., Debonnel, G., 2007. Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. Neuron 55, 712-725.

M

Maccari, S., Morley-Fletcher, S., 2007. Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. Psychoneuroendocrinology 32 Suppl 1, S10-15.

Madsen, K., Torstensen, E., Holst, K.K., Haahr, M.E., Knorr, U., Frokjaer, V.G., Brandt-Larsen, M., Iversen, P., Fisher, P.M., Knudsen, G.M., 2015. Familial risk for major depression is associated with lower striatal 5-HT(4) receptor binding. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 18.

Maeng, S., Zarate, C.A., Jr., Du, J., Schloesser, R.J., McCammon, J., Chen, G., Manji, H.K., 2008. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biological psychiatry 63, 349-352.

Magarinos, A.M., Deslandes, A., McEwen, B.S., 1999. Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. European journal of pharmacology 371, 113-122.

Malberg, J.E., Eisch, A.J., Nestler, E.J., Duman, R.S., 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 9104-9110.

Marchetti, E., Chaillan, F.A., Dumuis, A., Bockaert, J., Soumireu-Mourat, B., Roman, F.S., 2004. Modulation of memory processes and cellular excitability in the dentate gyrus of freely moving rats by a 5-HT4 receptors partial agonist, and an antagonist. Neuropharmacology 47, 1021-1035.

Marchetti, E., Jacquet, M., Jeltsch, H., Migliorati, M., Nivet, E., Cassel, J.C., Roman, F.S., 2008. Complete recovery of olfactory associative learning by activation of 5-HT4 receptors after dentate granule cell damage in rats. Neurobiology of learning and memory 90, 185-191.

Marin, P., Becamel, C., Dumuis, A., Bockaert, J., 2012. 5-HT receptor-associated protein networks: new targets for drug discovery in psychiatric disorders? Curr Drug Targets 13, 28-52.

Martinowich, K., Jimenez, D.V., Zarate, C.A., Jr., Manji, H.K., 2013. Rapid antidepressant effects: moving right along. Molecular psychiatry 18, 856-863.

Matsumoto, M., Togashi, H., Mori, K., Ueno, K., Ohashi, S., Kojima, T., Yoshioka, M., 2001. Evidence for involvement of central 5-HT(4) receptors in cholinergic function associated with cognitive processes: behavioral, electrophysiological, and neurochemical studies. The Journal of pharmacology and experimental therapeutics 296, 676-682.

Matuzany-Ruban, A., Avissar, S., Schreiber, G., 2005. Dynamics of beta-arrestin1 protein and mRNA levels elevation by antidepressants in mononuclear leukocytes of patients with depression. Journal of affective disorders 88, 307-312.

Mayberg, H.S., Lozano, A.M., Voon, V., McNeely, H.E., Seminowicz, D., Hamani, C., Schwalb, J.M., Kennedy, S.H., 2005. Deep brain stimulation for treatment-resistant depression. Neuron 45, 651-660.

McAllister, A.K., 1999. Subplate neurons: a missing link among neurotrophins, activity, and ocular dominance plasticity? Proceedings of the National Academy of Sciences of the United States of America 96, 13600-13602.

McEwen, B.S., Chattarji, S., Diamond, D.M., Jay, T.M., Reagan, L.P., Svenningsson, P., Fuchs, E., 2010. The neurobiological properties of tianeptine (Stablon): from monoamine hypothesis to glutamatergic modulation. Molecular psychiatry 15, 237-249.

McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. Current opinion in neurobiology 5, 205-216.
Mendez-David, I., David, D.J., Guilloux, J.-P., Hen, R., Gardier, A.M., 2015a. 5-HT4 Receptor Subtype, β-Arrestin Level, and Rapid-Onset Effects of Antidepressant Drugs, vol. 95, pp. 101-121.

Mendez-David, I., El-Ali, Z., Hen, R., Falissard, B., Corruble, E., Gardier, A.M., Kerdine-Romer, S., David, D.J., 2013. A method for biomarker measurements in peripheral blood mononuclear cells isolated from anxious and depressed mice: beta-arrestin 1 protein levels in depression and treatment. Frontiers in pharmacology 4, 124.

