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FACULTÉ DE PHARMACIE DE CHÂTENAY-MALABRY

UNIVERSITÉ PARIS-SUD 11

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par

M Gaël QUESSEVEUR

**Relations astrocytes-neurones et mécanisme d'action
des inhibiteurs sélectifs de recapture de la sérotonine :
rôle du BDNF et des récepteurs 5-HT_{2A}**

Directeur de thèse : Dr. Bruno GUIARD

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Membres du jury :

Dr. Nicole DÉGLON
Pr. Michel HAMON
Pr. Alain GARDIER
Dr. Bruno GUIARD

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Nguyen TH, Guiard BP, Bacq A, David D.J., **Quesseveur, G**, Gautron S. Sanchez C. & A.M. Gardier 2011. *Enhancement of cortical extracellular 5-HT and NE outflow by escitalopram in awake freely moving mice. Neuroscience 2011, Society for Neuroscience, Washington, USA (2011).*

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SSRIs are commonly prescribed for the treatment of major depressive episodes (MDE). However, 50% of patients do not respond to these medications. Although evidence incriminates the overactivation of 5-HT_{1A} autoreceptor in both (MDE) and poor antidepressant response, others serotonergic receptors could be recruited.

This study first examined the effects of 5-HT_{2A} receptor (5-HT_{2A}R) inactivation on the electrophysiological activity of 5-HT neurons in mice. Second, it evaluated the impact of two putatively functional SNPs of the 5-HT_{2A}R gene (T102C and His452Tyr) on the severity of depression in a sample of 485 Caucasians patients with MDE. Correlations between 5-HT_{1A}R and 5-HT_{2A}R activity was also investigated.

In 5-HT_{1A}R^{+/+} mice, acute administration of SSRIs decreased the firing rate of dorsal raphe (DR) 5-HT neurons, while 5-HT_{1A}R antagonism reversed this effect. Remarkably, such an inhibitory response persisted in mice displaying a pharmacological or genetic inactivation of 5-HT_{1A}R. These results along with the observation that 5-HT_{2A}R antagonism also reversed escitalopram-induced decrease in DR 5-HT neuronal activity suggest that the simultaneous blockade of 5-HT_{1A}R and 5-HT_{2A}R is required to prevent the inhibitory effects of SSRIs. Interestingly, although long-term SSRIs treatment was associated with a recovery to normal of DR 5-HT activity, this response was blunted in 5-HT_{2A}R^{-/-} mice due to 5-HT_{1A} autoreceptor hypersensitization. In patients, the C allele of T102C and the rare TT variant of His452Tyr were associated with a more severe depression.

This study demonstrates a role of 5-HT_{2A}R in chronic antidepressant drugs response that needs to be further investigated in patients with MDE.

Quesseveur G, David DJ, Nguyen TH, Gaillard M-C, Pla, P, Auregan G, Nicolas V, David I, Dranovski A, Wu M, Saudou F, Hen R, Déglon N, Gardier AM, Guiard BP, 2013. *BDNF overexpression in hippocampal astrocytes on anxiolytic-/antidepressant-like activities.* **International Astrocyte School, Bertinoro, Italie (2013).**

The therapeutic activity of selective serotonin (5-HT) reuptake inhibitors (SSRIs) relies on long-term adaptation at pre- and post-synaptic levels. The sustained administration of SSRIs increases the serotonergic neurotransmission in various brain regions involved in mood. In the hippocampus, the enhancement of 5-HT availability increases Brain Derived Neurotrophic Factor (BDNF) synthesis and signaling, a major event in the stimulation of adult neurogenesis. In physiological conditions, BDNF would be expressed at functionally relevant levels in neurons. However, the recent observation that SSRIs upregulate BDNF mRNA in primary cultures of astrocytes strongly suggest that the therapeutic activity of antidepressant drugs might result from an increase in BDNF synthesis in this cell type. In this study, by overexpressing BDNF in astrocytes, we balanced the ratio between astrocytic and neuronal BDNF raising the possibility that such manipulation could positively reverberate on anxiolytic-/antidepressant-like activities in treated mice. In this prospect, we used a novel BDNF-gene transfer strategy to shift the tropism of lentiviral vectors towards astrocytes coupled to a detargeting method with miRNA to eliminate residual BDNF expression in neurons.

Our results indicate that BDNF overexpression in hippocampal astrocytes produced anxiolytic-antidepressant-like activity in the novelty suppressed feeding in relation with the stimulation of hippocampal neurogenesis whereas it did not potentiate the effects of the SSRI fluoxetine on these parameters. Moreover, overexpressing BDNF revealed the anxiolytic-like activity of fluoxetine in the elevated plus maze while attenuating 5-HT neurotransmission in the hippocampus.

These results emphasize an original role of hippocampal astrocytes in the synthesis of BDNF, which can act through neurogenesis-dependent and -independent mechanisms to regulate different facets of anxiolytic-antidepressant-like responses.

We are currently investigating the impact of such a BDNF overexpression in hippocampal astrocytes on anxiolytic-antidepressant-like responses in a mouse model of depression based on chronic administration of corticosterone in the drinking water.

Together, our results illustrate the concept of the tripartite synapse in which astrocytes are essential for serotonergic neuronal activity and their reactive response may contribute to the regulation of mood and anxiolytic-antidepressant-like activities.

Quesseveur G, Petit AC, Gressier F, Colle R, Nguyen HT, David DJ, Guilloux JP, Sanchez C, Gardier AM, Verstuyft C, Corruble E, Guiard BP, 2013. *Role of the 5-HT_{2A} receptor in the mechanism of action of antidepressant drugs: a translational human-mouse study.* European College of Neuropsychopharmacology Workshop, Nice, France (2013).

Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed for the treatment of major depression. However, 50% of depressive patients do not respond adequately to these medications. Although evidence incriminates the overactivation of the 5-HT_{1A} autoreceptor in this poor response [1], others serotonergic receptors could be recruited to modulate the therapeutic activity of SSRIs [2]. In agreement with this hypothesis, growing arguments suggest that variants at gene encoding the 5-HT_{2A} receptor are associated with antidepressant responses [3] but the results of pharmacogenetic studies in human are still matter of debate.

The purpose of this translational study was to determine the effects of 5-HT_{2A} receptor inactivation on the electrophysiological, neurochemical and behavioral activity of SSRIs in mice. On the other hand, this work evaluated the impact of two putatively functional single nucleotide polymorphisms of the 5-HT_{2A} receptor gene (rs6313 and rs6314) [3] on SSRIs responses in depressed patients from the French cohort METADAP. In particular, we studied the percentage of responders and the improvement of their depression scores from various relevant scales.

In wild-type 5-HT_{1A}^{+/+} mice, the acute administration of the SSRIs escitalopram or fluoxetine decreased the firing rate of dorsal raphe (DR) 5-HT neurons, while the administration of the selective 5-HT_{1A} receptor antagonist WAY100635 reversed this effect. Remarkably, the electrophysiological response induced by both SSRIs persisted in 5-HT_{1A}^{+/+} mice pretreated with WAY100635 or in 5-HT_{1A}^{-/-} mice thereby demonstrating the involvement of another serotonergic receptor type in the inhibitory activity. The observation that the 5-HT_{2A} receptor antagonist MDL100907 also reversed escitalopram-induced decrease in DR 5-HT neuronal activity indicates that the simultaneous blockade of 5-HT_{1A} and 5-HT_{2A} receptors is required to prevent the acute inhibitory effects of SSRIs upon the serotonergic system. It also suggests that the activity of SSRIs might be enhanced in 5-HT_{2A}^{-/-} mice after chronic treatment. However, the genetic inactivation of the 5-HT_{2A} receptor significantly attenuated the ability of repeated administration of escitalopram or fluoxetine to increase the firing rate of DR 5-HT neurons and reduced their antidepressant-like effects in the tail suspension test or the novelty suppressed feeding paradigm. Finally, the enhancement of adult hippocampal neurogenesis induced by prolonged administration of SSRIs was blunted in 5-HT_{2A}^{-/-} mice.

In depressed patients, rs6313 and rs6314 genetic variants were not associated with SSRIs response. We extended this observation to the fact that separately analyzed neither escitalopram nor fluoxetine responses were altered for the rs6313. In marked contrast, a trend toward a lower percentage of improvement of depression score was detected after escitalopram treatment in homozygous individuals for the C allele of the rs6314 (p=0.06).

Altogether, these preclinical and clinical data indicate that a functional variant of the 5-HT_{2A} receptor gene may be associated with a poor SSRIs response resulting, at least in part, from an impairment of serotonergic neurotransmission. Although this study has to be completed by determining the consequence of the C allele on the function/expression of the 5-HT_{2A} receptor in the human brain, these results could be of particular importance to select appropriate antidepressant treatment according to the patients' genotype.

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Liste des abréviations :

[Ca²⁺]_i : Concentrations intracellulaires de calcium
[5-HT]_{ext} : Concentration extracellulaire de sérotonine
5-HT : Sérotonine
ATC : Antidépresseur tricyclique
ANLS : Astrocyte neuron lactate shuttle
AMPA : acide 2-amino-3-(5-méthyl-3-hydroxy-1,2-oxazol-4-yl)propanoïque
ARN : Acide ribonucléique
ATP : Adénosine triphosphate
BHE : Barrière hémato-encéphalique
BDNF : Brain-derived neurotrophic factor
CCL : Chemokin ligand
CMH : Complexe majeur d'histocompatibilité
CREB : C-AMP Response Element- binding protein
Cxs : Connexines
DA : Dopamine
DG : Gyrus dentelé
DBS : Stimulation cérébrale profonde
ECT : Thérapie électroconvulsive
EET : Acide epoxyeicosatétranoïque
EGF : Epidermal growth factor
EPM : Test du labyrinthe en croix surélevé (elevated plus maze)
ErK : Extracellular signal- regulated kinase
FGF : Fibroblast growth factor
GCL : Couche cellulaire granulaire
GDNF : Glial-derived neurotrophic factor
GFAP : Glial-fibrillary associated protein
GLT : Transporteur glial du glutamate
GLUT : Transporteur au glucose
GLAST : Transporteur glutamate/aspartate
GPCR : Récepteur couplé aux protéines G
IL : Interleukine
IP₃ : Inositol triphosphate
IGF : Inulin-like growth factor
ISRS : Inhibiteur sélectif de recapture de la sérotonine
JC : Jonction communicante
KO : Knock-out
LDH : Lactate deshydrogénase
LTD : Dépression à long terme
LTP : Potentialisation à long terme
MAPK : Mitogen-activated protein kinase
MCT : Transporteur aux monocarboxylates
NA : Noradrénaline
NAD : Nicotinamide adénine dinucléotide
NGF : Nerve growth factor
NMDA : N-Methyl-D aspartate
NSF : Test d'hypophagie induite par la nouveauté (novelty suppressed feeding)

NT : Neurotrophine
OF : Test du champ ouvert (open field)
PI3K : Inositol-triphosphate kinase
PGE : Prostaglandine
PLC γ : Phospholipase C γ
SNC : Système nerveux central
SGZ : Zone sous-granulaire
ST : Test d'éclaboussure (splash test)
TGF β : Transforming growth factor β
TNF : Tumor necrosis factor
TrKB : Récepteur tyrosine kinase B
TST : Test de suspension caudale (tail suspension test)
VGF : Vascular growth factor
VEGF : Vascular endothelial growth factor

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Résumé :

Les astrocytes joueraient un rôle central dans la physiopathologie des troubles anxio-dépressifs et dans l'activité thérapeutique des antidépresseurs. En effet, différentes études *in vitro* suggèrent que les inhibiteurs sélectifs de recapture de la sérotonine (ISRS) stimulent la synthèse de substances neuroactives par ces cellules gliales nécessaires à la prolifération, la maturation et la survie neuronale mais également au maintien de la plasticité synaptique. Le Brain-derived neurotrophic factor (BDNF) fait partie de ces substances mais son origine, notamment astrocytaire, doit encore être démontrée dans les systèmes intégrés tel que l'animal vivant. A partir de ce constat, dans une première partie de ce travail de thèse, nous avons donc voulu préciser si les astrocytes constituent une source cellulaire participant à la synthèse et/ou à la libération de BDNF en réponse à l'administration prolongée d'un ISRS, la fluoxétine d'une part chez des souris naïves et d'autre part chez des souris exposées à la corticostérone pendant plusieurs semaines (modèle « CORT »). Pour cela, nous avons utilisé une stratégie de transfection virale induisant la surexpression de BDNF spécifiquement dans les astrocytes de l'hippocampe. Nos résultats mettent en lumière que cette surexpression provoque des effets de types anxiolytiques-antidépresseurs dépendant de la neurogenèse hippocampique chez des souris naïves soumises au test d'hypophagie induite par la nouveauté mais pas dans le modèle CORT. Nous avons également mis en évidence que le BDNF pouvait agir en retour sur les cellules qui l'ont libéré pour renforcer le réseau astrocytaire (mécanisme autocrine) mais également sur les neurones sérotoninergiques pré-synaptiques (mécanisme paracrine) pour exercer un frein sur la libération de sérotonine dans l'hippocampe. Différents arguments de la littérature suggèrent d'ailleurs que ce dernier mécanisme pourrait être favorable à l'activité anxiolytique de la fluoxétine tel que nous l'avons observé dans ce travail.

Sachant que les astrocytes expriment à leur surface, une grande variété de récepteurs sérotoninergiques, nous nous sommes ensuite intéressé à la possibilité que le sous-type de récepteur 5-HT_{2A} pourrait être un élément clé dans la synthèse et/ou la libération de BDNF et de ce fait moduler la réponse au stress et celle à la fluoxétine. Lors de cette seconde étude nous avons appliqué une approche génétique utilisant des souris mutées, privées de manière constitutive du récepteur 5-HT_{2A} (5-HT_{2A}^{-/-}). A l'opposé de la surexpression de BDNF dans les astrocytes, nous avons montré que les souris 5-HT_{2A}^{-/-} sont plus sensibles au stress, modélisé par l'exposition chronique à la corticostérone, et semblent résistantes à la fluoxétine comparées aux souris 5-HT_{2A}^{+/+}. Afin de préciser le mécanisme pouvant rendre compte de ces observations, nous avons mis en évidence que l'inactivation du récepteur 5-HT_{2A} s'accompagne d'une hypersensibilité du frein inhibiteur exercé par les autorécepteurs 5-HT_{1A} somatodendritiques sur le tonus sérotoninergique.

L'ensemble de ces travaux de thèse suggère que les astrocytes participent, avec les neurones, à réguler l'humeur et la réponse aux ISRS, notamment grâce à leur capacité à synthétiser et/ou libérer le BDNF

suite à l'activation des récepteurs 5-HT_{2A}. De manière plus générale, nos résultats illustrent le concept de synapse tripartite au sein duquel la communication astrocytes/neurones monoaminergiques serait essentielle dans la régulation de fonctions cérébrales supérieures chez l'animal vivant.

Mots clés :

5-HT_{2A}, antidépresseur, anxiété, astrocyte, BDNF, corticostérone, dépression, neurogenèse et stress.

I. INTRODUCTION :

Les épisodes dépressifs majeurs sont des troubles mentaux qui touchent près de 350 millions de personnes ; ils représentent actuellement la première cause d'incapacité dans le monde (Li et al. 2013). Touchant deux fois plus de femmes que d'hommes, les différentes formes de dépressions (légère, modérée et sévère) se caractérisent par une humeur triste associée à une perte d'intérêt et/ou de plaisir, une dévalorisation de soi, une altération du sommeil et de l'appétit, ainsi qu'une réduction de l'activité psychomotrice. Dans le pire des cas, la dépression peut conduire au suicide. D'un point de vue physiopathologique, les troubles dépressifs majeurs pourraient reposer sur différentes hypothèses neurobiologiques telles que :

- une diminution de la neurotransmission monoaminergique centrale : sérotonine (5-HT), dopamine (DA) et/ou noradrénaline (NA),
- une diminution du processus de neurogenèse hippocampique chez l'adulte en rapport avec une réduction du volume de l'hippocampe et de l'expression de certains facteurs neurotrophiques,
- une sur-activation de l'axe Hypothalamo-hypophyso-surrénalien (HPA) responsable d'une augmentation des concentrations plasmatiques et cérébrales en glucocorticoïdes.

Sur la base de ces observations, différents modèles animaux de dépression ont été élaborés de manière à tester de nouveaux traitements antidépresseurs et d'en préciser leur(s) mécanisme(s) d'action. Au laboratoire, nous avons développé, en collaboration avec l'équipe du Pr René Hen, un modèle de souris présentant un phénotype de type anxio-dépressif : « le modèle CORT » (David et al. 2009). Basé sur l'hypothèse de la dérégulation de l'axe HPA, ce modèle précédemment décrit chez le rat (Hill et al. 2003; Murray et al. 2008), consiste à introduire de la corticostérone dans l'eau de boisson des souris pendant 4 à 5 semaines. Cette exposition induit un certain nombre d'anomalies cellulaires (diminution du processus de neurogenèse hippocampique adulte) et moléculaires (diminution de l'expression de facteurs neurotrophiques) cohérentes avec un état anxieux et résigné, mais également avec d'autres symptômes de la pathologie souvent moins caractérisés tel qu'un abaissement des seuils de douleur thermique et mécanique (David et al. 2009; Rainer et al. 2011; Hache et al. 2012). Sur le plan de la validité prédictive, ce modèle est également pertinent puisque les modifications comportementales induites par la corticostérone peuvent être corrigées par l'administration chronique d'antidépresseurs comme les inhibiteurs sélectifs de recapture de la sérotonine (ISRS) ou l'agomélatine (David et al. 2009; Rainer et al. 2011, Rainer et al. 2012).