Mendez-David, I., Tritschler, L., Ali, Z.E., Damiens, M.H., Pallardy, M., David, D.J., Kerdine-Romer, S., Gardier, A.M., 2015b. Nrf2-signaling and BDNF: A new target for the antidepressant-like activity of chronic fluoxetine treatment in a mouse model of anxiety/depression. Neuroscience letters 597, 121-126.

Meneses, A., 2015. Serotonin, neural markers, and memory. Frontiers in pharmacology 6, 143.

Meneses, A., Hong, E., 1997. Effects of 5-HT4 receptor agonists and antagonists in learning. Pharmacology, biochemistry, and behavior 56, 347-351.

Micale, V., Leggio, G.M., Mazzola, C., Drago, F., 2006. Cognitive effects of SL65.0155, a serotonin 5-HT4 receptor partial agonist, in animal models of amnesia. Brain research 1121, 207-215.

Miller, B.R., Hen, R., 2015. The current state of the neurogenic theory of depression and anxiety. Current opinion in neurobiology 30, 51-58.

Mineka, S., Watson, D., Clark, L.A., 1998. Comorbidity of anxiety and unipolar mood disorders. Annu Rev Psychol 49, 377-412.

Mineur, Y.S., Prasol, D.J., Belzung, C., Crusio, W.E., 2003. Agonistic behavior and unpredictable chronic mild stress in mice. Behav Genet 33, 513-519.

Mitra, R., Sapolsky, R.M., 2008. Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. Proceedings of the National Academy of Sciences of the United States of America 105, 5573-5578.

Mizoguchi, K., Shoji, H., Ikeda, R., Tanaka, Y., Maruyama, W., Tabira, T., 2008. Suppression of glucocorticoid secretion enhances cholinergic transmission in rat hippocampus. Brain research bulletin 76, 612-615.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Tabira, T., 2001. Effect of chronic stress on cholinergic transmission in rat hippocampus. Brain research 915, 108-111.

Mnie-Filali, O., Piñeyro, G., 2012. Desensitization and internalization mechanisms of the 5-HT4 receptors. WIRES Membr Transp Signal 1, 779-788.

Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. Br J Psychiatry 134, 382-389.

Murray, F., Smith, D.W., Hutson, P.H., 2008. Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. European journal of pharmacology 583, 115-127.

Musazzi, L., Cattaneo, A., Tardito, D., Barbon, A., Gennarelli, M., Barlati, S., Racagni, G., Popoli, M., 2009. Early raise of BDNF in hippocampus suggests induction of posttranscriptional mechanisms by antidepressants. BMC Neurosci 10, 48.

N

Nakagawa, S., Kim, J.E., Lee, R., Malberg, J.E., Chen, J., Steffen, C., Zhang, Y.J., Nestler, E.J., Duman, R.S., 2002. Regulation of neurogenesis in adult mouse hippocampus by cAMP and the cAMP response element-binding protein. The Journal of neuroscience : the official journal of the Society for Neuroscience 22, 3673-3682.
Nemeroff, C.B., 1994. Evolutionary trends in the pharmacotherapeutic management of depression. The Journal of clinical psychiatry 55 Suppl, 3-15; discussion 16-17.

Nestler, E.J., Gould, E., Manji, H., Buncan, M., Duman, R.S., Greshenfeld, H.K., Hen, R., Koester, S., Lederhendler, I., Meaney, M., Robbins, T., Winsky, L., Zalcman, S., 2002. Preclinical models: status of basic research in depression. Biological psychiatry 52, 503-528.

Nestler, E.J., Hyman, S.E., 2010. Animal models of neuropsychiatric disorders. Nature neuroscience 13, 1161-1169.

Neves, G., Cooke, S.F., Bliss, T.V., 2008. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nature reviews. Neuroscience 9, 65-75.

Nibuya, M., Morinobu, S., Duman, R.S., 1995. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. The Journal of neuroscience : the official journal of the Society for Neuroscience 15, 7539-7547.

Nikiforuk, A., 2015. Targeting the Serotonin 5-HT7 Receptor in the Search for Treatments for CNS Disorders: Rationale and Progress to Date. CNS drugs 29, 265-275.

Nirogi, R., Mohammed, A.R., Shinde, A.K., Bogaraju, N., Gagginapalli, S.R., Ravella, S.R., Kota, L., Bhyrapuneni, G., Muddana, N.R., Benade, V., Palacharla, R.C., Jayarajan, P., Subramanian, R., Goyal, V.K., 2015. Synthesis and SAR of Imidazo[1,5-a]pyridine derivatives as 5-HT4 receptor partial agonists for the treatment of cognitive disorders associated with Alzheimer's disease. European journal of medicinal chemistry 103, 289-301.

Nutt, D.J., Davidson, J.R., Gelenberg, A.J., Higuchi, T., Kanba, S., Karamustafalioglu, O., Papakostas, G.I., Sakamoto, K., Terao, T., Zhang, M., 2010. International consensus statement on major depressive disorder. The Journal of clinical psychiatry 71 Suppl E1, e08.

O

O'Keane, V., Frodl, T., Dinan, T.G., 2012. A review of Atypical depression in relation to the course of depression and changes in HPA axis organization. Psychoneuroendocrinology 37, 1589-1599.

O'Leary, T.P., Savoie, V., Brown, R.E., 2011. Learning, memory and search strategies of inbred mouse strains with different visual abilities in the Barnes maze. Behavioural brain research 216, 531-542.

Ohtsuki, T., Ishiguro, H., Detera-Wadleigh, S.D., Toyota, T., Shimizu, H., Yamada, K., Yoshitsugu, K., Hattori, E., Yoshikawa, T., Arinami, T., 2002. Association between serotonin 4 receptor gene polymorphisms and bipolar disorder in Japanese case-control samples and the NIMH Genetics Initiative Bipolar Pedigrees. Molecular psychiatry 7, 954-961.

Orsetti, M., Colella, L., Dellarole, A., Canonico, P.L., Ghi, P., 2007. Modification of spatial recognition memory and object discrimination after chronic administration of haloperidol, amitriptyline, sodium valproate or olanzapine in normal and anhedonic rats. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 10, 345-357.

Orsetti, M., Dellarole, A., Ferri, S., Ghi, P., 2003. Acquisition, retention, and recall of memory after injection of RS67333, a 5-HT(4) receptor agonist, into the nucleus basalis magnocellularis of the rat. Learning & memory 10, 420-426.

P

Parruti, G., Peracchia, F., Sallese, M., Ambrosini, G., Masini, M., Rotilio, D., De Blasi, A., 1993. Molecular analysis of human beta-arrestin-1: cloning, tissue distribution, and regulation of expression. Identification of two isoforms generated by alternative splicing. The Journal of biological chemistry 268, 9753-9761.

Pascual-Brazo, J., Castro, E., Diaz, A., Valdizan, E.M., Pilar-Cuellar, F., Vidal, R., Treceno, B., Pazos, A., 2012. Modulation of neuroplasticity pathways and antidepressant-like behavioural responses following the short-term (3 and 7 days) administration of the 5-HT(4) receptor agonist RS67333. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 15, 631-643.

Paul, E.S., Harding, E.J., Mendl, M., 2005. Measuring emotional processes in animals: the utility of a cognitive approach. Neuroscience and biobehavioral reviews 29, 469-491.

Pego, J.M., Morgado, P., Pinto, L.G., Cerqueira, J.J., Almeida, O.F., Sousa, N., 2008. Dissociation of the morphological correlates of stress-induced anxiety and fear. The European journal of neuroscience 27, 1503-1516.

Pellow, S., File, S.E., 1986. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacology, biochemistry, and behavior 24, 525-529.

Perez-Garcia, G.S., Meneses, A., 2005. Effects of the potential 5-HT7 receptor agonist AS 19 in an autoshaping learning task. Behavioural brain research 163, 136-140.