A l'heure actuelle, il existe une grande variété de traitements antidépresseurs. La classe des ISRS est sans nul doute l'une des plus utilisées en psychiatrie. Concernant le mécanisme d'action de ces agents pharmacologiques, on sait aujourd'hui que leur administration aiguë entraîne une augmentation du tonus sérotoninergique qui reste toutefois modérée en raison de la mobilisation d'un frein inhibiteur exercé par les autorécepteurs somatodendritiques 5-HT_{1A} (Hamon et al. 1990; Blier, 2001; Blier et al. 2003; Richardson-Jones et al. 2010; Ferrés-Coy et al. 2013). Lorsque le traitement se prolonge, ce frein est levé suite à un processus de désensibilisation au bout d'un temps compatible avec leur délai d'action thérapeutique (Gardier et al. 1996; Le Poul et al. 2000; Blier et al. 2003; Guiard et al. 2011). Cependant, différents arguments scientifiques suggèrent que d'autres récepteurs sérotoninergiques, situés sur des populations neuronales non-sérotoninergiques, pourraient également être recrutés pour limiter l'action à court- et long-terme des ISRS (Spindelegger et al. 2009). C'est le cas par exemple des hétérorécepteurs 5-HT_{2A} dont l'activation, en réponse à l'administration d'ISRS, diminue non seulement l'activité électrique des neurones sérotoninergiques (Boothman and Sharp, 2005; Cremers et al. 2007; Quéérée et al. 2009; Calcagno and Invernizzi, 2010) mais aussi celle des neurones noradrénergiques (Szabo and Blier, 2001; Dremencov et al. 2007). En accord avec ces observations, un travail réalisé au cours de ma thèse a permis de montrer que l'effet inhibiteur d'un ISRS sur l'activité des neurones sérotoninergiques persiste chez des souris présentant une inactivation pharmacologique des récepteurs 5-HT_{1A} tandis qu'elle peut être reversée par le MDL100907, un antagoniste des récepteurs 5-HT_{2A} (Quesseveur et al. 2013). Ces résultats pourraient ainsi apporter une explication au fait que les antipsychotiques (présentant des propriétés d'antagonistes des récepteurs 5-HT_{2A}) s'avèrent efficaces chez les patients résistant aux ISRS (Hamon et Blier, 2013). Ils mettent également en évidence la nécessité de bloquer simultanément les autorécepteurs 5-HT_{1A} pré-synaptique et les hétérorécepteurs 5-HT_{2A} pour renforcer la libération intra-synaptique de 5-HT en réponse à l'administration d'un ISRS.

Malgré ces résultats, la question que l'on peut se poser est de savoir comment des événements synaptiques peuvent entraîner des modifications de la plasticité cérébrale nécessaires à l'activité thérapeutique de ces antidépresseurs (Santarelli et al. 2003) ?

On connaît désormais un certain nombre de voies de signalisations aboutissant à la régulation de l'expression de gènes qui seraient impliquée dans les différentes formes de dépressions et la réponse aux ISRS. En effet, au niveau post-synaptique, il est désormais admis qu'une partie des effets thérapeutiques de ces molécules passe par la synthèse de facteurs neurotrophiques et la stimulation du processus de neurogenèse hippocampique dans le cerveau mature (Russo-Neustadt et al. 2003; Sen et al. 2003). Parmi les neurotrophines synthétisées et sécrétées, le Brain-Derived Neurotrophic Factor (BDNF) joue un rôle clé dans la stimulation des étapes de prolifération, maturation et survie

neuronale, mais également dans la formation et le maintien des synapses, processus qui seraient fortement altérés en conditions de stress (Duman et al. 2001; Waterhouse et al. 2009). Pourtant, malgré les nombreuses études cliniques et précliniques étayant ce concept, on ne sait pas dans quelle mesure les astrocytes participent à la synthèse de BDNF et le(s) sous-type(s) de récepteur(s) sérotoninergique(s) impliqués dans les propriétés neurotrophiques d'un traitement chronique d'ISRS. Les récepteurs 5-HT_{2A} font partie des cibles potentielles car en plus de leur rôle avéré dans la régulation de l'activité des neurones sérotoninergiques suite à l'administration d'ISRS, ils pourraient également contribuer à l'activité des ces antidépresseurs grâce à une action post-synaptique.

Ces dernières années ont été marquées par l'hypothèse expérimentale et clinique du rôle des cellules gliales, et plus particulièrement des astrocytes, dans la physiopathologie de la dépression et les effets bénéfiques des antidépresseurs actuellement disponibles. Par exemple, des diminutions du nombre et de la densité des astrocytes ont été décrites dans le cortex frontal, l'hippocampe et l'amygdale de patients dépressifs (Rajkowska et Stockmeier, 2013). Ceci a également été décrit dans différents modèles animaux de la pathologie (Czeh et al. 2007; Banasr et al. 2008 ; Gosselin et al. 2009) tandis que les stratégies antidépressives (non)-pharmacologiques préviennent ou corrigent ces altérations (Czeh et al. 2006; Cabras et al. 2010 ; Czeh et Benedetto, 2012). Ces observations peuvent s'expliquer par le fait que les astrocytes participent à de nombreuses modifications morphologiques et fonctionnelles modulant l'activité neuronale. En effet, de par leur contact avec les capillaires sanguins et leur organisation en réseau, ces cellules gliales assurent l'apport nutritif nécessaire au bon fonctionnement des neurones. Par ailleurs, en réponse à la libération de neurotransmetteurs, les astrocytes assurent à leur tour la synthèse et la libération de gliotransmetteurs (ATP-adénosine, D-sérine, glutamate) mais aussi de facteurs neurotrophiques participant à la communication bidirectionnelle entre neurones et cellules gliales au sein de la synapse tripartite (Araque et al. 1999). En outre, des études récentes montrent que les antidépresseurs augmentent de manière significative la synthèse de BDNF dans des cultures primaires d'astrocytes de rongeurs issues de différentes régions cérébrales telles que l'hippocampe et le cortex (Allaman et al. 2011; Prickaerts et al. 2012; Kittel-Schneider et al. 2012; Takano et al. 2012)

Durant ce travail de thèse, et à la lumière de ces données bibliographiques, nous avons tenté de répondre aux questions suivantes :

- La synthèse de BDNF observée dans des cultures d'astrocytes suite à l'application d'ISRS peut-elle rendre compte, du moins en partie, des effets thérapeutiques de ces antidépresseurs *in vivo*, chez des souris naïves ou stressées de manière chronique par l'administration de corticostérone?
- Quels mécanismes pourraient être responsables de la synthèse et de la libération de BDNF par les astrocytes? Nous avons notamment émis l'hypothèse que la synthèse de ce facteur

neurotrophique par les astrocytes résulterait de l'activation du sous-type de récepteur 5-HT_{2A} de la sérotonine.

Ces questions nous ont conduit à aborder dans **une première partie de ce travail**, l'étude des effets comportementaux, électrophysiologiques et neurochimiques de la surexpression de BDNF dans les astrocytes de l'hippocampe chez la souris adulte non stressée. Sur le plan expérimental, ces recherches ont été possibles grâce à une collaboration avec le Dr Nicole Déglon (CEA de Fontenay-aux-Roses, France) qui nous a fourni un outil original permettant le transfert du gène codant pour le BDNF majoritairement dans les astrocytes, tout en réprimant l'expression résiduelle dans les neurones (Colin et al. 2009). Le choix de cette approche reposait, d'une part sur l'échec d'une étude préliminaire menée en 2007-2008 avec un virus présentant un effet anxiogénique/dépressiogène propre qui nous a empêché d'exploiter les données expérimentales, et d'autre part, en raison de l'intérêt croissant pour les astrocytes dans la physiopathologie des troubles de l'humeur. Notre première étude a été publiée dans « Translational Psychiatry » en 2013, montrant les effets bénéfiques de la surexpression de BDNF dans les astrocytes de l'hippocampe sur le comportement anxio-dépressif des souris et/ou la réponse à l'ISRS (la fluoxétine) (**Article 1**). Toutefois, notre approche lentivirale souligne la suffisance mais pas la nécessité du BDNF astrocytaire dans la manifestation des effets thérapeutiques des antidépresseurs sérotoninergiques. Pour palier à ce problème, il aurait fallu tester en parallèle l'effet de l'inactivation du BDNF dans les astrocytes, ce qui était techniquement impossible jusqu'en 2013, faute d'outils viraux valides. Par ailleurs, comme la surexpression a été testée initialement chez des animaux naïfs, c'est à dire en conditions non pathologiques, nous avons ensuite exploré les effets de cette surexpression chez des souris exposées à la corticostérone pendant 4 semaines. Cet aspect nous a paru pertinent dans la mesure où, comme nous l'avons mentionné plus haut, le stress chronique et la corticostérone réduisent l'activité et la densité des astrocytes dans différentes régions cérébrales impliquées dans la régulation de l'humeur. Enfin, à la faveur d'une discussion au cours de mon comité de mi-thèse avec le Dr Christian Giaume (INSERM U840, Collège de France), nous avons entrepris une collaboration visant à déterminer l'impact de la surexpression de BDNF dans les astrocytes de l'hippocampe chez des souris traitées à la corticostérone sur le réseau astrocytaire reposant sur des protéines de jonctions telles que les connexines 30 et 43. En effet, l'hypothèse selon laquelle les effets bénéfiques de cette stratégie virale résulterait d'une amélioration du dialogue entre neurones et cellules gliales au travers de ce réseau astrocytaire, nous a parue possible et originale (**Article 2 : en préparation**).

Compte tenu de la présence des récepteurs 5-HT_{2A} sur les astrocytes (Wu et al. 1999) et des données récentes suggérant que leur activation *in vitro* pourrait entraîner la synthèse de facteurs neurotrophiques (Hisoaka et al. 2007 ; Martin et al. 2013), nous avons émis et testé l'hypothèse qu'au contraire, des souris déficientes en récepteurs 5-HT_{2A} pourraient présenter une augmentation de la

vulnérabilité au stress et/ou une atténuation de la réponse aux ISRS. Ainsi, dans **une seconde partie de ce travail de recherche**, nous avons étudié le phénotype de type anxio-dépressif de souris knock-out pour le récepteur 5-HT_{2A} (mutation constitutive) suite à l'administration prolongée de corticostérone ainsi que leur réponse à la fluoxétine. En parallèle de cette approche préclinique, nous nous sommes rapprochés d'une équipe de psychiatres de l'Université Paris Sud XI dont le Pr Emmanuelle Corruble (INSERM U769, Hôpital de Bicêtre) qui fait partie du comité de pilotage d'une cohorte prospective de 630 patients souffrant d'épisodes dépressifs majeurs (METADAP, ClinicalTrial.gov Id : NCT00526383). Outre le nombre élevé de patients, l'un des avantages de cette cohorte repose sur le fait qu'environ 50% d'entre eux étaient traités par des ISRS au moment de leur inclusion. Nous avons donc tiré profit des données collectées sur la sévérité de l'épisode dépressif à l'inclusion et au cours du traitement pour tester deux polymorphismes fonctionnels du récepteur 5-HT_{2A}, le T102C (rs6313) et le Hys452Tyr (rs6314). Grâce à cette étude translationnelle, nous avons proposé que la diminution de la signalisation médiée par ce sous-type de récepteur sérotoninergique chez la souris et chez le patient dépressif était responsable d'une vulnérabilité accrue au stress (**Article 3 : en préparation**) et d'une diminution de la réponse à la fluoxétine (**Article 4 : en préparation**).

Au moment de la rédaction de ce mémoire, mon nom est cité dans 5 publications (dont un article de recherche et deux revues en premier auteur en rapport avec le sujet de ma thèse) tandis que 3 autres publications sont en cours de rédaction. Nous avons choisi de présenter les travaux qui s'inscrivent directement dans la mise en évidence des liens éventuels entre le BDNF astrocytaire et le sous-type de récepteur 5-HT_{2A} dans la physiopathologie de la dépression et la réponse aux ISRS. Au cours de ma thèse, d'une durée de 3 ans, les deux parties ont été menées de manière indépendante.

Je termine cette introduction en soulignant le fait que ce travail a fait l'objet de différentes collaborations avec :

- N. Déglon, A. Bemelmans, MC. Gaillard, G. Auregan et P. Hantraye, du laboratoire Mircen du CEA de Fontenay-aux-Roses,
- P. Plat et F. Saudou, de l'Unité U1005 de l'Institut Curie d'Orsay,
- E. Corruble, F. Gressier, C. Verstuylft, AC. Petit et B. Fallissard), de l'Unité INSERM U769 du Kremlin Bicêtre,
- A. Roumier et L. Maroteaux, de l'Unité INSERM Mixte de Recherche (UMR)-S 8399 à Paris,
- P. Ezan et C. Giaume, de l'Unité CNRS U7241 du Collège de France à Paris,

- M. Wu, A. Dranovski et R. Hen, département de psychiatrie et neurosciences de l'Université de Columbia à New-York.

Ces collaborations nous ont permis de compléter la batterie de tests déjà présents au laboratoire EA 3544, grâce à des tests *in vitro* ou l'accès à des cohortes de patients dépressifs, et de confirmer nos hypothèses mais aussi d'ouvrir la voie à de nouvelles perspectives qui seront détaillées dans la discussion générale de ce mémoire.

En raison d'une littérature abondante et des thèses précédentes du laboratoire, nous avons choisi de ne pas centrer la partie bibliographique de ma thèse sur les mécanismes physiopathologiques de la dépression et les propriétés pharmacologiques des antidépresseurs mais sur les astrocytes et les évidences scientifiques suggérant leur rôle dans le domaine de la neuropsychopharmacologie. L'introduction de ce manuscrit récapitule également les connaissances actuelles sur les propriétés du BDNF et son récepteur à haute affinité TrkB en insistant plus particulièrement sur les liens réciproques qu'entretient ce facteur neurotrophique avec le système sérotoninergique central. La seconde partie de ce mémoire est organisée sous la forme d'articles (publiés ou en cours de préparation) à l'intérieur desquels les "matériel et méthodes" ainsi que nos "résultats" expérimentaux peuvent être consultés. Enfin, la discussion générale tente de réconcilier l'ensemble de nos données et de les confronter à celles de la littérature de manière à proposer un mécanisme d'action original des ISRS.

Partie I : Les astrocytes : Fonctions physiologiques, organisation anatomique et implication dans les troubles de l'humeur et le mécanisme d'action des antidépresseurs.

Les astrocytes appartiennent à la grande famille des cellules gliales décrites pour la première fois en 1824 par René Dutrochet, puis identifiées et nommées "névroglies" en 1858 par Rudolf Virchow (Parpura et al. 2012). Le terme névroglie faisait référence à l'hypothèse selon laquelle ces cellules non-neuronales assuraient la cohésion du système nerveux (*glie*, mot grec signifiant "glu"). Par la suite, trois types de cellules gliales ont été identifiées : les oligodendrocytes dont l'une des fonctions principales est la myélinisation des axones ; les cellules microgliales qui jouent le rôle de cellules immunitaires du système nerveux et qui sont impliquées dans les processus inflammatoires ; et les astrocytes. Ces derniers représentent plus de la moitié des cellules du cerveau chez l'Homme (Azevedo et al. 2009). Les astrocytes présentent 3 principales caractéristiques :

- la présence de filaments intermédiaires, les gliofilaments, qui sont groupés en faisceaux et composés d'une protéine spécifique : la "glial fibrillary acidic protéin" ou protéine acide gliofibrillaire (GFAP),
- la présence de grains de glycogène, absents des autres cellules nerveuses, disséminés dans le corps cellulaire et les prolongements cytoplasmiques dont les extrémités distales sont appelées "pieds astrocytaires",
- la présence de jonctions communicantes qui permettent la signalisation intercellulaire entre astrocytes adjacents. Ces jonctions sont le support anatomique de réseaux fonctionnels astrocytaires.

D'un point de vue fonctionnel on peut distinguer deux types d'astrocytes : les astrocytes de type I, en contact avec les capillaires sanguins, qui jouent un rôle clé dans le métabolisme et l'apport nutritif des neurones ; les astrocytes de type II situés autour des neurones et des synapses où ils participent au fonctionnement et à la plasticité synaptique. On peut également différencier les astrocytes en fonction de leur morphologie : les astrocytes protoplasmiques présents dans la substance grise et qui sont dotés d'une multitude de courtes ramifications ; les astrocytes fibreux localisés dans la substance blanche et qui possèdent des processus moins nombreux mais plus longs et plus étroits.

A. Fonctions de support des neurones.

Longtemps assimilés à de simples cellules de soutien, des études démontrent que les astrocytes possèdent de très nombreuses fonctions au sein du système nerveux central (SNC).

- Tout d'abord, les astrocytes jouent un rôle dans la migration et le guidage des neurones au cours des stades précoces du développement cérébral, notamment via la production de molécules

d'adhésion et des protéines de matrice, servant d'échafaudage à l'architecture cérébrale (Clarke and Barres, 2013).

- Ils participent aussi à la formation et au maintien de la barrière hémato-encéphalique (BHE) qui protège le cerveau contre les toxines et agents pathogènes circulant. La BHE participe également au passage des nutriments nécessaires à l'activité neuronale (Ballabh and Nedergaard, 2004; Nico and Ribatti, 2012).

Les astrocytes sont en contact étroit avec les capillaires sanguins et les neurones ce qui leur confèrent un rôle majeur dans le couplage neurovasculaire et neurométabolique (Martin et al. 2013). Ces cellules gliales sont des acteurs majeurs dans la capture et le métabolisme du glucose et du glutamate (Bélanger et al. 2011; Oberheim et al. 2012). De nombreuses études soulignent ainsi que plus de 99% de la surface cérébrovasculaire est enveloppée par les processus astrocytaires (Kacem et al. 1998; Simard et al. 2003; Haydon et al. 2006). Ainsi, les astrocytes participent à la régulation du débit sanguin cérébral via la sécrétion de prostaglandine E2 (PGE₂) et d'acide epoxyeicosatétranoïque (EETs) (Zonta et al. 2003; Takano et al. 2006; Koehler et al. 2009).

- Ces cellules gliales ont également la capacité de libérer de nombreuses substances neuroactives qui contribuent à réguler la neurotransmission. Par ce mécanisme, les astrocytes possèdent des fonctions trophiques via la synthèse de facteurs neurotrophiques tels que le Glial-derived neurotrophic factor (GDNF), le Epidermal growth factor (EGF), le Fibroblast growth factor (FGF), le Nerve growth factor (NGF) et le neurotrophine-3 et 4/5 (NT-3 et NT-4/5). Des études *in vitro* chez le rongeur soulignent que ces facteurs sont nécessaires à la prolifération, maturation et survie neuronale mais également à la plasticité synaptique (Lim et al. 1999; Song et al. 2002; Juric et al. 2006; Rusakov et al. 2011; Parpura et al. 2012). Les astrocytes libèrent des gliotransmetteurs dont le glutamate, l'ATP/Adénosine et la D-sérine, (Parpura et al. 2012; Santello et al. 2012; Zorec et al. 2012). Ces cellules gliales exercent un rôle prépondérant dans les processus immunitaires et inflammatoires via la synthèse de facteurs pro-inflammatoires tels que les interleukines-3/-6 (IL-3 et IL-6) et le Tumor necrosis factor (TNF) mais également anti-inflammatoires tels que le Transforming growth factor β (TGF β) (Eyre et al. 2012).

- Enfin, les astrocytes contribuent au maintien des conditions optimales pour la conduction nerveuse (Hertz et al. 2004) grâce à deux transporteurs actifs, la pompe Na⁺/K⁺ -ATPase et le cotransporteur Na⁺/K⁺, impliqués dans la clairance des ions potassium.