Pfister, C., Chabre, M., Plouet, J., Tuyen, V.V., De Kozak, Y., Faure, J.P., Kuhn, H., 1985. Retinal S antigen identified as the 48K protein regulating light-dependent phosphodiesterase in rods. Science 228, 891-893.

Picciotto, M.R., Lewis, A.S., van Schalkwyk, G.I., Mineur, Y.S., 2015. Mood and anxiety regulation by nicotinic acetylcholine receptors: A potential pathway to modulate aggression and related behavioral states. Neuropharmacology 96, 235-243.

Pittenger, C., Duman, R.S., 2008. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 33, 88-109.

Ponimaskin, E.G., Heine, M., Joubert, L., Sebben, M., Bickmeyer, U., Richter, D.W., Dumuis, A., 2002. The 5-hydroxytryptamine(4a) receptor is palmitoylated at two different sites, and acylation is critically involved in regulation of receptor constitutive activity. The Journal of biological chemistry 277, 2534-2546.

Popoli, M., Yan, Z., McEwen, B.S., Sanacora, G., 2012. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nature reviews. Neuroscience 13, 22-37.

Porsolt, R.D., Chermat, R., Lenegre, A., Avril, I., Janvier, S., Steru, L., 1987. Use of the automated tail suspension test for the primary screening of psychotropic agents. Archives internationales de pharmacodynamie et de therapie 288, 11-30.

Pothion, S., Bizot, J.C., Trovero, F., Belzung, C., 2004. Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behavioural brain research 155, 135-146.

Preskorn, S.H., Othmer, S.C., 1984. Evaluation of bupropion hydrochloride: the first of a new class of atypical antidepressants. Pharmacotherapy 4, 20-34.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. European journal of pharmacology 463, 3-33.

Q

Qanbar, R., Bouvier, M., 2003. Role of palmitoylation/depalmitoylation reactions in G-protein-coupled receptor function. Pharmacol Ther 97, 1-33.

Quiedeville, A., Boulouard, M., Hamidouche, K., Da Silva Costa-Aze, V., Nee, G., Rochais, C., Dallemagne, P., Fabis, F., Freret, T., Bouet, V., 2015. Chronic activation of 5-HT4 receptors or blockade of 5-HT6 receptors improve memory performances. Behavioural brain research 293, 10-17.

R

Rainer, Q., Xia, L., Guilloux, J.P., Gabriel, C., Mocaer, E., Hen, R., Enhamre, E., Gardier, A.M., David, D.J., 2012. Beneficial behavioural and neurogenic effects of agomelatine in a model of depression/anxiety. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 15, 321-335.

Ramirez, M.J., Lai, M.K., Tordera, R.M., Francis, P.T., 2014. Serotonergic therapies for cognitive symptoms in Alzheimer's disease: rationale and current status. Drugs 74, 729-736.

Ramos, A., 2008. Animal models of anxiety: do I need multiple tests? Trends in pharmacological sciences 29, 493-498.

Reagh, Z.M., Yassa, M.A., 2014. Object and spatial mnemonic interference differentially engage lateral and medial entorhinal cortex in humans. Proceedings of the National Academy of Sciences of the United States of America 111, E4264-4273.

Reierson, G.W., Mastronardi, C.A., Licinio, J., Wong, M.L., 2009. Chronic imipramine downregulates cyclic AMP signaling in rat hippocampus. Neuroreport 20, 307-311.

Riedel, G., Platt, B., Micheau, J., 2003. Glutamate receptor function in learning and memory. Behavioural brain research 140, 1-47.

Rochais, C., Lecoutey, C., Gaven, F., Giannoni, P., Hamidouche, K., Hedou, D., Dubost, E., Genest, D., Yahiaoui, S., Freret, T., Bouet, V., Dauphin, F., Sopkova de Oliveira Santos, J., Ballandonne, C., Corvaisier, S., Malzert-Freon, A., Legay, R., Boulouard, M., Claeysen, S., Dallemagne, P., 2015. Novel multitarget-directed ligands (MTDLs) with acetylcholinesterase (AChE) inhibitory and serotonergic subtype 4 receptor (5-HT4R) agonist activities as potential agents against Alzheimer's disease: the design of donecopride. Journal of medicinal chemistry 58, 3172-3187.