L'ensemble de ces fonctions attribuées aux astrocytes a conduit au concept de synapse tripartite qui repose sur l'intercommunication de 3 éléments: une terminaison pré- et une terminaison post-synaptique neuronale entourée par un processus astrocytaire (Araque et al. 1999) ([Figure 1](#)). Chaque astrocyte peut ainsi envelopper plus de 100.000 synapses et des études récentes suggèrent par exemple qu'environ 50% des synapses de l'hippocampe sont des synapses tripartites (Bushong et al. 2002; Fellin et al. 2006). Le rôle des astrocytes dans la synapse tripartite est de moduler l'activité des

neurones pré- et post-synaptiques et donc de participer activement à la régulation de la neurotransmission. Cela peut se faire grâce à la présence d'une grande variété de transporteurs pour les neurotransmetteurs, tels que du glutamate, des monoamines (sérotonine, noradrénaline, dopamine), ou encore du GABA (Perea et al. 2005; Perea et al. 2009). Ces cellules gliales expriment également à leur surface de nombreux récepteurs pour les neurotransmetteurs, incluant les récepteurs ionotropiques (AMPA, NMDA) et métabotropiques du glutamate, des récepteurs GABAergiques, purinergiques, cholinergiques, histaminergiques, mais aussi monoaminergiques (Perea et al. 2005). Leur stimulation induit, entre autre, une augmentation des concentrations intracellulaires de calcium ($[Ca^{2+}]_i$) responsable de la libération de glucose, de ses métabolites, de facteurs neurotrophiques et de gliotransmetteurs (Parpura et al. 1994; Pascual et al. 2005; Haydon et al. 2006) qui régulent en retour l'excitabilité des éléments pré- et post-synaptiques neuronaux et donc la libération des neurotransmetteurs (Aguilhon et al. 2008).

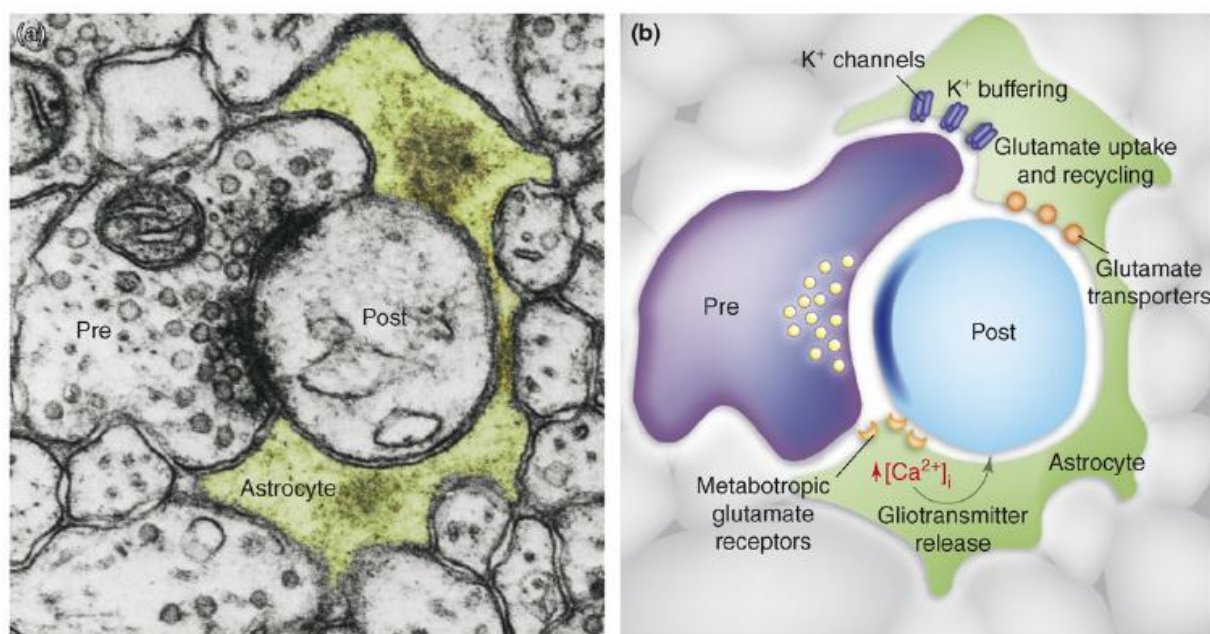


Figure 1 : Représentation d'une synapse tripartite.

D'après Halassa et al. 2007

(a) Photographie de microscopie électronique représentant des terminaisons pré- et post-synaptiques entourées par un processus astrocytaire (en vert), formant la synapse tripartite. (b) Représentation schématique de la photographie de microscopie électronique révélant l'étroite interaction anatomique et fonctionnelle entre les terminaisons neuronales et le processus astrocytaire.

a) La signalisation calcique

On a longtemps considéré que les astrocytes ne possédaient que des propriétés passives tel qu'un support trophique et métabolique pour les neurones. En effet, des études électrophysiologiques révélaient que les cellules gliales n'étaient pas électriquement excitables et dans

l'incapacité de produire un potentiel d'action. Ce n'est qu'au début des années 90 que l'on démontra la capacité des astrocytes à répondre à de nombreux stimuli. Cornell-Bell et ses collaborateurs observèrent une élévation des $[Ca^{2+}]_i$ dans une culture astrocytaire suite à une stimulation glutamatergique (Cornell-Bell et al. 1990). Cette élévation de $[Ca^{2+}]_i$ locale pouvait se propager aux cellules voisines grâce à la présence de jonctions communicantes entre astrocytes ou "GAP jonctions". Cette propagation de la réponse calcique porte aujourd'hui le nom de "vagues calciques" et elles ont été observées *in situ* sur des coupes de cerveau (Schipke et al. 2002). En réalité, ce n'est pas le Ca^{2+} qui se propage de cellules en cellules mais l'inositol triphosphate (IP3). En effet, l'IP3 diffuse dans les astrocytes voisins où il va induire une élévation du $[Ca^{2+}]_i$, qui à son tour va stimuler la production et la diffusion d'IP3 aux cellules adjacentes (Allbritton et al. 1992; Hofer et al. 2002). En plus de la voie intracellulaire utilisant l'IP3, il existe une voie extracellulaire, utilisant cette fois-ci l'ATP, permettant la diffusion de vagues calciques intercellulaires entre astrocytes non connectés (Hassinger et al. 1996).

Des études *in vitro* et *in vivo* ont démontré que les astrocytes exprimaient à leur surface une grande variété de canaux ioniques (Barres, 1991) mais également de récepteurs couplés à des protéines G (GPCRs) (Murphy et al. 1987; Porter et al. 1997; Volterra and Meldolesi, 2005). La stimulation des ces récepteurs induit une grande variété de réponses de la part des astrocytes, notamment une élévation des $[Ca^{2+}]_i$ et la propagation de vagues calciques (Perea et al. 2005; Agulhon et al. 2008). Cette stimulation intracellulaire calcique aboutit à la libération de substances neuroactives telles que les gliotransmetteurs, les facteurs neurotrophiques et les médiateurs pro- ou anti-inflammatoires.

b) L'apport en glucose et glycogène

Malgré le fait qu'il ne représente que 2% de la masse totale du corps, le cerveau consomme 20 à 25% du glucose circulant. L'activité neuronale nécessite donc un apport énergétique constant car les neurones ne disposent que de très peu de réserves énergétiques. L'activité cérébrale est ainsi hautement dépendante de l'apport sanguin en nutriments.

Les astrocytes possèdent à leur surface des transporteurs pour le glucose tel que le transporteur au glucose 1 (GLUT1) (Figure 2). Le glucose capté est stocké sous forme de glycogène ou métabolisé en pyruvate puis en lactate par la lactate déshydrogénase (LDH). Les astrocytes métabolisent 50% du glucose passant la BHE et sont les principaux sites de stockage du glycogène cérébral (Magistretti, 2006; Hertz et al. 2007; Bélanger et al. 2011). Le lactate est ensuite libéré dans le milieu extracellulaire par des transporteurs aux monocarboxylates (MCTs) puis capté par les neurones où il alimentera le cycle de Krebs mitochondrial afin de produire de l'ATP (Tsacopoulos et al. 1996; Bouzier-Sore et al. 2006). Le lactate représente donc la principale source d'énergie sous-tendant l'activité neuronale (Bouzier-Sore et al. 2006; Bélanger et al. 2011). De manière remarquable, le

glutamate joue un rôle important dans le couplage du métabolisme astrocytaire et l'activité neuronale. En effet, le glutamate libéré lors de l'activité neuronale est principalement recapté par les astrocytes où il va stimuler la capture du glucose et le métabolisme du glycogène. Ce processus, appelé la navette du lactate entre astrocytes et neurones (ou Astrocyte Neuron Lactate Shuttle, ANLS), permet de synchroniser la production de lactate astrocytaire selon les besoins de l'activité neuronale (Pellerin et al. 1994; Magistretti, 2006).

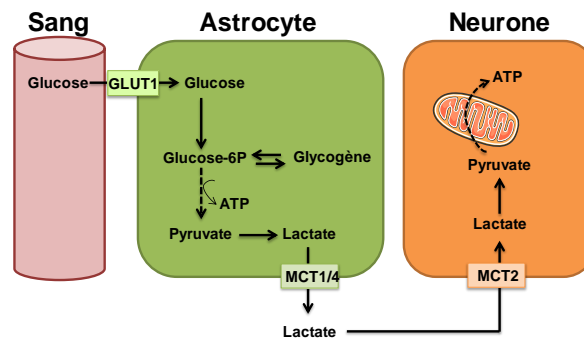


Figure 2 : Schéma du métabolisme du glucose dans la communication neurone-astrocyte.

Le glucose est capté par les astrocytes grâce au transporteur GLUT1. Il est alors métabolisé en glucose-6 phosphate (Glucose-6P) puis stocké sous forme de glycogène ou métabolisé en pyruvate et lactate. Ce dernier va alors être libéré dans le milieu extracellulaire et recapté par les neurones par l'intermédiaire des transporteurs aux monocarboxylates (MCT) 1/4 et 2, respectivement. Dans les neurones, le lactate permettra la production de pyruvate qui à son tour alimentera le cycle de Krebs mitochondrial afin de synthétiser de l'ATP.

c) La libération de gliotransmetteurs.

1. Le Glutamate.

Le glutamate est le principal neurotransmetteur exciteur du cerveau. Les astrocytes jouent un rôle clé dans la neurotransmission glutamatergique puisque ces cellules expriment à leur surface des transporteurs de haute affinité pour cet acide aminé, tels que le transporteur glutamate/aspartate (GLAST) et le transporteur glial du glutamate (GLT1) (ou EAAT1, EAAT2 chez l'Homme). Ces transporteurs assurent 90% de la recapture du glutamate et sont fortement exprimés dans la matière grise du cortex (Oberheim et al. 2012). Le glutamate capté par les astrocytes est métabolisé en glutamine par la glutamine synthase puis libéré dans le milieu extracellulaire (Figure 3). La glutamine est alors captée par les neurones et transformée en glutamate par la glutaminase. Les astrocytes jouent donc un rôle clé dans le recyclage du glutamate nécessaire à la neurotransmission glutamatergique mais également dans la protection de ses effets excitotoxiques. En effet, il a été démontré que le blocage de l'expression des ces transporteurs chez le rat provoquait une élévation des concentrations extracellulaires de glutamate, induisant des effets neurotoxiques (Rothstein et al. 1996; Rao et al. 2001). En plus de cette fonction de recapture, les astrocytes assurent la libération du

glutamate qui est l'un des premiers gliotransmetteurs libérés par les astrocytes à avoir été identifié. En 1994, des études démontrent pour la première fois que les astrocytes, en libérant du glutamate, étaient capables de moduler l'activité neuronale via la stimulation des récepteurs N-Méthyl-D aspartate (NMDA) des neurones adjacents (Nedergaard, 1994; Parpura et al. 1994). Des études ont par la suite démontré que le glutamate astrocytaire induisait différents types d'effets sur l'activité des éléments pré- et post-synaptiques neuronaux. Par exemple, le glutamate astrocytaire provoque l'apparition de courants entrants de type lent (Slow inward currents, SICs) dans les neurones pyramidaux du CA1 de l'hippocampe à travers l'activation des récepteurs NMDA post-synaptiques (Angulo et al. 2004; Fellin et al. 2004; Perea et al. 2005). Le glutamate agit aussi sur l'élément pré-synaptique neuronal (Perea et al. 2010) en potentialisant ou réprimant la transmission synaptique par l'activation respective des récepteurs pré-synaptiques du type kainate ou métabotrope (mGlu II/III) (Liu et al. 2004a; Liu et al. 2004b). Le glutamate astrocytaire semble donc avoir différents effets au niveau de l'activité neuronale en fonction du type de récepteur impliqué, ceci démontrant le haut degré de complexité de la communication neurone-astrocyte au travers de cet exemple.

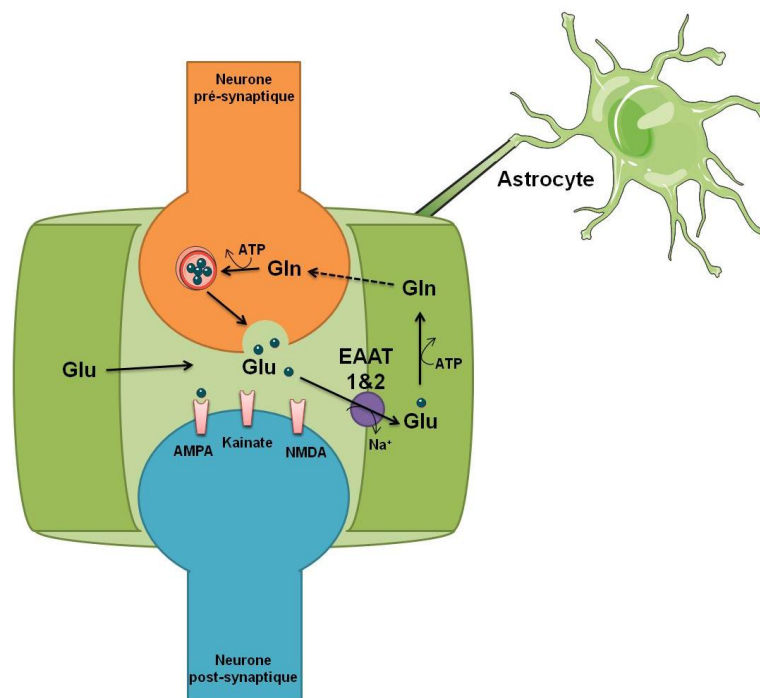


Figure 3 : Schéma du cycle glutamate-glutamine dans l'interaction neurone-astrocyte.

Le glutamate, libéré lors de l'activité neuronale, est recapté par les transporteurs au glutamate EAAT1&2 présents sur la membrane plasmique des astrocytes. Dans ces cellules, le glutamate est transformé en glutamine par la glutamine synthase. La glutamine est alors libérée par les astrocytes dans le milieu extracellulaire puis recaptée par les neurones où elle servira de précurseur pour la synthèse de glutamate nécessaire à la neurotransmission. Le glutamate peut également être libéré par les astrocytes dans l'espace synaptique.

2. L'ATP/Adénosine.

Peu de temps après l'observation que des astrocytes en culture pouvaient libérer du glutamate, d'autres groupes de recherche ont démontré que l'ATP était également libérée par ces

cellules . Des données *in vitro* et *in vivo* révèlent que l'ATP est utilisée par les astrocytes comme signal assurant la propagation de vagues calciques entre astrocytes non connectés via des jonctions communicantes (Guthrie et al. 1999). Une fois libérée dans le milieu extracellulaire, l'ATP est rapidement hydrolysé (environ 200 ms) en adénosine par les ectonucléotidases (Newman et al. 2003). Il est désormais admis qu'une partie des effets dus à la libération d'ATP astrocytaire passe par l'adénosine (Halassa et al. 2010). Les cellules astrogliales utilisent ainsi l'ATP/adénosine pour moduler l'activité synaptique à travers la stimulation des récepteurs purinergiques neuronaux mais les effets semblent dépendre de la région cérébrale impliquée. En effet, des études montrent que la libération d'ATP/adénosine par les astrocytes augmente l'expression membranaire des récepteurs AMPA post-synaptiques mais également l'amplitude des courants excitateurs post-synaptiques (Gordon et al. 2005). A l'inverse dans la région du CA1 de l'hippocampe, la libération d'ATP/adénosine module également le processus de potentialisation à long terme (LTP) et induit une dépression de la transmission synaptique (Zhang et al. 2003; Pascual et al. 2005; Serrano et al. 2006).

3. La D-sérine.

La D-sérine fait également partie des gliotransmetteurs libérés par les astrocytes. Synthétisée par les astrocytes à partir de la L-sérine, des études *in vitro* et *in situ* démontrent que la D-sérine est un co-agoniste des récepteurs NMDA (Mothet et al. 2000; Mothet et al. 2005). En se liant simultanément avec le glutamate au niveau de la sous-unité NR1, ce gliotransmetteur permet l'activation des récepteurs NMDA (Dalkara et al. 1992; Panatier et al. 2006). D'ailleurs, une étude *in vitro* sur des tranches d'hippocampe indique que le blocage de la libération de D-sérine par les astrocytes inhibe l'activité des récepteurs NMDA et l'induction de LTP (Henneberger et al. 2010). En accord avec cette étude, il a été rapporté que la dégradation de la D-sérine par l'enzyme D-amino acide oxydase, inhibe l'activation des récepteurs NMDA mais également perturbe l'induction de la LTP enregistrée dans la région CA1 de l'hippocampe (Yang et al., 2003). Par ce mécanisme la D-sérine astrocytaire est ainsi capable de moduler l'action de la neurotransmission glutamatergique (Yang et al. 2003; Panatier et al. 2006; Scolari et al. 2007).

d) La libération de facteurs neurotrophiques.

Les astrocytes sont impliqués dans la survie neuronale, la croissance dendritique et la synaptogenèse grâce aux facteurs neurotrophiques et aux facteurs de croissance qu'ils synthétisent, stockent et/ou libèrent, tels que le Brain-derived neurotrophic factor (BDNF), le nerve growth factor (NGF), la neurotrophine-3 (NT-3), le ciliary neurotrophique factor (CNTF), le fibroblast growth factor 2 (FGF-2), le glial cell line-derived neurotrophic factor (GDNF), le insulin-like growth factor 1 (IGF-

1) et les vascular (endothelial) growth factor (VGF et VEGF) (Ballotti et al. 1987; Schaar et al. 1993; Allaman et al. 2011).