Roose, S.P., Glassman, A.H., Attia, E., Woodring, S., 1994. Comparative efficacy of selective serotonin reuptake inhibitors and tricyclics in the treatment of melancholia. The American journal of psychiatry 151, 1735-1739.

Roozendaal, B., McEwen, B.S., Chattarji, S., 2009. Stress, memory and the amygdala. Nature reviews. Neuroscience 10, 423-433.

Rosel, P., Arranz, B., Urretavizcaya, M., Oros, M., San, L., Navarro, M.A., 2004. Altered 5-HT2A and 5-HT4 postsynaptic receptors and their intracellular signalling systems IP3 and cAMP in brains from depressed violent suicide victims. Neuropsychobiology 49, 189-195.

Roychowdhury, S., Haas, H., Anderson, E.G., 1994. 5-HT1A and 5-HT4 receptor colocalization on hippocampal pyramidal cells. Neuropharmacology 33, 551-557.

S

Sahay, A., Scobie, K.N., Hill, A.S., O'Carroll, C.M., Kheirbek, M.A., Burghardt, N.S., Fenton, A.A., Dranovsky, A., Hen, R., 2011. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472, 466-470.

Samuels, B.A., Leonardo, E.D., Gadient, R., Williams, A., Zhou, J., David, D.J., Gardier, A.M., Wong, E.H., Hen, R., 2011. Modeling treatment-resistant depression. Neuropharmacology 61, 408-413.

Sandi, C., 2011. Glucocorticoids act on glutamatergic pathways to affect memory processes. Trends in neurosciences 34, 165-176.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., Hen, R., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805-809.

Sapinho, D., Chan-Chee, C., Briffault, X., Guignard, R., Beck, F., 2008. Mesure de l'épisode dépressif majeur en population générale : apports et limites des outils, in: INV (Ed.), Bulletin Epidémiologique Hebdomadaire, Institut de Veille Sanitaire ed. Institut de Veille Sanitaire, France, pp. 314-317.

Schatzberg, A.F., Nemeroff, C.B., 2009. The American Psychiatric Publishing textbook of psychopharmacology, 4th ed. American Psychiatric Pub., Washington, D.C.

Schmidt, E.F., Warner-Schmidt, J.L., Otopalik, B.G., Pickett, S.B., Greengard, P., Heintz, N., 2012. Identification of the cortical neurons that mediate antidepressant responses. Cell 149, 1152-1163.

Schreiber, G., Golan, M., Avissar, S., 2009. Beta-arrestin signaling complex as a target for antidepressants and as a depression marker. Drug News Perspect 22, 467-480.

Schreiber, R., Melon, C., De Vry, J., 1998. The role of 5-HT receptor subtypes in the anxiolytic effects of selective serotonin reuptake inhibitors in the rat ultrasonic vocalization test. Psychopharmacology 135, 383-391.

Seib, L.M., Wellman, C.L., 2003. Daily injections alter spine density in rat medial prefrontal cortex. Neuroscience letters 337, 29-32.

Sen, S., Duman, R., Sanacora, G., 2008. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biological psychiatry 64, 527-532.

Shukla, A.K., Manglik, A., Kruse, A.C., Xiao, K., Reis, R.I., Tseng, W.C., Staus, D.P., Hilger, D., Uysal, S., Huang, L.Y., Paduch, M., Tripathi-Shukla, P., Koide, A., Koide, S., Weis, W.I., Kossiakoff, A.A., Kobilka, B.K., Lefkowitz, R.J., 2013. Structure of active beta-arrestin-1 bound to a G-protein-coupled receptor phosphopeptide. Nature 497, 137-141.

Silvestre, J.S., Fernandez, A.G., Palacios, J.M., 1996. Effects of 5-HT4 receptor antagonists on rat behaviour in the elevated plus-maze test. European journal of pharmacology 309, 219-222.

Siopi, E., Denizet, M., Gabellec, M.M., de Chaumont, F., Olivo-Marin, J.C., Guilloux, J.P., Lledo, P.M., Lazarini, F., 2016. Anxiety- and Depression-Like States Lead to Pronounced Olfactory Deficits and Impaired Adult Neurogenesis in Mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 36, 518-531.

Southwick, S.M., Vythilingam, M., Charney, D.S., 2005. The psychobiology of depression and resilience to stress: implications for prevention and treatment. Annual review of clinical psychology 1, 255-291.
Srinivas, S., Watanabe, T., Lin, C.S., William, C.M., Tanabe, Y., Jessell, T.M., Costantini, F., 2001. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC developmental biology 1, 4.

Stahl, S.M., Lee-Zimmerman, C., Cartwright, S., Ann Morrissette, D., 2013. Serotonergic drugs for depression and beyond. Curr Drug Targets 14, 578-585.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367-370.

Stimmel, G.L., Dopheide, J.A., Stahl, S.M., 1997. Mirtazapine: an antidepressant with noradrenergic and specific serotonergic effects. Pharmacotherapy 17, 10-21.

Sun, D., Ma, J.Z., Payne, T.J., Li, M.D., 2008. Beta-arrestins 1 and 2 are associated with nicotine dependence in European American smokers. Molecular psychiatry 13, 398-406.

Sun, M.Y., Yetman, M.J., Lee, T.C., Chen, Y., Jankowsky, J.L., 2014. Specificity and efficiency of reporter expression in adult neural progenitors vary substantially among nestin-CreER(T2) lines. The Journal of comparative neurology 522, 1191-1208.

T

Terry, A.V., Jr., Buccafusco, J.J., Jackson, W.J., Prendergast, M.A., Fontana, D.J., Wong, E.H., Bonhaus, D.W., Weller, P., Eglen, R.M., 1998. Enhanced delayed matching performance in younger and older macaques administered the 5-HT4 receptor agonist, RS 17017. Psychopharmacology 135, 407-415.

Thierry, B., Steru, L., Simon, P., Porsolt, R.D., 1986. The tail suspension test: ethical considerations. Psychopharmacology 90, 284-285.

Tollefson, G.D., 1983. Monoamine oxidase inhibitors: a review. The Journal of clinical psychiatry 44, 280-288.

Torres, G.E., Chaput, Y., Andrade, R., 1995. Cyclic AMP and protein kinase A mediate 5-hydroxytryptamine type 4 receptor regulation of calcium-activated potassium current in adult hippocampal neurons. Mol Pharmacol 47, 191-197.

Trivedi, M.H., Rush, A.J., Wisniewski, S.R., Nierenberg, A.A., Warden, D., Ritz, L., Norquist, G., Howland, R.H., Lebowitz, B., McGrath, P.J., Shores-Wilson, K., Biggs, M.M., Balasubramani, G.K., Fava, M., Team, S.D.S., 2006. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. The American journal of psychiatry 163, 28-40.

Tsang, S.W., Keene, J., Hope, T., Spence, I., Francis, P.T., Wong, P.T., Chen, C.P., Lai, M.K., 2010. A serotoninergic basis for hyperphagic eating changes in Alzheimer's disease. J Neurol Sci 288, 151-155.
Van den Wyngaert, I., Gommeren, W., Verhasselt, P., Jurzak, M., Leysen, J., Luyten, W., Bender, E., 1997. Cloning and expression of a human serotonin 5-HT4 receptor cDNA. Journal of neurochemistry 69, 1810-1819.
Vaswani, M., Linda, F.K., Ramesh, S., 2003. Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. Progress in neuro-psychopharmacology & biological psychiatry 27, 85-102.
Vidal, R., Valdizan, E.M., Mostany, R., Pazos, A., Castro, E., 2009. Long-term treatment with fluoxetine induces desensitization of 5-HT4 receptor-dependent signalling and functionality in rat brain. Journal of neurochemistry 110, 1120-1127.