1. Le BDNF.

Le Brain-derived neurotrophic factor (BDNF) est un facteur neurotrophique impliqué dans de nombreux processus dont la survie, le développement et le fonctionnement neuronal mais également dans la formation et la plasticité synaptique, ainsi que la dendritogenèse au cours du développement du cerveau (Martin et al. 2011; Martin et al. 2013). Plusieurs études soulignent que ce facteur est impliqué dans l'éthiologie et les traitements de la dépression (Duman et al. 2006; Castren and Rantamaki, 2010; Autry and Monteggia, 2012). Cependant, les types cellulaires impliqués dans la synthèse et la libération de BDNF en réponse à un traitement antidépresseur restent à être identifiés. En effet, bien que les neurones semblent être une source majeure de BDNF (Schmidt-Kastner et al. 1996), des études récentes *in vitro* démontrent que les astrocytes pourraient également jouer un tel rôle (Zafra et al. 1992; Miklic et al. 2004). Ainsi, différentes classes d'antidépresseurs (les ISRS, Fluoxétine et paroxétine, mais également les antidépresseurs tricyclique (ATCs), imipramine et amitriptyline) induisent une augmentation du taux d'ARN messager codant pour le BDNF dans des cultures primaires d'astrocytes de rongeurs (Allaman et al. 2011; Kajitani et al. 2012; Kittel-Schneider et al. 2012; Takano et al. 2012). Une confirmation *in vivo* de ces données reste toutefois nécessaire.

2. Les autres facteurs neurotrophiques.

Le VGF et le VEGF sont également impliqués dans de nombreuses fonctions cérébrales telles que la prolifération des cellules progénitrices, la stimulation de la neurogenèse hippocampique ainsi que la plasticité neuronale au niveau du cortex préfrontal et de l'hippocampe (Alder et al. 2003; Wada et al. 2006; Licht et al. 2011; Fournier and Duman, 2012). A l'instar du BDNF, des études récentes utilisant des cultures primaires d'astrocytes démontrent que les antidépresseurs stimulent l'expression de la protéine et de l'ARNm codant pour le VGF et le VEGF (Allaman et al. 2011; Kajitani et al. 2012).

Le FGF-2 est impliqué dans les mécanismes d'apprentissage et de mémoire, mais également dans la neurogenèse hippocampique (Zechel et al. 2010). Cette neurotrophine, localisée dans les neurones et les astrocytes, pourrait aussi jouer un rôle dans l'étiologie de la dépression et l'activité des antidépresseurs. Des études *in vitro* et *in vivo* révèlent que certains antidépresseurs stimulent l'expression de FGF-2 neuronale et astrocytaire (Evans et al. 2004; Bachis et al. 2008; Allaman et al. 2011; Kajitani et al. 2012).

Enfin, le GDNF est clairement impliqué dans la survie des neurones dopaminergiques et dans le guidage et la croissance de leurs axones (Airaksinen et al. 2002; Paratcha et al. 2008). L'implication de cette neurotrophine dans la dépression reste toutefois controversée. En

effet, bien que de nombreuses études chez l'animal soulignent son rôle dans l'activité des antidépresseurs, rares sont les études cliniques ayant démontré son implication dans la dépression (Uchida et al. 2011; Kajitani et al. 2012).

e) Les médiateurs inflammatoires.

La microglie est considérée comme le type cellulaire participant aux réponses immunitaires et inflammatoires du SNC. Toutefois, des études cliniques et précliniques suggèrent que les astrocytes jouent également un rôle clé dans ces processus (Sofroniew et al. 2009; Najjar et al. 2013). Par exemple, les astrocytes sécrètent des cytokines pro-inflammatoires, tels que les interleukine 6 et 1 (IL-6/IL-1), le tumor necrosis factor α (TNF α), l'interferon β (IFN β) mais également des agents anti-inflammatoires comme l'IL-4, IL-5 et l'IL-10 (Najjar et al. 2013) ou encore le transforming growth factor β (TGF β) qui régule la prolifération et l'activation des cellules immunitaires comme les lymphocytes (Trajkovic et al. 2004; Farina et al. 2007). Enfin, les cellules astrogliales produisent des chimiokines (le chemokin ligand (CCL) 2, CCL5, CCL20, CXCL8 et CXCL10) responsables du recrutement des cellules immunitaires lors du processus inflammatoire (Farina et al. 2007). De nombreuses évidences suggèrent un lien entre inflammation et dépression, et notamment le fait qu'une stimulation des processus inflammatoires prédisposerait à l'apparition de troubles dépressifs (Dantzer et al. 2008; Leonard et al. 2009; Anisman and Hayley, 2012). Ainsi, différentes études cliniques démontrent une augmentation d'IL-6 et TNF α chez des patients dépressifs (Janelidze et al. 2011; Liu et al. 2012) tandis qu'un traitement chronique d'antidépresseurs permet de normaliser ces taux (McNally et al. 2008). Cette hypothèse est corroborée par des études démontrant une diminution des symptômes dépressifs chez des patients traités par un inhibiteur de la cycloxygénase-2 (Cox-2) (Müller et al. 2006; Akhondzadeh et al. 2009). Enfin, des expériences *in vivo*, utilisant des modèles animaux de neuro-inflammation, révèlent que les ISRS sont capables de réduire le processus de gliose et l'expression des ARNm codant pour des facteurs pro-inflammatoires tels que COX-2, IL-1 et TNF α (Jin et al. 2009; Lim et al. 2009) (Figure 4) suggérant qu'une partie des effets thérapeutiques des ISRS passerait par la normalisation de l'activité astrocytaire, et notamment le blocage de leurs propriétés pro-inflammatoires.

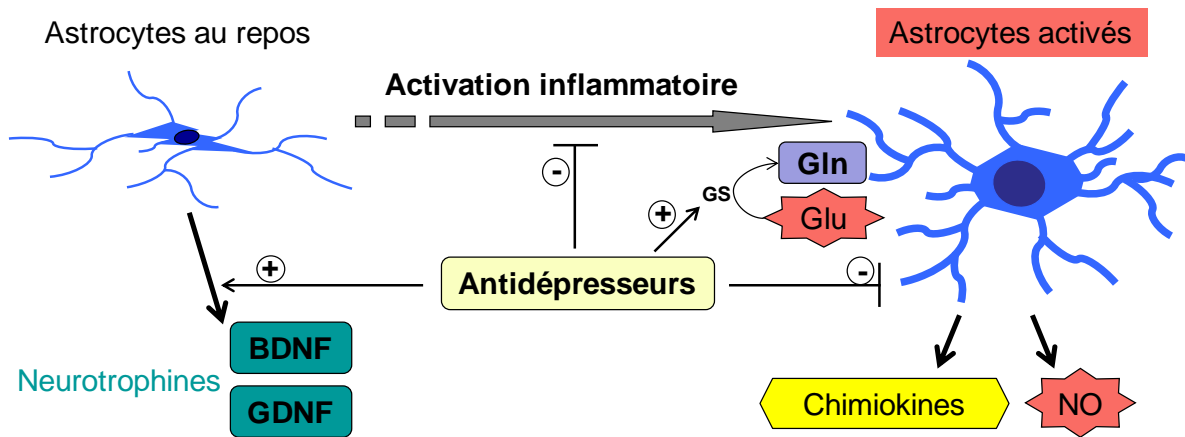


Figure 4 : Schéma des effets présumés des antidépresseurs sur l'activité astrocytaire lors du processus inflammatoire.

Adapté d'après Hashioka et al. 2013

Les antidépresseurs peuvent exercer des effets anti-inflammatoires à travers l'inhibition du processus de gliose induit lors de l'inflammation. Les antidépresseurs peuvent également induire des effets anti-neurodégénératifs à travers la stimulation de la libération de facteurs neurotrophiques astrocytaires et de l'activité de la glutamine synthase (GS), prévenant ainsi les effets excitotoxiques du glutamate. Gln : glutamine ; Glu : glutamate.

L'ensemble des données bibliographiques exposées jusqu'ici souligne la capacité des astrocytes à synthétiser et/ou libérer des substances neuroactives permettant à ces cellules de répondre à l'activité synaptique et de moduler la neurotransmission. Le prochain chapitre a pour but de rappeler que ces cellules sont organisées en réseaux, reposant sur l'expression de protéines appelées connexines (Cxs).

B. Les réseaux astrocytaires.

A l'instar des neurones, les astrocytes possèdent également leur propre réseau, leur permettant de communiquer entre eux. C'est au début des années 50, avec l'avènement de la microscopie électronique, que l'on observa pour la première fois la communication intercellulaire entre astrocytes. A la différence de la communication neuronale basée sur la propagation de signaux électriques, la communication astrocytaire repose sur la diffusion de molécules chimiques à travers des jonctions dites communicantes.

a) Les jonctions communicantes.

Les jonctions communicantes (JC) sont issues de l'apposition de deux membranes plasmiques séparées par une fente de 2 à 3 nm où l'on observe la présence de nombreux canaux. Cette organisation porte le nom de plaque jonctionnelle. Des études de microscopie électronique et de

cryofracture ont ainsi permis d'observer la présence au niveau de ces plaques, de plusieurs milliers de canaux intercellulaires (Lauf et al. 2002). Ces canaux sont ainsi formés par la juxtaposition de structures appelées connexons, ou hemi-canaux, présents sur chacune des membranes se faisant face (Figure 5). Les connexons sont eux-mêmes formés de 6 protéines transmembranaires appelées connexines (Cxs). Les hemi-canaux peuvent ainsi être formés de connexines identiques (homodimères) ou différentes (hétérodimères). L'arrangement de ces protéines permet la création d'un canal hydrophile de 1,5 à 2 nm de diamètre au centre du connexon (Meda et al. 1996). Les connexines sont des protéines possédant 4 domaines transmembranaires ainsi que 2 boucles extracellulaires et une boucle intracellulaire (Figure 5). Le domaine C-terminal et la boucle intracellulaire des connexines modulent l'ouverture et la fermeture du canal (Sosinsky and Nicholson, 2005). À ce jour, on dénombre 21 gènes codant pour les Cxs chez l'Homme et 20 chez la souris. On retrouve dans les astrocytes majoritairement les Cxs 30 et 43 (Giaume et al. 2010). Chez l'Homme il existe des polymorphismes de la Cx 43, notamment la mutation GJA1 (Paznekas et al. 2009) mais aucun lien n'a encore été établi entre ces SNPs et des pathologies psychiatriques.

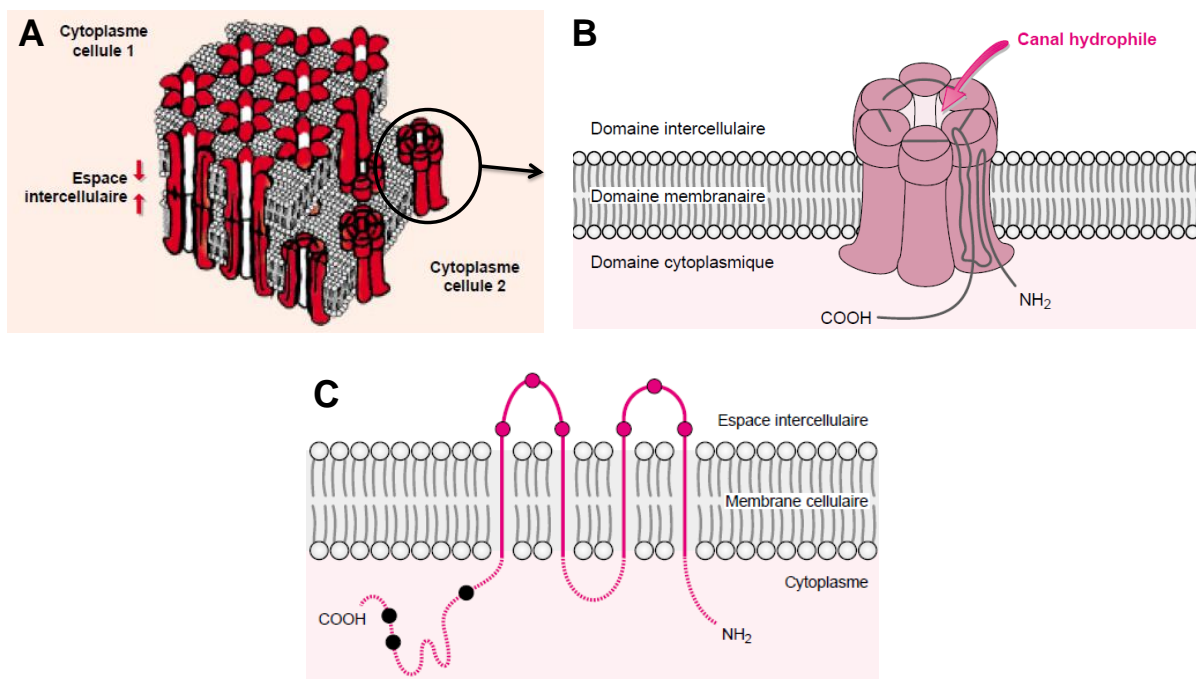


Figure 5 : Représentation schématique de l'organisation et de la structure des jonctions communicantes astrocytaires.

D'après Meda et al. 1996

A) Représentation schématique de l'organisation des plaques communicantes entre deux astrocytes. Ces plaques sont constituées de l'association de plusieurs jonctions communicantes, elles mêmes formées par la juxtaposition de structures appelées connexons, ou hemi-canaux, présents sur chacune des membranes se faisant face. B) Les connexons sont constitués de 6 sous-unités appelées connexines. C) Les connexines sont des protéines possédant 4 domaines transmembranaires ainsi que 2 boucles extracellulaires et une boucle intracellulaire. La liaison entre connexons repose sur la formation de ponts disulfures entre les chaînes extracellulaires des connexines.

La fonction principale des jonctions communicantes est la diffusion d'ions et de petites molécules ayant un poids moléculaire inférieur à 1,2 KDa. Ce sont les propriétés des connexines qui permettent une diffusion sélective des molécules à travers les JC (Giaume et al. 2010). Ainsi dans les astrocytes, les Cxs 30 et 43 sont sélectives pour de nombreuses molécules endogènes telles que des seconds messagers (AMPC, IP3 and Ca^{2+}), des acides aminés (glutamate, aspartate), des nucléotides (ADP, ATP, NAD), mais aussi des métabolites énergétiques (glucose, glucose-6-phosphate, lactate). Les états d'ouverture et de fermeture des canaux sont régulés par de nombreuses molécules comme les ions, certains seconds messagers, les neurotransmetteurs (glutamate et noradrénaline) (Giaume et al. 1991; De Pina-Benabou et al. 2001; Rouach et al. 2002; Rouach et al. 2008) mais également par des modifications post-traductionnelles telle que leurs phosphorylations. Ainsi, la Cx 43 peut être inactivée par phosphorylation, ce qui induit le découplage des cellules et bloque la propagation de signaux intracellulaires à travers les JC (Zlomuzica et al. 2012).

Les propriétés des Cxs 30 et 43 confèrent aux JC de nombreuses fonctions dans le réseau astrocytaire. Ainsi, les JC sont impliquées dans la propagation des vagues calciques intracellulaires via la diffusion de Ca^{2+} et d'IP3 (Scemes and Giaume, 2006). Des études ont également démontré l'importance de ces structures et des Cxs 30/43 dans le métabolisme cérébral. En effet, en assurant le passage de glucose et de lactate entre les astrocytes, les JC participent à un approvisionnement en énergie plus large et plus étendu aux neurones (Taberner et al. 1996; Rouach et al. 2008).

b) Les héli-canaux.

En plus d'être impliquées dans la formation de JC, les Cxs 30 et 43 participent à la mise en place d'héli-canaux. En effet, ces canaux sont organisés sur le même principe que les connexons des JC, c'est-à-dire un assemblage de 6 sous-unités organisées autour d'un canal hydrophile mais ne se connectent pas à un connexon d'une cellule voisine (Bennett et al. 2003; Sáez et al. 2003) (Figure 6). Il existe deux types de protéines permettant la formation d'héli-canaux : les Cxs (notamment l'isoforme 43) et les pannexines. Ces dernières ont une topologie très proche de celle des Cxs mais elles ont la particularité de ne pouvoir former que des héli-canaux (Scemes et al. 2009; Sáez et al. 2010).

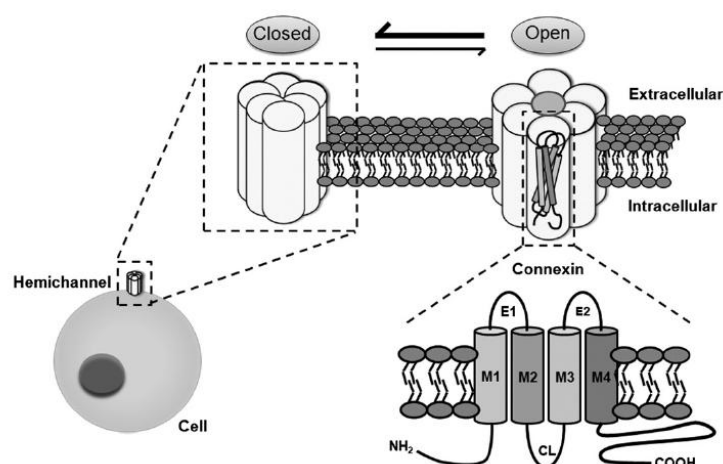


Figure 6 : Représentation schématique de l'organisation structurale des héli-canaux.

D'après Sáez et al. 2010

A l'instar des connexons constituant les jonctions communicantes, les héli-canaux sont formés de 6 sous-unités organisées autour d'un canal hydrophile. Les héli-canaux peuvent être formés de sous-unités de pannexines ou connexines, deux protéines ayant une topologie très proche. Les héli-canaux permettent la communication entre le compartiment intracellulaire des astrocytes et le milieu extracellulaire.

Les héli-canaux sont principalement impliqués dans la capture et la libération de molécules. Des études montrent que ces héli-canaux sont très peu perméables et permettent la libération d'ATP et de glutamate, induisant à leur tour des effets paracrines ou autocrines (Stout et al. 2002; Sáez et al. 2003; Spray et al. 2006; Iglesias et al. 2009). L'ouverture de ces canaux est contrôlée par de nombreux facteurs, incluant la concentration extracellulaire de calcium ($[Ca^{2+}]_{ext}$) et l'état de phosphorylation des Cxs (Sáez et al. 2003). De plus, il a été observé que certaines conditions pathologiques induiraient l'ouverture des héli-canaux, occasionnant des effets délétères pour la survie cellulaire probablement via la libération de facteurs pro-inflammatoires ou de glutamate dont l'augmentation des concentrations peut être excitotoxique (Sáez et al. 2010).

c) Les outils permettant d'étudier le rôle des connexines 30 et 43.

1. Les stratégies pharmacologiques.

Divers composés pharmacologiques sont capables de moduler l'activité des JC. Les agents les plus utilisés sont des bloqueurs de JC, comprenant des molécules telles que la quinidine, la mefloquine, l'heptanol, l'octanol, l'anandamide, l'oleamide et le carbenoxolone (Juszczack and Swiergiel, 2009). Le carbenoxolone, qui est le bloqueur le plus utilisé, est un dérivé d'hémisynthèse de l'acide glycérrhétique (ou acide glycérrhizinique) bloquant rapidement, et de manière réversible, les JC de cellules en culture (Blomstrand et al. 2004). Ainsi, l'emploi de ces composés dans des études *in vitro* a récemment permis de démontrer l'importance des JC dans la synchronisation et la plasticité synaptique. Cependant, bien que les effets de ces molécules soient bien détaillés dans les études *in vitro*, leur utilisation *in vivo* est beaucoup moins documentée. Ceci peut s'expliquer par le fait que ces

produits possèdent une faible sélectivité vis-à-vis des différentes Cxs et qu'ils induisent de nombreux effets secondaires notamment au niveau des organes vitaux périphériques (Hervé and Sarrouilhe, 2005; Juszczack and Swiergiel, 2009). Une autre limite du carbenoxolone dans les études *in vivo* vient du fait que l'intérêt de son administration systémique est limité par la faible perméabilité de la BHE (Leshchenko et al. 2006) et que la micro-infusion de ce composé au niveau cérébral induit des effets toxiques sur les mitochondries (Pivato et al. 2006).

Des peptides ont été développés pour bloquer de manière rapide et réversible les canaux connexines (Evans and Leybaert, 2007). Parmi ces peptides, on retrouve les gap26 et gap27 qui se fixent sur les boucles extracellulaires des Cxs 37, 40 et 43, diminuant ainsi la conductance des canaux (Evans and Leybaert, 2007). Une étude récente a également caractérisé un peptide TAT-Cx43L2 dirigé contre la boucle intracellulaire de la Cx 43 comme bloqueur de cette protéine (Stehberg et al. 2012) et dont la particularité serait de bloquer sélectivement les hémicanaux sans interférer sur les JC (Iyyathurai et al. 2013).

Enfin, il existe des outils pharmacologiques permettant l'étude des Cxs 30 et 43, tels que les inducteurs d'ouverture des JC. La triméthylamine, qui est un inducteur d'ouverture des JC, a notamment été utilisée dans l'étude du rôle des JC dans la synchronisation de l'activité du réseau neuronal et dans la plasticité synaptique au niveau de l'hippocampe (Chepkova et al. 2008; Bocian et al. 2011).

2. Stratégies génétiques.

Les modèles génétiques, et notamment les souris transgéniques, permettent d'étudier le rôle des Cxs 30 et 43 *in vivo*. Les stratégies génétiques présentent l'avantage de pouvoir étudier spécifiquement chacune des Cxs dans des fonctions cérébrales supérieures du SNC. Ainsi, des études *in vivo* révèlent par exemple une augmentation du phénotype anxieux chez les souris knock-out (KO) constitutive pour la Cx 30 (Cx30^{-/-}) (Dere et al. 2003). De manière intéressante et en adéquation avec ces observations, la délétion constitutive de la Cx 43 (Cx43^{-/-}) induit des malformations cardiaques provoquant la mort prématurée des nouveau-nés (Reaume et al. 1995) en rapport avec une diminution du couplage des JC et de la propagation des vagues calciques (Naus et al. 1997; Scemes et al. 1998). Des souris KO conditionnelles, comme par exemple les souris KO pour la Cx 43 spécifiquement dans les astrocytes (Cx43^{fl/fl}:GFAP-Cre) sont disponibles et permettent d'étudier de manière plus précise l'impact de la Cx 43 dans différentes fonctions cérébrales *in vivo* (Theis et al. 2003; Wallraff et al. 2006). Enfin, des souris double KO pour les Cx 30 et 43 (Cx30^{-/-} / Cx43^{fl/fl}:GFAP-Cre) ont été créées et des études révèlent une diminution du couplage astrocytaire et de la clairance du potassium extracellulaire au niveau de l'hippocampe chez ces animaux (Wallraff et al. 2006). Il est important de souligner qu'une des limites de ces souris réside dans le fait que la mutation affecte l'ensemble du cerveau.

3. Stratégies virales.

L'avènement des techniques de transfection virale a permis l'élaboration de nouvelles approches plus spécifiques permettant l'étude des Cxs 30 et 43 dans des régions spécifiques du cerveau. Ainsi, l'utilisation d'ARN interférant (ARNsi) a permis la création de vecteurs viraux permettant le blocage de l'expression de l'ARNm codant pour différentes Cxs et notamment la Cx 43 astrocytaire (Cx43-ARNsi) (Jacobas et al. 2008). Des études *in vitro* utilisant le Cx43-ARNsi mettent en évidence le rôle de la connexine 43 dans la prolifération astrocytaire et la capture du glucose (Herrero-Gonzalez et al. 2009) mais également dans l'organisation du cytosquelette (Olk et al. 2010). Une des perspectives intéressantes de l'utilisation de la stratégie virale serait la combinaison de vecteurs viraux et d'animaux transgéniques. En effet, à la lumière de ces données, il serait intéressant d'injecter, de manière locale dans différentes régions cérébrales, un virus AAA-GFAP-Cre chez des souris Cx43^{fl/fl} afin de contrôler l'inactivation temporelle et spatiale de la Cx 43 dans les astrocytes, un aspect qui n'est malheureusement pas possible avec les souris Cx30^{-/-} et/ou Cx43^{fl/fl}:GFAP-Cre.

Les astrocytes organisés en réseaux grâce aux Cxs sont impliqués dans de nombreux processus physiologiques laissant supposer qu'une atteinte de l'activité de ces cellules pourrait engendrer des perturbations du fonctionnement neuronal (excitabilité pré- et post-synaptique, neurotransmission,...) et être ainsi à l'origine de certains troubles du SNC, tels que des troubles de la cognition et des émotions mobilisant l'hippocampe.

C. Astrocytes, physiopathologie de la dépression et traitements antidépresseurs.

Il est désormais admis que l'exposition à un stress chronique prédispose à l'apparition d'épisodes dépressifs majeurs. Parallèlement à cette observation, plusieurs études démontrent des modifications des cellules gliales chez les patients déprimés mais également dans différents modèles animaux de la pathologie. De manière remarquable, certaines de ces anomalies peuvent être corrigées par des traitements antidépresseurs.

a) La dépression.

1. Modifications anatomiques.

Des analyses réalisées dans différents modèles animaux de dépression révèlent une diminution de la densité astrocytaire dans le cortex préfrontal, l'hippocampe et l'amygdale (Czeh et al. 2006; Banasr and Duman, 2007; Banasr et al. 2007; Czeh et al. 2007; Gosselin et al. 2009; Banasr et al. 2010). En accord avec un lien possible entre astrocyte et dépression, il a été observé que

l'administration d'une gliotoxine, la L-alpha-aminoadipic acid (L-AAA) dans le cortex de rat, induit une diminution de la densité de cellules GFAP-positives (GFAP pour "glial fibrillary associated protein", protéine fibrillaire associée à la glie) associée à un phénotype de type dépressif chez le rat (Banasr et al. 2008). Chez l'Homme, plusieurs études post-mortem rapportent également une diminution de la densité et du nombre de cellules gliales chez des patients souffrant de dépression majeure. Cette perte de cellules concerne les mêmes régions que celles décrites plus haut chez le rongeur (Ongur et al. 1998; Rajkowska et al. 2001; Bowley et al. 2002; Cotter et al. 2002). Des études morphométriques indiquent que la taille et la forme des cellules gliales sont aussi affectées chez des sujets dépressifs (Rajkowska et al. 1999). De manière intéressante, chez les patients âgés, l'analyse morphométrique ne révèle aucune modification de la densité astrocytaire (Khundakar et al. 2011a; Khundakar et al. 2011b; Schroeter et al. 2011), probablement en raison du fait que la densité astrocytaire augmente au cours du temps (Michel-Hidalgo et al. 2000), compensant ainsi une éventuelle perte liée à l'état dépressif.

2. Modifications fonctionnelles.

D'autres analyses post-mortem chez des patients dépressifs ont mis en évidence une diminution de l'expression de la GFAP, du transporteur du glutamate exprimé par les astrocytes et de la glutamine synthétase (GS) (Si et al. 2004; Choudary et al. 2005; Bernard et al. 2011). Des études d'imagerie ont en effet permis d'identifier un dysfonctionnement de la neurotransmission glutamatergique au cours d'épisodes dépressifs (Auer et al. 2000) suggérant ainsi que les anomalies structurelles des cellules gliales sont également associées à des modifications fonctionnelles chez les patients dépressifs.

Les astrocytes jouent un rôle clé dans le métabolisme et la neurotransmission glutamatergique, mais aussi GABAergique. Les modifications fonctionnelles induites par un stress chronique peuvent influencer la neurotransmission synaptique. C'est ainsi que de nouvelles hypothèses concernant la physiopathologie de la dépression ont vu le jour (Sapolsky, 2000; Rajkowska and Miguel-Hidalgo, 2007; Hashimoto, 2011). Ces postulats ont été étayés par l'observation d'une augmentation des concentrations de glutamate au sein du cortex préfrontal des patients dépressifs (Kugaya and Sanacora, 2005; Hashimoto et al. 2007). Ainsi, l'implication des cellules gliales dans la modulation des fonctions de ces neurotransmetteurs semble être particulièrement importante dans la régulation de l'humeur (Hertz et al. 1999; Schousboe and Waagepetersen, 2006). En effet, l'altération des fonctions gliales (diminution du transporteur du glutamate, altération du cycle glutamate-glutamine) pourrait être associée à une augmentation du taux de glutamate extracellulaires responsables d'effets excitotoxiques (Soriano and Hardingham, 2007). Ces effets pourraient alors contribuer aux altérations morphologiques observées dans les régions limbiques chez les patients déprimés.

Comme nous l'avons vu précédemment, les astrocytes sont impliqués dans la régulation du métabolisme cérébral. Des observations récentes montrent une altération du métabolisme du glucose chez des patients souffrant d'épisodes dépressifs majeurs (Drevets et al. 2000a; Drevets et al. 2000b). Les régions cérébrales les plus affectées par ces modifications sont le cortex préfrontal, le cortex cingulaire antérieur, l'amygdale et l'hippocampe (Drevets et al. 1998; Drevets et al. 2000a; Drevets et al. 2000b; Videbech et al. 2000; Drevets et al. 2008). De plus, ces mêmes études révèlent une corrélation entre la sévérité de la dépression (calculée grâce à l'échelle d'Hamilton) et l'altération de la capture du glucose sanguin dans certaines de ces régions et plus particulièrement dans l'amygdale (Drevets et al. 1999).

L'intégrité du réseau astrocytaire pourrait jouer un rôle majeur dans la physiopathologie de la dépression. En effet, différentes données précliniques suggèrent l'implication des Cxs 30 et 43 dans la régulation du stress et de l'humeur. En effet, un phénotype de type anxieux a été observé chez des souris KO pour la Cx 30 (Dere et al. 2003). D'autres études *in vivo* démontrent que l'exposition à un stress diminue l'expression des Cxs 43 dans l'hippocampe et le couplage astrocytaire (Irwin et al. 2001; Sun et al. 2012) tandis que l'infusion locale (cortex préfrontal) de carbenoxolone ou des peptides gap26 et gap27 induit l'apparition de symptômes de la dépression (Sun et al. 2012). De manière contradictoire d'autres observations révèlent que la délétion conditionnelle de la Cx 43 astrocytaire chez la souris induirait un effet de type anxiolytique (Frisch et al. 2003; Theis et al. 2003) ce qui laisse penser que des caractérisations plus approfondies du phénotype de ces souris sont nécessaires. De manière intéressante, une étude récente démontre que les effets psychostimulants du modafinil, un inhibiteur de la recapture de la dopamine (entre autre), passe par la stimulation de l'expression (protéine et ARNm) de la Cx 43 au niveau cortical (Liu et al. 2013).

L'implication des Cxs et des JC dans le processus de neurogenèse hippocampique chez l'adulte a été abordée (Kunze et al. 2009). Dans ce travail, les auteurs démontrent que les cellules souches hippocampiques (la glie radiale) sont connectées entre elles via des JC issues des Cxs 43 et que l'inactivation génétiques des Cxs 30 et 43 chez les souris $Cx30^{-/-}/Cx43^{fl/fl};GFAP-Cre$, bloque la prolifération cellulaire et diminue le nombre de cellules souches et de cellules granulaires (Kunze et al. 2009). De plus, dans cette études les auteurs observent également une diminution du processus de neurogenèse hippocampique suite au blocage de l'expression des Cxs 30 et 43 au niveau de l'hippocampe grâce à l'injection intra-hippocampique d'un virus exprimant Cre chez des souris $Cx30^{-/-}/Cx43^{fl/fl}$ (Kunze et al. 2009). Ce rôle des Cxs 43 dans la régulation de la neurogenèse hippocampique chez l'adulte renforce l'idée de l'implication de cette Cx dans la régulation du phénotype anxio-dépressif des souris et probablement la réponse aux antidépresseurs.

En accord avec les données précliniques, des études récentes chez l'Homme démontrent une diminution de l'expression des Cxs 30 et 43, dans le cortex préfrontal de patients suicidés (Ernst et al. 2011).

Enfin, les astrocytes participent à la libération de facteurs neurotrophiques nécessaires à la prolifération, maturation et survie neuronale, mais également au maintien de l'activité synaptique. Compte tenu du rôle du stress sur la neurogenèse hippocampique (Hanson et al. 2011), il est tentant de penser que la diminution d'astrocytes pourrait participer à l'apparition des symptômes dépressifs suite à une diminution de la libération de facteurs neurotrophiques. Le lien entre Cxs et la libération de neurotrophines constitue également une piste intéressante qui reste à être explorée.

b) Les traitements antidépresseurs.

1. Modifications anatomiques.

Chez le rongeur, un traitement chronique à la fluoxétine corrige l'effet délétère du stress sur le nombre et la morphologie des astrocytes dans l'hippocampe (Czeh et al. 2006; Cabras et al. 2010). De même chez l'animal, la fluoxétine stimule le processus de gliogenèse dans le cortex préfrontal (Czeh et al. 2007). Ces propriétés ne sont pas exclusives des stratégies antidépressives pharmacologiques mais peuvent également être retrouvées lors de traitements physiques telles que la thérapie électroconvulsive (ECT), la stimulation magnétique transcraniale (TMS), la stimulation du nerf vague (VNS) et la stimulation cérébrale profonde (DBS) (Oosterhof et al. 2013). En effet, une étude récente *in vivo* chez le rat montre que les électrochocs induisent une modification de la morphologie des astrocytes hippocampiques, notamment un raccourcissement et un affinement de leurs processus (Jansson et al. 2009).

2. Modifications fonctionnelles.

Des études *in vitro*, utilisant des cultures primaires d'astrocytes, rapportent qu'une administration de fluoxétine ou de paroxétine, stimule le processus de glycogénolyse et la libération de lactate (Zhang et al. 1993; Allaman et al 2011). Lors d'un stress, ce processus pourrait contribuer à restaurer un métabolisme cérébral déficient et ainsi normaliser l'activité neuronale. Ces études *in vitro* révèlent également que cette administration d'ISRS stimule la synthèse de BDNF, VGF et VEGF par les astrocytes (Allaman et al. 2011; Martin et al. 2013). De plus, des études précliniques montrent une restauration de l'expression de GFAP (ARNm et protéine) mais également du transporteur du glutamate et de la glutamine synthase en réponse à une traitement chronique d'antidépresseurs chez le rat (Czeh et al. 2006; Liu et al. 2009). L'ensemble de ces données suggère que ces traitements agissent sur les astrocytes en renforçant leur rôle de support pour l'activité neuronale et synaptique.

Le renforcement de ce support pourrait reposer sur une amélioration du réseau astrocytaire puisqu'une étude récente révèle que les antidépresseurs tels que la fluoxétine et la duloxétine augmentent l'expression des Cxs 30 et 43 (Sun et al. 2012). Enfin, les astrocytes semblent

participer aux effets thérapeutiques des traitements antidépresseurs non pharmacologiques tels que les ECT. Par exemple, la DBS module le débit sanguin cérébral au niveau local (Perlmutter et al. 2002; Kefalopoulou et al. 2010). En raison du rôle des astrocytes dans la régulation de ce processus, ces données suggèrent que la DBS influencerait sur le débit sanguin cérébral via la stimulation de l'activité astrocytaire (Vedam-Mai et al. 2012). De plus, une étude *in vivo*, utilisant la microscopie deux photons, montre que la DBS induit une augmentation $[Ca^{2+}]_i$, conduisant à la libération d'ATP/adénosine dans le thalamus (Bekar et al. 2008) mais également de glutamate (Tawfik et al. 2010), deux processus qui pourraient contribuer à ces effets bénéfiques dans la population de patients résistants aux ISRS.

L'ensemble de ces données suggère un rôle possible des astrocytes dans la modulation de la neurotransmission monoaminergique basale ou stimulée par les thérapies antidépressives. Le prochain chapitre de cette thèse est constitué de la revue 1 de ce mémoire bibliographique, synthétisant les évidences scientifiques laissant entrevoir que le modèle de synapse tripartite, régulièrement associé au glutamate, pourrait être transposé aux monoamines et de ce fait être la cible des traitements antidépresseurs actuellement disponible.

D. Astrocytes et systèmes monoaminergiques.

Revue 1

The monoaminergic tripartite synapse: a putative target for currently available antidepressant drugs

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Current Drug Targets, Epub2013

RÉSUMÉ :

La dépression communément associée à une diminution de la neurotransmission sérotoninergique, à une baisse de l'expression de facteurs neurotrophiques et à une diminution du processus de neurogenèse dans l'hippocampe mature, pourrait également résulter d'une diminution de la densité, du nombre et de l'activité des astrocytes.

Différentes études *in vitro* suggèrent que les astrocytes seraient capables de moduler la neurotransmission monoaminergique, en raison de l'expression des transporteurs sérotoninergiques, noradrénergiques et dopaminergiques, sur ces cellules gliales. Par ailleurs, les astrocytes sont sensibles aux variations extracellulaires des monoamines puisqu'ils expriment également à leur surface différents sous-types de récepteurs dont la stimulation entraîne des cascades de signalisations intracellulaires responsables de la synthèse/libération de substances neuroactives.

Cette revue bibliographique apporte les éléments supposant qu'une partie des propriétés thérapeutiques des antidépresseurs pourrait passer par une activation des propriétés de supports nutritifs et neurotrophiques des astrocytes aux neurones de manière à améliorer la communication entre ces cellules. Les évidences *in vivo* de telles propriétés doivent toutefois être démontrées notamment sur la synthèse/libération de BDNF, un facteur largement décrit pour son rôle dans la réponse aux antidépresseurs appartenant à la classe des inhibiteurs sélectifs de recapture de la sérotonine (ISRS).