V

Vidal, R., Valdizan, E.M., Vilaro, M.T., Pazos, A., Castro, E., 2010. Reduced signal transduction by 5-HT4 receptors after long-term venlafaxine treatment in rats. Br J Pharmacol 161, 695-706.

Vilaro, M.T., Cortes, R., Gerald, C., Branchek, T.A., Palacios, J.M., Mengod, G., 1996. Localization of 5-HT4 receptor mRNA in rat brain by in situ hybridization histochemistry. Brain Res Mol Brain Res 43, 356-360.

Vilaro, M.T., Cortes, R., Mengod, G., 2005. Serotonin 5-HT4 receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions. The Journal of comparative neurology 484, 418-439.

Vilaro, M.T., Domenech, T., Palacios, J.M., Mengod, G., 2002. Cloning and characterization of a novel human 5-HT4 receptor variant that lacks the alternatively spliced carboxy terminal exon. RT-PCR distribution in human brain and periphery of multiple 5-HT4 receptor variants. Neuropharmacology 42, 60-73.

W

Waeber, C., Sebben, M., Grossman, C., Javoy-Agid, F., Bockaert, J., Dumuis, A., 1993. [3H]-GR113808 labels 5-HT4 receptors in the human and guinea-pig brain. Neuroreport 4, 1239-1242.

Waeber, C., Sebben, M., Nieoullon, A., Bockaert, J., Dumuis, A., 1994. Regional distribution and ontogeny of 5-HT4 binding sites in rodent brain. Neuropharmacology 33, 527-541.

Wang, H., Warner-Schmidt, J., Varela, S., Enikolopov, G., Greengard, P., Flajolet, M., 2015. Norbin ablation results in defective adult hippocampal neurogenesis and depressive-like behavior in mice. Proceedings of the National Academy of Sciences of the United States of America 112, 9745-9750.

Wang, J.W., David, D.J., Monckton, J.E., Battaglia, F., Hen, R., 2008. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. The Journal of neuroscience : the official journal of the Society for Neuroscience 28, 1374-1384.

Warner-Schmidt, J.L., Flajolet, M., Maller, A., Chen, E.Y., Qi, H., Svenningsson, P., Greengard, P., 2009. Role of p11 in cellular and behavioral effects of 5-HT4 receptor stimulation. The Journal of neuroscience : the official journal of the Society for Neuroscience 29, 1937-1946.

Watanabe, Y., Gould, E., Cameron, H.A., Daniels, D.C., McEwen, B.S., 1992. Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. Hippocampus 2, 431-435.

Wilkinson, D., Windfeld, K., Colding-Jorgensen, E., 2014. Safety and efficacy of idalopirdine, a 5-HT6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial. The Lancet. Neurology 13, 1092-1099.

Willner, P., 1984. The validity of animal models of depression. Psychopharmacology 83, 1-16.

Willner, P., 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52, 90-110.

Wilson, D.I., Watanabe, S., Milner, H., Ainge, J.A., 2013. Lateral entorhinal cortex is necessary for associative but not nonassociative recognition memory. Hippocampus 23, 1280-1290.

Wong, M.L., Licinio, J., 2001. Research and treatment approaches to depression. Nature reviews. Neuroscience 2, 343-351.

Wu, N., Song, L., Yang, X., Yuan, W., Liu, Z., 2013. NMDA receptor regulation of levodopa-induced behavior and changes in striatal G protein-coupled receptor kinase 6 and beta-arrestin-1 expression in parkinsonian rats. Clinical interventions in aging 8, 347-352.

Y

Yamaguchi, T., Suzuki, M., Yamamoto, M., 1997. Facilitation of acetylcholine release in rat frontal cortex by indeloxazine hydrochloride: involvement of endogenous serotonin and 5-HT4 receptors. Naunyn Schmiedebergs Arch Pharmacol 356, 712-720.