The Monoaminergic Tripartite Synapse: A Putative Target for Currently Available Antidepressant Drugs

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Abstract: Antidepressant drugs such as the serotonin (5-HT)/norepinephrine (NE) and dopamine (DA) reuptake inhibitors activate monoaminergic neurotransmission in various brain regions, such as the amygdala, the frontal cortex or the hippocampus. Although this property is well established, the post-synaptic mechanisms by which these pharmacological agents exert therapeutic activity in major depressive disorders (MDD) is not fully understood. Recent clinical and preclinical studies have indicated that the density and reactivity of glia and more particularly of astrocytes is reduced in MDD patients. These data along with the fact that astrocytes express monoaminergic transporters and receptors make these cells putative targets for antidepressant treatments. Accordingly, *in vitro* evidence has demonstrated that the application of various classes of antidepressant drugs on rodent primary astrocyte cultures elicits a wide spectrum of responses, from the rise in cytosolic calcium concentrations, as a marker of cellular activity, to the release of glucose metabolites, gliotransmitters and neurotrophic factors. Remarkably, antidepressant drugs also attenuate the release of inflammatory molecules from reactive astrocytes or microglia, suggesting that part of the beneficial effects in depressed patients or animal models of depression might result from the ability of antidepressants to regulate the synthesis and release of psychoactive substances acting on both pre- and post-synaptic neurons. Among the many long-term targets of antidepressant drugs, brain-derived neurotrophic factor (BDNF) has been well studied because of the positive influence on adult hippocampal neurogenesis, synaptogenesis and the local serotonergic tone. This review will illustrate how the concept of the tripartite synapse, which is classically associated with different forms of plasticity involving glutamate, could be expanded to the monoaminergic systems to regulate antidepressant drug responses. The recent *in vivo* data supporting that hippocampal astrocytes act in concert with neurons to release BDNF under pharmacological conditions and thereby regulating different facets of anxiolytic/antidepressant-like activities through neurogenesis-dependent and independent mechanisms will be emphasized.

Keywords: Antidepressants, astrocytes, depression, gliotransmitters, neurotrophic factors, tripartite synapse.

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) block serotonin (5-HT) reuptake, leading to the increase in synaptic 5-HT. This increase in synaptic 5-HT activates a feedback mechanism mediated by 5-HT_{1A} autoreceptors in the dorsal raphe (DR), which reduces 5-HT neuronal activity and limits the release of this monoamine at the nerve terminals in regions such as the amygdala (AMY), frontal cortex (FC) and hippocampus (HP). Long-term treatment with these antidepressant drugs desensitizes the inhibitory 5-HT_{1A} autoreceptors, and 5-HT neurotransmission is enhanced [1]. Similar observations have been made with noradrenergic and dopaminergic reuptake inhibitors, whose therapeutic activities also depend on the ability of these drugs to desensitize inhibitory α -₂ and D₂ autoreceptors located in the locus coeruleus (LC) and ventral tegmental area (VTA), respectively [2]. Despite these well documented properties, the post-synaptic mechanisms by which antidepressant drugs,

more particularly SSRIs, exert beneficial effects in depressed patients or relevant animal models remains largely unknown. In particular, the nature of the post-synaptic receptors and cell types targeted by monoamine reuptake inhibitors or monoamine oxidase blockers has yet to be understood.

There is increasing evidence that astrocytes play a significant role in major depressive disorders (MDD). A recent review by Rajkowska and Stockmeier (2013) [3] examined human post-mortem studies that demonstrated that MDD is associated with a decrease in the density of astrocytes in the AMY, FC or HP. Such a decrease has also been reported in rodents subjected to stressful conditions [4-6], whereas the gliotoxin L-alpha amino adipic acid-induced reduction in the number of glial fibrillary acidic protein (GFAP)-positive cells in the preFC, also produces behavioral anomalies indicative of a depressive-like phenotype [7]. Despite these findings, whether the modifications of astrocytic density and activity are the causes or consequences of chronic stress and related symptoms/disorders is still unclear. However, these findings raise the possibility that part of antidepressant drug activity could rely on the ability of these treatments to modulate astrocytes at different levels. Several arguments support this assumption. First, the identification of the monoaminergic

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gic transporters, catabolic enzymes and receptors in primary astrocyte cultures [8-16] makes these cells putative partners of neurons during acute and long-term antidepressant drug treatment. Second, *in vitro* studies have recently shown that SSRIs stimulate the synthesis of neurotrophic factors in cultured astrocytes [17]. These factors are necessary *in vivo* to stimulate hippocampal neurogenesis in the mature brain, a process that is specifically relevant to the behavioral effects of antidepressant drugs in rodents [18]. Finally, antidepressant drugs have been shown to reverse glial cell loss and atrophy induced by chronic stress [4, 5, 19], suggesting a non-neuronal mechanism of action of these pharmacological agents. These observations must be considered in the context of the "tripartite synapse" within the central nervous system (CNS) [20] in which astrocytes respond to intracellular calcium (Ca^{2+}) elevations during synaptic activity to regulate intrasynaptic monoamine levels under basal conditions or in response to antidepressant drugs. In addition to this property, astrocyte responses can be adapted to these changes by regulating the release of gliotransmitters, inflammatory molecules or neurotrophic/growth factors [21-23], which could influence the activity of pre- and post-synaptic neurons (paracrine mechanism) or astrocytes themselves (autocrine mechanism) to modify the emotional state.

The bidirectional dialogue between astrocytes and neurons is not restricted to the signaling exchange between both cell types within a single synapse and also includes the astrocytic modulation of relatively distant synapses through the intracellular signal spread within an astrocyte or the intercellular signaling among astrocytic networks. Astrocytes are connected to each other through gap junctions formed by the two main connexins (Cx) 30 and 43 that align between adjacent astrocytes to form intercellular channels [24, 25]. These Cx proteins mediate direct intercellular communication that involves the exchange of ions and small signaling molecules. Interestingly, Cx proteins that are not engaged in gap junctions can also operate as hemichannels, mediating the exchange between the astrocyte cytoplasm and the extracellular medium [26]. These proteins are important in neuroglial interactions and could be of particular interest in the context of MDD and related treatments. Consistent with this hypothesis, a new theory stipulates that the dysregulation of Cx expression in the astrocytic syncytium could account for psychiatric disorders [27]. A first echo in a recent preclinical study in the rat chronic unpredictable stress model (UCMS) showed that structural and functional alterations of astrocytic gap junctions is restored by chronic SSRI fluoxetine or SNRI duloxetine treatment [28]. In addition, Cx 43 function is decreased in this model, whereas the non-selective Cx 43 blocker carbenoxolone elicits depressive-like behaviors [28].

Given the above-mentioned anatomical, morphological and functional considerations, one may wonder whether astrocytic activation contributes to the therapeutic activity of the various classes of antidepressant drugs. To answer this question, the present review will explore the current knowledge about the monoaminergic key elements expressed by astrocytes and will describe the effects of antidepressant drugs on related intracellular signaling pathways. Also, this review will propose an overview of the influence of these medications on the synthesis and release of neuroactive sub-

stances by astrocytes, which could play an important role in the antidepressant response. At the end of this review, the example of the brain-derived neurotrophic factor (BDNF) is addressed to illustrate the first *in vivo* results supporting that astrocytes might participate in the synthesis and release of this factor to regulate the serotonergic neurotransmission and related behaviors. The experimental arguments in favor of 5-HT-mediated BDNF synthesis by astrocytes are based on data collected from cell cultures, and *in vivo* evidence is lacking.

1. ASTROCYTIC MONOAMINERGIC TRANSPORTERS

1.1. Monoamine Uptake1

Historically, the monoamine uptake1 site was the first type of monoamine transporter observed in primary astrocyte cultures [8, 29]. This reuptake mechanism is a high-affinity Na^{+} -dependent system that allows astrocytes to participate in the clearance of 5-HT, NE and DA in the synaptic cleft [29].

1.1.1. The Serotonin Transporter (SERT)

The first demonstration of the 5-HT uptake in astrocytes was in *in vitro* studies showing that such a process in GFAP-positive cells is Na^{+} -dependent and fluoxetine-sensitive [8]. Other studies have confirmed these initial observations by detecting SERT mRNA and protein in primary rodent astrocyte cultures [9, 30-35]. Additionally, antidepressant drugs, such as citalopram, clomipramine, fluoxetine, fluvoxamine, paroxetine and sertraline, have been shown to inhibit 5-HT uptake through the blockade of astrocytic SERT [9, 33, 36]. The finding that the primary sequence of glial SERT is similar to the neuronal homologue [9] strongly suggests that SSRIs do not necessarily discriminate between both cell types to increase extracellular 5-HT levels. Interestingly, antidepressant drugs have been shown to decrease the expression of SERT in the mouse and rat HP after prolonged administration [37-39]. Although, this mechanism is favorable to the enhancement of the serotonergic neurotransmission, the cell type in which such a functional adaptation would occur remains unknown.

1.1.2. The Norepinephrine Transporter (NET)

The expression of the norepinephrine (NE) transporters was also initially observed in primary cultures of rat astrocytes [40] and was further confirmed by RT-PCR and western-blot analyses [41]. Although the Na^{+} -dependent or -independent uptake of NE by astrocytes has led to heterogeneous results [32, 40, 42, 43], it is now well accepted that selective and non-selective NE reuptake inhibitors, such as nisoxetine, milnacipran or desipramine, inhibit the *in vitro* Na^{+} -dependent NE uptake from cultured rat astrocytes [41]. Remarkably, a heterologous uptake of DA from glial NET has been observed [44, 45], suggesting that the antidepressant activity of NE reuptake inhibitors lies, at least in part, in the ability to simultaneously inactivate catecholamine uptake from astrocytes.

1.1.3. The Dopamine Transporter (DAT)

In a marked contrast to the SERT and NET, the expression of the DAT on astrocytes is poorly documented. Recent

RT-PCR analyses in primary cultures of astrocytes suggest the presence of the DAT on this cell type but its expression would depend on the brain region from which the cell cultures derive [45, 46]. Moreover, although evidence suggests that astrocytes can uptake DA through a high-affinity Na⁺-dependent process [29, 40], other studies in rat primary cultures of astrocytes failed to unveil such a mechanism [42, 47, 48]. More recently, a study in cultured rat cortical astrocytes showed that the high-affinity DAT inhibitor GBR12935 had no effect on DA uptake [45], whereas nisoxetine or higher concentrations of GBR12935, which blocks both the DAT and NET, inhibit DA uptake₁ activity [44, 45]. These results point out the putative ability of astrocytic NET to uptake DA. Finally, it has been proposed that bFGF and EGF increase the DA uptake in rat astrocyte cultures, probably through the regulation of DAT expression [49]. This interesting finding warrants further investigation.

1.2. Monoamine Uptake₂

The uptake₂ transporters, such as the extraneuronal monoamine transporters (EMT), display distinct pharmacological profiles compared to the monoamine uptake₁ transporters and were identified several years after the monoamine uptake₁ [50, 51]. Also named organic cation transporters (OCTs), these transporters act through a Na⁺-independent, corticosterone-sensitive process [52] with transporters that have a low affinity but a high-capacity for monoamines in the brain [53, 54]. *In vitro*, functional studies have demonstrated the presence of OCT3 in rodent and human primary astrocyte cultures [41, 45, 49], as these transporter mediated-uptake₂ processes appear to be insensitive to antidepressant drugs, such as amitriptyline or fluoxetine [43]. However this notion has been recently challenged by studies suggesting that these transporters would be secondary targets of antidepressant drugs [54, 55]. A particular characteristic of OCT3 relies on the ability to uptake all three monoamines from non-neuronal cells [56, 57], not only in the FC or HP, but also in the main monoaminergic brain nuclei, including the DR or LC [58-60]. These observations open the way for new investigations aimed at determining the influence of OCT3 on the homeostasis of the monoaminergic systems and the regulation of OCT3 neuronal activities.

2. ASTROCYTIC MONOAMINERGIC CATABOLIC ENZYMES

Glia cells do not express tryptophan hydroxylase. However, the catabolic isoenzymes responsible for the degradation of monoamines (i.e. monoamine oxidase A and B) were clearly identified in this cell type. Several studies report the presence of both isoforms in cultures of astrocytes [47, 61, 62], suggesting that monoamine oxidase inhibitors could exert some therapeutic activity by acting on these cells. Catechol-*O*-methyltransferase (COMT) mRNA and protein, another metabolic enzyme regulating monoamine concentration, was also detected in astrocytes using microscopic, biochemical and pharmacological approaches [63-66]. These observations emphasize the fact that astrocytes can regulate the extracellular monoamine levels by modulating the expression and function of MAO-A, MAO-B and COMT.

3. ASTROCYTIC MONOAMINERGIC RECEPTORS, RELATED SIGNALING PATHWAYS AND ENERGETIC METABOLISM

Astrocytes express several monoaminergic receptor subtypes at the surface that make these cells sensitive to increases in the extracellular 5-HT, NE and DA levels in response to the pharmacological inactivation of the glial or neuronal monoaminergic transporters and catabolic enzymes.

3.1. Astrocytic 5-HT Receptors

3.1.1. The 5-HT₁ Receptor Subtypes

The 5-HT_{1A} receptor subtype has been detected in both cultured astrocytes and *in situ* in the HP or entorhinal cortex [67] (Table 1). Despite the controversial coupling of the 5-HT_{1A} receptor to adenylate cyclase in astrocytes [12, 34], several studies have shown that the 5-HT_{1A} receptor agonist 8-OHDPAT stimulates the synthesis and release of the Ca²⁺-binding protein s100beta from astrocytes [68-70] (Fig. 1). This protein regulates cell shape, energy metabolism, cell to cell communication and cell growth [71]. Remarkably, at low concentrations, s100beta positively regulates neuronal and astrocytic differentiation while reducing neuronal death and astrogliosis in response to brain injury [69, 70, 72, 73]. However, high concentrations of s100beta induce apoptosis, suggesting that the regulation of this neurotrophic factor by the 5-HT_{1A} receptor could play a significant role in both the physiopathology of MDD and the antidepressant activity of SSRIs [74].

3.1.2. The 5-HT₂ Receptor Subtypes

The presence of the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor subtypes in primary astrocyte cultures from various brain regions, including the HP [12, 75, 76], and also from *in situ* analyses [77] has been repeatedly documented. The 5-HT₂ receptors are G_{q/11} protein-coupled receptors (Table 1), and the activation of these receptors leads to the activation of the phospholipase C (PLC) production of diacylglycerol (DAG) and inositol triphosphate (IP3). The ultimate step of this intracellular cascade is an increase in cytosolic calcium concentrations ([Ca²⁺]_i). This signaling pathway is of particular importance because astrocyte excitability depends on this rise of [Ca²⁺]_i, and such an increase is the starting point to other events, such as the release of neuroactive substances. As expected, the stimulation of the 5-HT₂ receptors by 5-HT and the SSRIs fluoxetine or citalopram in rodent primary cultures of cortical astrocytes increases [Ca²⁺]_i and protein kinase C (PKC) activity, which is responsible for the extracellular signal-regulated kinase1/2 (ERK_{1/2}) activation [78, 79] (Fig. 1). In the dentate gyrus of the HP, ERK_{1/2} has been implicated in the regulation of mood as supported by the blunted activation and expression of ERK_{1/2} in a mouse model of depression [80]. Similarly, western-blot analyses showed decreased levels of ERK in the postmortem FC of individuals with MDD [81]. Because a downregulation of ERK_{1/2} has been associated with an impairment of the adult hippocampal neurogenesis during brain development [82], it is possible that such an effect in the adulthood could also dampen this process in the adult HP. Conversely, these results suggest that antidepressant drugs exert therapeutic activity by reversing these alterations. Consistent with this

Table 1. Serotonergic Receptor Subtypes Identified on Primary Cultures of Astrocytes.

Receptor	G Protein	Brain region	Experimental conditions	References
5-HT _{1A}	-	Hippocampus Entorhinal cortex	<i>In vitro</i> <i>In situ</i>	[12, 263] [67]
5-HT _{2A}	G _q	Cortex Striatum Hippocampus	<i>In vitro</i>	[75] [263]
5-HT _{2B}	G _q			[12, 263]
5-HT _{2C}	G _q			[34] [264]
5-HT ₄	G _s	Mesencephalon	<i>In vitro</i> <i>In vitro</i>	[86] [265]
5-HT _{5A}	G _i	-	<i>In vitro</i>	[87]
5-HT ₇	G _s	Frontal cortex Hypothalamus	<i>In vitro</i>	[89]
			<i>In situ</i>	[88]
			<i>In situ</i>	[12]

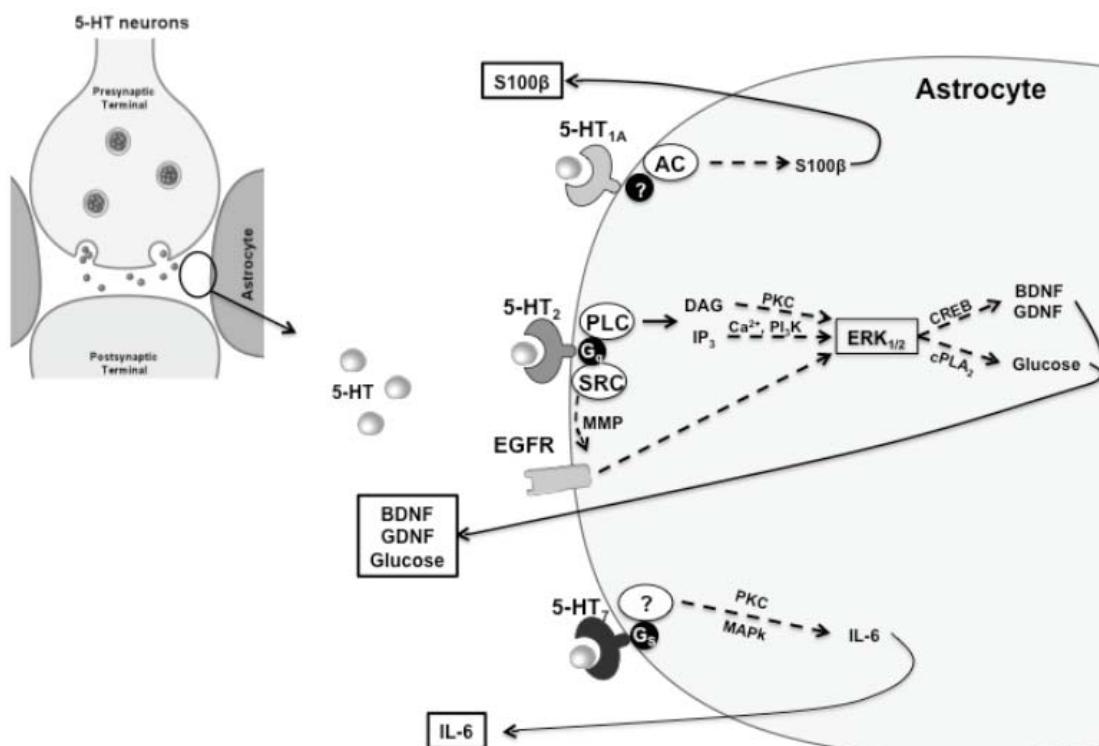


Fig. (1). Regulation of brain astrocytic activity by 5-HT and related receptor subtypes identified on this cell type.

assumption, the SSRI fluoxetine stimulates the astrocytic 5-HT_{2A} and 5-HT_{2B} receptors to promote the synthesis of fibroblast growth factor (FGF) and epidermal growth factor (EGF), two molecules known to favor ERK_{1/2} pathway in primary cultures of mouse astrocytes [79, 83]. In addition to these effects, the activation of the 5-HT₂ receptor types has been proposed to stimulate the synthesis glial-derived neurotrophic factor (GDNF) in C6 glioma cells, a model of astrocytes, and this effect could result from ERK phosphorylation/activation [84, 85]. As detailed in the next chapter of this review, all of these factors play a role in adult hippocampal neurogenesis, making these 5-HT₂ receptor subtypes

molecular elements potentially involved in the therapeutic action of serotonergic antidepressant drugs.