Yoshikawa, T., Yoshida, N., Mine, Y., Hosoki, K., 1998. Affinity of mosapride citrate, a new gastroprokinetic agent, for 5-HT4 receptors in guinea pig ileum. Japanese journal of pharmacology 77, 53-59.

université PARIS-SACLAY | ÉCOLE DOCTORALE Innovation thérapeutique : du fondamental à l'appliqué

Titre : Rôle du récepteur 5-HT_4 et de la protéine β-arrestine 1 dans la modulation des processus émotionnels et cognitifs dans un modèle d'anxiété/dépression

Mots clés : récepteur 5-HT_4, β-arrestine 1, cognition, modèle d'anxiété/dépression, neurogenèse

Résumé : Les troubles cognitifs constituent des symptômes quasi constants parmi les patients souffrant de dépression caractérisée. De récentes études indiquent que ces troubles mentaux pourraient bénéficier de la modulation de la signalisation du récepteur sérotoninergique 5-HT_4. Dans ce travail de thèse, nous avons dans un premier temps caractérisé les troubles cognitifs dans un modèle d'anxiété/dépression chez la Souris, le modèle CORT. Nous avons ensuite évalué l'efficacité d'un traitement chronique par un agoniste du récepteur 5-HT_4, le RS 67333, en comparaison à la fluoxetine, un antidépresseur monoaminergique de référence sur les altérations émotionnelles et cognitives. D'autre part, des données de la littérature indiquent que la cascade de signalisation de la protéine β-arrestine 1 (impliquée dans la désensibilisation et l'internalisation du récepteur 5-HT_4) serait un biomarqueur potentiel préclinique/clinique des états dépressifs et de la réponse au traitement antidépresseur. Nous avons donc cherché à définir le phénotype anxio/dépressif et cognitif chez des souris tissus-spécifiques conditionnelles, dont l'expression de la protéine β-arrestine 1 dans les cellules souches du gyrus dentelé a été supprimée. L'ensemble de ces travaux de thèse met en avant le rôle prépondérant de l'activation du récepteur 5-HT_4 non seulement dans sa capacité à corriger les symptômes d'anxiété/dépression mais aussi les troubles cognitifs associés à la dépression dans un modèle d'anxiété/dépression chez la Souris. D'un point de vue mécanistique, ces données confirment l'implication de la protéine β-arrestine 1 dans la réponse à la fluoxetine au niveau comportemental et directement au sein du processus de neurogenèse hippocampique chez la souris adulte. Enfin, l'expression de la protéine β-arrestine 1 dans les jeunes neurones de l'hippocampe se révèle aussi être un acteur déterminant dans les processus cognitifs et la réponse à la fluoxetine et au RS 67333.

Title : Role of 5-HT_4 receptor and β-arrestin 1 protein in the modulation of emotional and cognitive processes in an anxiety/depression model.

Keywords : 5-HT_4 receptor, β-arrestin 1, cognition, anxiety/depression model, neurogenesis

Abstract: Cognitive disturbances are often reported as serious incapacitating symptoms by patients suffering from major depressive disorders. Recent studies showed that these mood disorders could benefit from the modulation of 5-HT_4 receptor pathway. Here, we performed a complete characterization of cognitive functions in a neuroendocrine mouse model of depression, the CORT model. We then evaluated emotional and cognitive effects of either a chronic 5-HT_4 receptor agonist treatment, RS 67333 or fluoxetine, a classical monoaminergic antidepressant. Recent data indicate that β-arrestins proteins could be an important molecular determinant in depressive states and in the effects of antidepressants. We determined emotional and cognitive phenotypes in conditional tissue-specific mice in which expression of β-arrestin 1 in adult hippocampal stem cells was deleted. This work suggests that the 5-HT_4 receptor plays a major role to correct not only anxiety/depression-related symptoms but also cognitive alterations in a mouse model of anxiety/depression model. Moreover, these data confirm the involvement of β-arrestine 1 protein in behavioral and neurogenic responses to fluoxetine treatment in adult mice. Finally, β-arrestin 1 protein expression in adult born neuron is critical for cognition and also to fluoxetine and RS 67333 responses.