3.1.3. Other 5-HT Receptor Subtypes

Recent immunohistochemical studies using double immunostaining with antibodies directed against the GFAP demonstrate that astrocytes from mesencephalic rat cultures express the 5-HT₄ receptors [86] (Table 1). The 5-HT_{5A} mRNA and proteins have also been detected in primary astrocyte cultures [87], but the precise role of 5-HT_{5A} in these cells remains undetermined. Finally, growing evidence emphasizes the presence of 5-HT₇ receptors in rat cultured as-

trocytes from the FC, thalamus (TH) and hypothalamus (HYP) [12, 88-90] (Table 1). The 5-HT₇ receptors are positively coupled to AC through G_s proteins [12] (Table 1). Long-term treatment with tricyclics elicits a time- and concentration-dependent increase in the production of cAMP in rat cultured astrocytes, which is attenuated by a pretreatment with 5-HT₇ receptors antisense oligonucleotides [89]. Although the functional consequences of the activation of astrocytic 5-HT₇ receptors have been poorly documented, *in vitro* studies suggest that the 5-HT-induced release of the proinflammatory interleukin-6 (IL-6) in a human astrocytoma cell line is mediated by this receptor subtype [91] (Fig. 1).

3.2. Astrocytic Adrenoceptors

3.2.1. The Alpha Adrenoceptor Subtypes

In vitro and *in situ* experiments have demonstrated that both the α_1 - and α_2 -adrenoceptors are expressed on astrocytes from various brain regions [92-96]. In particular, α_1 -adrenergic receptors have been detected on astrocytes in the LC and DR and also at the monoaminergic nerve terminal in regions such as the FC and nucleus accumbens (NAcc) [97]. The stimulation of these receptors, through coupling with G_{q/11} proteins, activates PLC and the production of DAG and IP₃, which in turn increases the activity of PKC and the [Ca²⁺]_i concentration, respectively [16] (Table 2; Fig. 2). In the FC, the local release of NE induced by the electrical stimulation of the LC neurons increases astrocytic [Ca²⁺]_i [98]. The activation of the PKC favors the synthesis of some neurotrophic factors, including BDNF in rat neonatal cortical primary cultures of astrocytes [99, 100]. This synthesis might involve specific neurotransmitter systems because the cellular content of BDNF is upregulated by the catecholamines but not by the kainic acid. In a marked contrast, the stimulation of NGF synthesis is mediated by histamine

[100]. These data are of particular interest because these differences also exist between the levels of NGF and BDNF *in vivo*, with BDNF being the most abundant in the adult rat central nervous system [101, 102]. Evidence also demonstrates that the α_1 -adrenoceptors regulate the energetic metabolism of astrocytes because the stimulation of these receptors promotes astrocytic glycolysis, resulting in the synthesis of energetic substrates, such as ATP or lactate, which are necessary for the neuronal activity [103, 104]. The α_2 -adrenoceptors are G_{i/o} protein-coupled receptors that are negatively coupled to the AC [16]. The activation of these receptors decreases cAMP levels, leading to glucose incorporation into glycogen, whereas α_2 -adrenergic receptor antagonists increase glycogenolysis [96]. The α_2 -adrenoceptors are also coupled to the PKC through the $\beta\gamma$ subunit, inducing transient [Ca²⁺]_i in response to the glycogenolysis [105]. Given that astrocytes are the main cerebral source of glycogen and that the glycogenogenesis and glycogenolysis are key processes for the rapid production of energetic substrates [106-109], it is likely that the stimulation of the astrocytic α_1 - and α_2 -adrenoceptors plays a major role in the metabolism necessary for the neuronal activity.

3.2.2. The Beta Adrenoceptor Subtypes

In vitro and *in situ* studies have shown the presence of the β_1 -, β_2 - and β_3 -adrenoceptors mRNA in astrocytes [92, 96, 110-113]. These receptors were primarily described as being positively coupled to AC through G_s proteins [114] (Table 2), but recent data have demonstrated that the β_3 -adrenoceptors are coupled to AC through G_i proteins in CHO cells [115], chick astrocytes [116] and mice [113, 117]. All the three β -adrenoceptors are implicated in astrocytic energetic metabolism. For example, the β_1 -, β_2 - and β_3 -adrenoceptors are involved in the uptake of glucose from astrocytes [113, 116], and the β_1 - and β_2 -adrenoceptors

Table 2. Adrenoceptors Subtypes Identified on Primary Cultures of Astrocytes.

Receptor	G Protein	Brain region	Experimental conditions	References
α_1	G _q	Locus coeruleus Dorsal raphe Nucleus accumbens	<i>In vitro</i> <i>In situ</i> <i>In vivo</i>	[92] [97] [98] [95] [96]
α_2	G _{i/o} G $\beta\gamma$	Locus coeruleus	<i>In vitro</i> <i>In situ</i> <i>In vivo</i>	[92] [94] [105] [97] [98] [95] [96]
β_1 β_2 β_3	G _s	Cortex Hippocampus Hypothalamus	<i>In vitro</i> <i>In situ</i>	[92] [110] [111] [112] [96] [113]

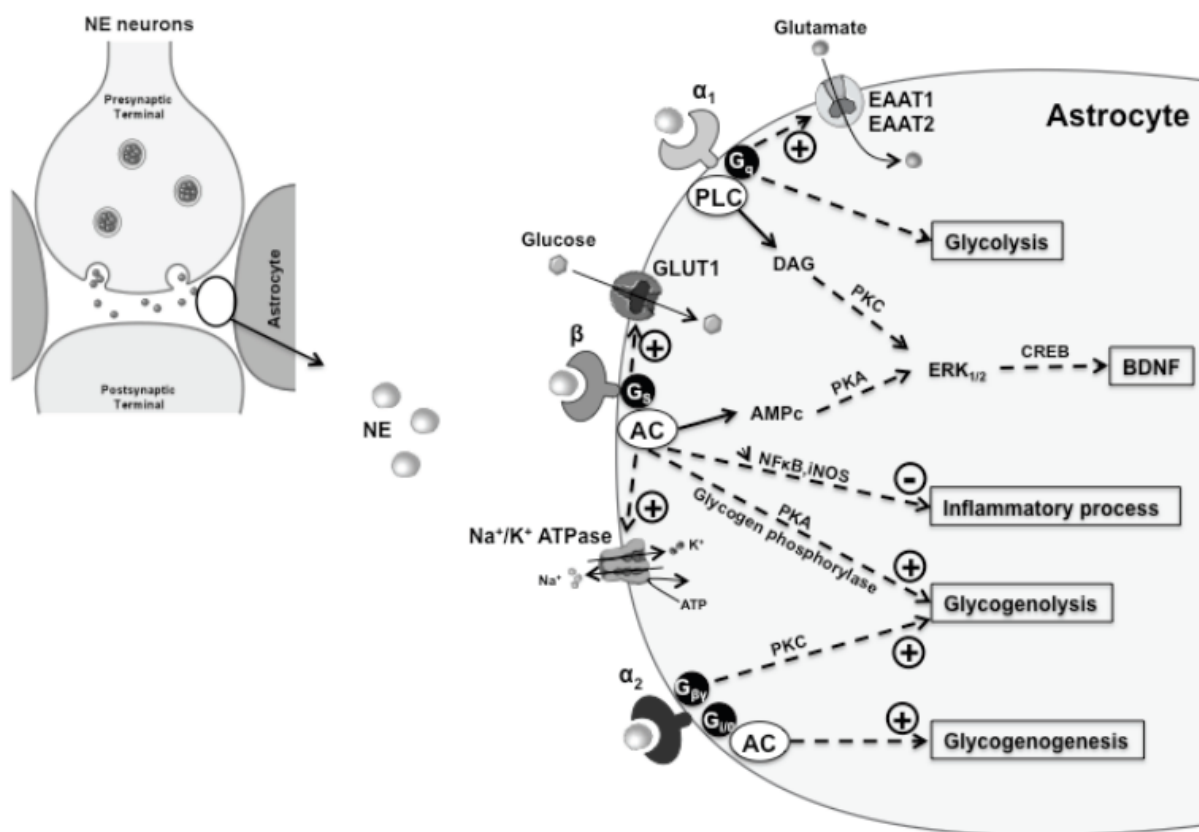


Fig. (2). Regulation of brain astrocytic activity by NE and related receptor subtypes identified on this cell type.

stimulate protein kinase A (PKA) through the stimulation of AC activity, leading to the stimulation of the glycogen phosphorylase that is required for glycogenolysis [114, 118, 119] (Fig. 2). In addition to the modulation of astrocytic metabolic activities, β -adrenergic receptors have been implicated in the neurotrophic support of neurons. Studies have shown that the application of NE in cultured neonatal rat astrocytes increases the synthesis of BDNF, an effect partially inhibited by specific β_1 - and β_2 -adrenoceptors antagonists [99, 120]. Through positive coupling with AC, the β_1 - and β_2 -adrenoceptors increase cAMP, leading to the activation of cAMP responsive element binding protein (CREB) and the regulation of BDNF synthesis [121] (Fig. 2). As an example of the physiological relevance of these mechanisms, BDNF has been proposed to have a neuroprotective effect on NE neurons in the LC [99, 122-124]. Finally, it has been well documented that astrocytes play a crucial role in the control and maintenance of ionic extracellular homeostasis, including K^+ [125]. Other studies have demonstrated that the β -adrenoceptor agonist isoproterenol stimulates the astrocytic Na^+/K^+ -ATPase activity, thereby facilitating the extracellular clearance of K^+ [96, 126, 127]. Due to the importance of the extracellular K^+ concentrations on neuronal firing, these findings suggest that NE could optimize astrocytic Na^+/K^+ -ATPase activity during high neuronal activity.

3.3. Astrocytic Dopaminergic Receptors

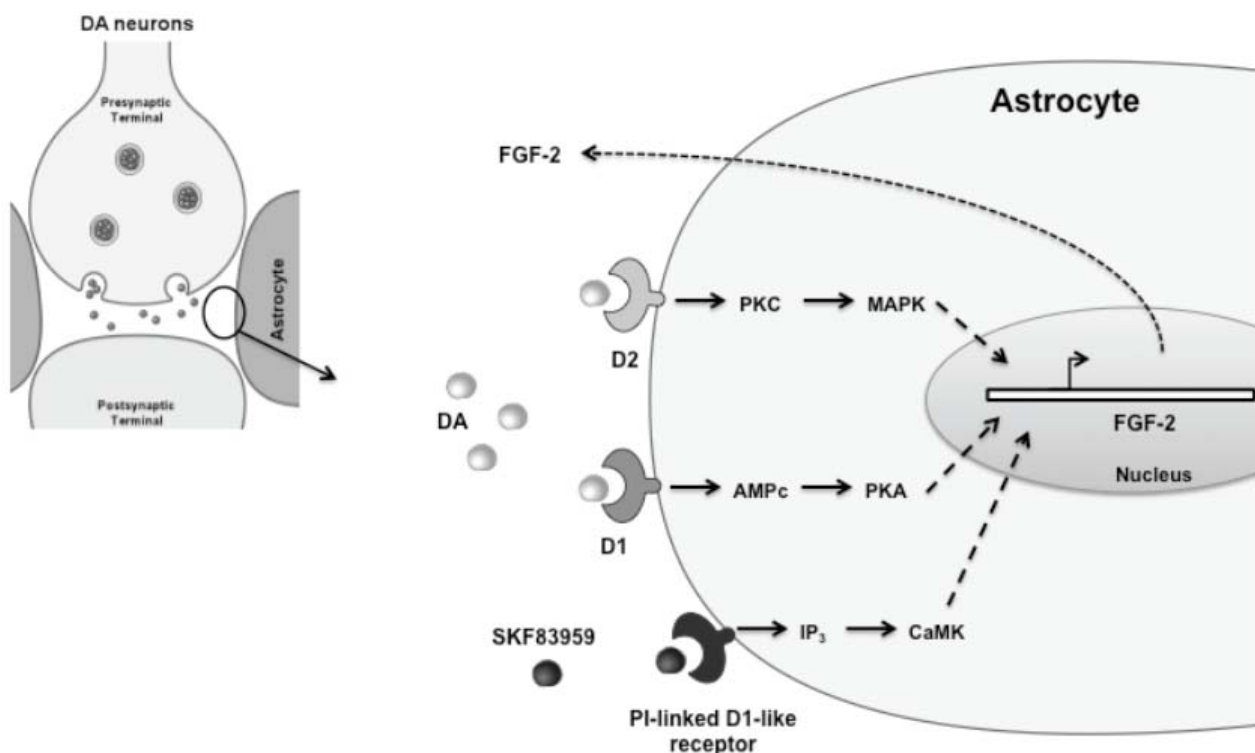
3.3.1. The D_1 - and D_2 -Like Receptors

Historically, the DA receptors were divided in two types: the D_1 receptor superfamily, positively coupled to AC

through the G_s proteins, and the D_2 receptor superfamily, negatively coupled to AC through the G_i proteins (Table 3). Thereafter, other subtypes of dopaminergic receptors were discovered: the D_3 and D_4 subtypes joined the D_2 receptor superfamily, and D_5 now belongs to the D_1 receptor superfamily. Several studies have reported the presence of all of the DA receptor subtype mRNAs in primary astrocyte cultures from the mouse striatum, and these observations were confirmed by western-blot and *in situ* analyses [15, 128, 129]. Interestingly, the application of DA in primary astrocyte cultures induces cAMP accumulation and p-CREB expression. This effect is inhibited by a D_4 receptor antagonist. Due to the implication of p-CREB in the transcriptional activation of some neurotrophic factors, this observation is consistent with previous reports demonstrating that DA up-regulates BDNF and NGF in the mouse astrocyte cultures [130, 131]. Similarly, the application of D_2 and D_3 receptor agonists, such as apomorphine and ropinirol, increases the synthesis and secretion of NGF and GDNF, but not of BDNF, in cultured mouse astrocytes [132, 133]. Moreover, apomorphine stimulates the synthesis and release of FGF-2 through the cAMP/PKA and PKC/MAPK signaling cascades in striatal primary cultures of astrocytes [134, 135] (Fig. 3). Such a release promotes the survival of dopaminergic neurons, thus demonstrating the potential therapeutic role of the astrocytes in neurodegenerative diseases, such as Parkinson disease [134], or in psychiatric disorders, such as MDD, which are characterized by specific symptoms involving this system. *In situ* and *in vitro* experiments also demonstrated the presence of the D_2 receptor in cortical astrocytes cultures [136], and these results must be considered in the

Table 3. Dopaminergic Receptor Subtypes Identified on Primary Cultures of Astrocytes.

Receptor	G Protein	Brain region	Experimental conditions	References
D ₁	G _s	Striatum	<i>In vitro</i>	[129] [15]
D ₂	G _i	Striatum Cortex	<i>In situ</i> <i>In vitro</i> <i>In situ</i>	[33] [48] [266] [136] [267]
D ₃	G _i	Striatum Mesencephalon	<i>In vitro</i> <i>In vitro</i>	[15]
D ₄	G _i	Striatum	<i>In vitro</i>	[15]
D ₅	G _s	Striatum	<i>In vitro</i>	[15]
PI-linked D ₁ -like	G _q	Striatum	<i>in vitro</i>	[140] [141]

**Fig. (3).** Regulation of brain astrocytic activity by DA and related receptor subtypes identified on this cell type.

development of a new class of antidepressant drugs that simultaneously increase all the three monoamines [137]. The triple reuptake inhibitor DOV216303 has been recently shown to stimulate the synthesis of BDNF from hippocampal and cortical primary cultures of astrocytes [138], emphasizing a possible role of DA on glial cells to promote neurotrophic effects.

3.3.2. The Phosphatidylinositol-Linked D₁-Like Receptor

Finally, studies have revealed the existence of a new dopamine D₁ receptor that is not coupled to AC. This receptor,

named non-cyclase-coupled D₁ receptor or phosphatidylinositol-linked D₁-like receptor (PI-linked D₁-like), is coupled to G_q-proteins and induces phosphatidylinositol (PI) hydrolysis by PLC β [139, 140]. *In vitro*, the selective agonist to this receptor, SKF83959 ([3-methyl-6-chloro-7,8-hydroxy-1-(3-methylphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine]), activates the synthesis and release of FGF-2 in rat astrocyte cultures through IP₃ and Ca²⁺/CaMK (Fig. 3). The activation of this receptor is believed to favor the survival of the dopaminergic neurons in the striatum [141, 142].

4. NEUROACTIVE SUBSTANCES RELEASED BY ASTROCYTES IN RESPONSE TO ANTIDEPRESSANT DRUGS

The above section sheds some light on the ability of antidepressant drugs to modulate astrocytic activity in response to increased extracellular monoamines levels. Indeed, 5-HT, NE and DA promote the synthesis of various neuroactive substances and favor the production of metabolic substrates that derive from glycogen and that are essential for neuronal activity. Martin and collaborators examined the regulation of energy metabolism by astrocytes in response to antidepressant drugs [143], and we will detail in this section the most recent findings that support a role for the gliotransmitters, neurotrophic factors or inflammatory molecules that are released by astrocytes in the mechanism of action of these pharmacological treatments.

4.1. Gliotransmitters

Astrocytes release gliotransmitters, such as the adenosine triphosphate (ATP), adenosine, D-serine and glutamate. The *in vivo* relevance of this process on the improvement of mood remains largely unexplored, although postmortem brain analyses and imaging studies in the late 90s reported glial dysfunction in the prefrontal and cingulate cortex of depressed patients [144-146]. Moreover, recent clinical and preclinical evidence has emphasized the putative role of astrocytes in the regulation of mood. Additionally, glial cells loss induced by the intra-cortical infusion of the gliotoxin L-alpha-amino adipic acid in rats is sufficient to induce anxiodepressive-like behaviors, such as anhedonia and anxiety [7], while the enhancement of glial cells activity, particularly through the release of gliotransmitters, promotes antidepressant responses.

4.1.1. ATP

It is well established that various classes of antidepressant drugs increase the levels of ATP in cultured astrocytes [147]. A recent study demonstrated that the astrocytic release of ATP is involved in the mood regulation and antidepressant response [148]. Indeed, mice susceptible to chronic social defeat or subjected to the forced swimming test exhibit low ATP brain levels. Moreover, mice with deficiencies in astrocytic ATP release induced by the inactivation of vesicular gliotransmission (dn-SNARE mice) display depressive-like behaviors that are rescued following the administration of ATP [148]. Conversely, an increased astrocytic ATP release is sufficient to elicit antidepressant-like effects in mouse models of depression (chronic social defeat; chronic mild stress), demonstrating for the first time that gliotransmitters positively reverberates on mood through the stimulation of adult hippocampal neurogenesis [149]. ATP is extracellularly hydrolyzed to adenosine, and several studies have also emphasized the possible role of this molecule in antidepressant-like responses. For example, the systemic or intracerebroventricular administration of adenosine induces antidepressant-like effects in forced swimming and tail suspension tests through the activation of the adenosine A₁ and A_{2A} receptors [150-154]. However, non-pharmacological antidepressant interventions, such as electroconvulsive ther-

apy (ECT) or sleep deprivation, are associated with pronounced increases in adenosine levels in the mouse whole brain [155]. Also, adenosine transmission in an antidepressant response could be important in humans because an up-regulation of the glial adenosine A₁-receptors has been observed in the brain of depressed patients [156]. In addition to these data, it has been reported that 12 hours of sleep deprivation induces an increase in the hippocampal extracellular adenosine levels and in the adenosine receptor A₁ signaling in the mouse brain, whereas the central administration of a potent and selective A₁ receptor agonist mimics the antidepressant-like effect of sleep deprivation in the forced swimming and tail suspension tests [157]. Paradoxically, *in vivo* microdialysis data indicate that a non-selective A₁ receptor antagonist increases the basal 5-HT release in the rat HP, thereby suggesting that an activation of this receptor subtype could suppress 5-HT release [158, 159]. The explanation could involve another adenosine receptor subtype because a selective A₂ receptor agonist also increases basal 5-HT release under these experimental conditions [159]. Accordingly, pre-synaptic activity-dependent release of adenosine, through activation of adenosine A_{2A} receptors, facilitates the excitatory BDNF modulation of synaptic neurotransmission in the CA1 area of the rat HP slices [160]. Thus, the HP seems to be modulated by adenosine through the activation of the inhibitory A₁ and excitatory A_{2A} receptors [158].

4.1.2. D-serine and Glutamate NMDA Receptors

The role of D-serine, a potent agonist at the glycine site of the NR1 subunit of glutamatergic NMDA receptors [161] in MDD and antidepressant response, has been recently addressed. The systemic administration of D-serine produces antidepressant-like effects in various paradigms in rats as indicated by the ability of D-serine to reduce the immobility time in the forced swimming test and to rescue the 5-HT depletion-induced learned helplessness behaviors [162]. Although the mechanisms underpinning the antidepressant-like effects of D-serine are unknown, it is possible that the facilitatory action at the NMDA receptors plays a significant role. However, growing evidence suggests that the expression of the major glutamate transporter EAAT2 is impaired in rat models of depression [163, 164]. Similar observations were made post-mortem in the cortical regions [165] and in the LC [166] of MDD patients in which microarray analyses showed a down-regulation of members of the glial glutamate/neutral amino acid transporter protein family. Such an astroglial distribution of glutamate transporters suggests a link between key components of glial cells and the pathophysiology of depression. It is hypothesized that these changes could elevate the levels of extracellular glutamate considerably, which is potentially neurotoxic [165]. Long-term treatment with fluoxetine significantly increases the expression of the glutamate transporter GLT1 in the rat HP and cortex [163]. In summary, it is difficult to reconcile the fact that an increase in synaptic extracellular glutamate levels would be associated with mood disorders, whereas D-serine, which stimulates glutamatergic NMDA receptor transmission, can produce antidepressant-like effects. Further preclinical studies are therefore warranted to help understand the putative link between the regulation of synaptic glutamate transmission and D-serine release by astrocytes.

4.2. Inflammatory Molecules

Pro-inflammatory agents such as lipopolysaccharide (LPS) are known to promote depressive-like behaviors in rodents [167], likely through the recruitment of various events, including the activation of microglia, the synthesis of reactive oxygen species (ROS) and pro-inflammatory molecules in the brain [i.e. interleukin 1 (IL-1), interleukin 2 (IL-2) and tumor necrosis factor- α (TNF- α), chemokines] [168]. These processes are also believed to decrease hippocampal neurogenesis [169], which could account for the negative impact on mood. The initial evidence demonstrating that activated microglia impairs hippocampal neurogenesis was reported ten years ago [170]. In this study, the authors demonstrated that the intraperitoneal injection of LPS significantly decreased new cell survival and the differentiation of new cells into neurons as evidenced by a reduction in the expression of the early neuron marker doublecortin in LPS treated rats [170]. In this study, the survival of new neurons was negatively correlated with the number of activated microglia. Remarkably, the acute administration of tricyclics and of paroxetine, fluoxetine or desipramine prevented the release of inflammatory mediators in the rat cortex, in primary cultured forebrain microglia and in astrocytes from newborn mice [171, 172] suggesting that antidepressant drug activity could rely on action on microglia. This is confirmed by the observation that fluoxetine prevents LPS-induced inhibition of adult hippocampal neurogenesis in rats [173] by attenuating microglia activation [174]. Additionally, antidepressant drugs also inhibit the synthesis and release of pro-inflammatory cytokines in the peripheral immune cells in patients suffering from MDD [175, 176] and in inflammatory models of depression [177, 178], leading to the theory that psychiatric disorders might have peripheral causes and consequences. In addition to the ability to inhibit the synthesis and release of pro-inflammatory molecules by peripheral immune cells, reactive astrocytes or microglia, antidepressant drugs elicit the astrocytic expression of neurotrophic factors. This is of particular interest because BDNF has anti-inflammatory properties [179] as demonstrated, for example, by the ability to reduce *in vitro* TNF- α mRNA from activated microglia in an animal model of sclerosis [180]. It is therefore possible that in the HP, astrocytic or neuronal BDNF maintains microglia in a resting state, which is an essential step for enhancing adult neurogenesis by removing the apoptotic new cells through phagocytosis [181]. Notably, all of these pro-inflammatory processes could also have deleterious effects on synaptogenesis, which is associated with MDD. In a recent review, Di Benedetto *et al.* (2013) described how antidepressant drugs might influence the dynamic of neuronal circuits through the impact on microglial cells and, consequently, on neuronal synapses [182, 183]. However, these considerations do not preclude the possibility of a positive effect of inflammation on brain plasticity. A recent study conducted in the adult zebrafish brain suggested that inflammation is required and sufficient for enhancing the proliferation of neuronal progenitors and subsequent neurogenesis [184]. This notion should also be integrated to better understand the impact of antidepressant drugs on the functions of the monoaminergic tripartite synapse.

4.3. Neurotrophic and Growth Factors

As described in the previous chapter, antidepressant drugs may modify the release of various neurotrophic and growth factors from cultured astrocytes. We will describe in this section the putative role of these factors in the mechanism of action of these agents.

4.3.1. S100beta

Among these factors, it has been shown that the SSRI fluoxetine stimulates s100beta synthesis in hippocampal astrocyte cultures [185] and *in vivo* in the HP of rats and mice subjected to the chronic mild stress [185-187]. These beneficial effects have been attributed to a facilitatory effect and the paracrine neurotrophic activity of s100 β on neuroplasticity [74] and more specifically on hippocampal neurogenesis [188]. Moreover, higher serum levels of s100beta have been found to efficiently predict the response to antidepressant drugs in MDD patients after 4 or 6 weeks of treatment [189, 190]. Nevertheless, the impact of central administration of s100beta on the behavioral paradigms relevant to antidepressant-like activity has never been studied and requires further attention. Interestingly, SERT is a target of microRNA-16 (miR-16), which is expressed at higher levels in noradrenergic cells than in serotonergic cells. S100 β has been reported to trigger miR-16 in noradrenergic neurons of the LC to stimulate the expression of SERT by this neuronal population [191]. The role of miR-16 could be to limit the accumulation of 5-HT in the synaptic cleft in the LC, a process potentially favorable to the activation of the NE neurons [192, 193].

4.3.2. GDNF

GDNF is another neurotrophic factor that modulates neuronal survival in the central and peripheral nervous systems. Additionally, GDNF affects the proliferation, migration, differentiation and survival of neuroblasts [194-196]. Human studies supporting a role for GDNF in MDD are scarce. However, preclinical studies have reported that the expression of GDNF is significantly decreased in the ventral striatum by chronic ultra-mild stress in mice but is increased after a prolonged treatment with imipramine [197]. In another study, animals exposed to the chronic unpredictable stress display a decreased GDNF expression in the HP, while chronic treatment with clomipramine reverses these behavioral deficits [198]. These results strongly suggest that epigenetic changes in GDNF gene expression contribute to behavioral stress responses. Consistent with the latter study, *in vitro* data showed that different classes of antidepressant drugs, including TCAs and SSRIs, modulate GDNF expression in cultured cortical rat astrocytes and in C6 glioma cells [199-201]. The role of 5-HT in the synthesis of GDNF has been recently proposed from *in vitro* studies showing that this monoamine induced GDNF mRNA expression in rat C6 glioma cells *via* 5-HT₂ receptor-mediated FGFR2 transactivation [84, 85].

4.3.3. VEGF

VEGF, a pleiotropic factor that possesses multiple activities in blood vessels and neurons, has been shown to increase the proliferation of neuronal progenitors [202], to stimulate

the neurogenesis [203, 204] and to modulate the neuronal plasticity in the adult HP [205]. The chronic administration of antidepressant drugs increases the expression of VEGF in the HP [206, 207], and the intracerebroventricular infusion of VEGF stimulates adult hippocampal neurogenesis. This process likely explains why VEGF elicits antidepressant-like activities in different paradigms, predicting the antidepressant-like activity of pharmacological agents in rodents and in another animal model of depression [206, 207]. There is also evidence that VEGF signaling is required for the effects of fluoxetine in different behavioral paradigms and that the chronic administration of this SSRI increases the expression of VEGF in neurons and endothelial cells of the HP [208]. In addition to neurons and endothelial cells, fluoxetine, paroxetine and TCA amitriptyline upregulate VEGF in cortical primary cultures of astrocytes [17, 199]. Given that VEGF increases astroglial proliferation [209], it is suspected that the upregulation of VEGF by antidepressant drugs contributes to the reversal of structural abnormalities detected in the preFC of depressed patients.

4.3.4. FGF2, FGFR1

FGF2 is a growth factor that is essential for the proper formation of the synaptic connections in the cerebral cortex, the maturation and survival of catecholamine neurons, and the enhancement of neurogenesis. It has been suggested that FGF-2 is involved in the regulation of mood because the expression of FGF-2 is decreased in the HP of patients suffering from MDD [210]. Moreover, treatment with antidepressants reduces this decreased expression of FGF-2 in MDD patients [211]. Animal studies have also shown that the expression of FGF-2 is reduced notably in the HP of rats subjected to the social defeat model of depression [212], whereas antidepressant drugs produced the opposite effects in the HP and in the FC [213, 214]. These observations are consistent with the fact that the intracerebroventricular administration of FGF-2 induces antidepressant-like effects in various animal models of depression [212]. FGF2 proteins predominantly increase in neurons of layer V throughout cortical areas and in some neurofilament-positive cells of the HP. FGFR1 mRNA also increases in the dentate gyrus of the HP 24 h post-FGF2 injection, confirming the role of this factor in neuronal modeling as well as in antidepressant drug responses. Indeed, cortical FGF-2/FGFR signaling is necessary for mediating the antidepressant-like effects of imipramine and fluoxetine in the chronic unpredictable stress model of depression in rats [215].

5. EXAMPLE OF BDNF AT THE TRIPARTITE SYNAPSE – LINK WITH 5-HT NEUROTRANSMISSION

A large body of evidence has emphasized the role of BDNF in the pathophysiology and treatment of MDD [216]. The role of serotonergic antidepressant drugs in the regulation of this neurotrophic factor is complicated because the outcome is largely dependent on the length of treatment. Although the acute administration of various SSRIs or the monoamine oxidase inhibitor tranylcypromine decreases the expression of BDNF in the HP [217-220], notably exon VI and V mRNA [219, 221, 222], the repeated administration (2-3 weeks) of these drugs produces the opposite effect [123, 219, 220, 222, 223] and reverses stress-induced BDNF

downregulation in the adult rat brain [223, 224]. It is therefore suspected that a prolonged increase in extracellular 5-HT levels results in the long-term activation of second messenger signal transduction systems, such as cAMP/PKA and DAG/PKC, leading to increased expression of the CREB gene [225, 226]. This transcription factor would then enhance BDNF expression [227, 228]. Because BDNF expression, identified in rat cultured embryonic cortical neurons, can be stimulated by the activation of the cAMP system and voltage sensitive Ca^{2+} channels [229], it is now accepted that mature hippocampal neurons represent the major source of BDNF. Accordingly, *in situ* hybridization studies in the HP of the adult rat brains reported that cells expressing the BDNF gene are found in pyramidal and granule neurons and in target cells receiving projections from BDNF-synthesizing neurons [101]. There is, however, a paucity of direct *in vivo* evidence supporting the specific involvement of neurons in BDNF synthesis, while growing arguments suggest that astrocytes might participate in the production of this factor. For example, the BDNF transcription factor CREB has been identified in cortical or hippocampal cultured astrocytes [230], and antidepressant drugs, such as imipramine, stimulate CREB activity [231].

Despite the fact that the precise source of BDNF remains somewhat equivocal, preclinical studies have clearly indicated that the intracerebroventricular and intra-hippocampal infusion of BDNF produces antidepressant-like activity in naive and stressed rodents [232-235], thereby demonstrating that the therapeutic activity of antidepressant drugs depends, at least in part, on BDNF synthesis and TrkB signaling in neurons. Accordingly, as the high affinity receptor of BDNF, TrkB is autophosphorylated in response to the chronic administration of various classes of antidepressant drugs in the mouse HP and cingulate cortex [236, 237], whereas transgenic mice with the non-functional truncated TrkB are less sensitive to these treatments [238]. Conversely, the ability of acute administration of SSRIs to produce antidepressant-like effects [239] and to increase hippocampal extracellular 5-HT levels is blunted in mice lacking 50% of BDNF [137]. Although the link between BDNF and 5-HT neurotransmission has not been completely elucidated, evidence suggests that BDNF favors the expression and activity of SERT, thereby increasing 5-HT clearance in the synaptic cleft [137]. The role of BDNF and the related TrkB receptor in adult hippocampal neurogenesis has stimulated number of studies showing that this factor promotes the *in vivo* proliferation and differentiation of neuronal progenitor cells [240-243], accelerates the maturation of new neurons and facilitates neuronal survival [242-244]. In human post-mortem studies, the expression of BDNF is increased in the HP of depressed patients treated with antidepressant drugs compared to untreated individuals [245].

Together, these data strongly suggest that BDNF is predominantly synthesized by neurons [246], but reactive astrocytes could also produce this factor under particular conditions, such as in neurodegenerative diseases or after the exposure of cultured astrocytes to kainic acid [247, 248]. Although the extremely low levels of BDNF in neurons have greatly complicated attempts to reliably localize this factor [249], the presence of SERT and 5-HT receptor subtypes on astrocytes, along with the recent observations that fluoxetine,

imipramine, amitriptyline and TRIs upregulate BDNF in primary cultures of astrocytes [17, 138, 231], suggest that glial cells represent a permissive microenvironment for the production of new neurons and neuronal integration into existing functional networks in the dentate gyrus of the HP. To address the question of the *in vivo* contribution of astrocytic BDNF to antidepressant drug actions, we applied a new lentiviral strategy [250] aimed at overexpressing this neurotrophic factor in the hippocampal astrocytes of adult mice. Our results show that such a manipulation produces anxiolytic-/antidepressant-like activity in mice subjected to novelty suppressed feeding (NSF), a behavioral paradigm in relation to the stimulation of adult hippocampal neurogenesis [251]. However, BDNF overexpression did not potentiate the effect of chronic fluoxetine, suggesting that SSRIs and astrocytic BDNF could act through a similar downstream mechanism. Interestingly, neurochemical data showed that the overexpression of BDNF within astrocytes did not modify hippocampal extracellular 5-HT levels, suggesting that the behavioral and neurogenic effects of astrocytic BDNF were not dependent on the modulation of serotonergic presynaptic activity. In a marked contrast, although BDNF overexpression or chronic fluoxetine alone are devoid of behavioral activity, the combination of both viral and pharmacological interventions elicited anxiolysis in behavioral paradigms used to unveil anxiolytic-like activity such as the elevated plus maze (EPM). At the neurochemical level, such a combination attenuated the ability of fluoxetine to enhance extracellular hippocampal 5-HT levels, a mechanism that could be favorable to anxiolysis. These results illustrate the concept of the tripartite synapse in which the increase in the 5-HT neurotransmission induced by the SSRI fluoxetine could promote the synthesis and release of BDNF from astrocytes. In turn, once released in the synaptic cleft, BDNF would exert a negative feedback upon extracellular hippocampal 5-HT release to limit the 5-HT neurotransmission and favor anxiolysis. Several explanations could be advanced to explain how BDNF released from astrocytes attenuates the extracellular 5-HT levels in HP. It is possible that BDNF promotes the sprouting of the serotonergic terminals, as previously described in adult animals [252]. Alternatively, hippocampal BDNF could be transported retrogradely to the DR, which could result in the remodeling and growth of 5-HT dendrites at somatodendritic levels, thereby increasing the density of the inhibitory 5-HT_{1A} autoreceptors in response to chronic fluoxetine treatment. In addition to these paracrine effects on the neuronal cell population in the HP, BDNF might also produce autocrine effects on astrocytes. This possibility should be further explored because TrkB [253, 254] and pan-neurotrophin receptor p75 on hippocampal astrocytes [255-257] are both required for BDNF-mediated effects. Interestingly, in cultured astrocytes, the p75 receptor has been shown to participate in pro-BDNF internalization, and this recycling suggests the existence of a specialized form of bidirectional communication between neurons and glia [258].

Although interesting, the above-mentioned results suggest some sufficiency but do not investigate the necessity of astrocytic BDNF in antidepressant drugs activity. The appropriate tool to decipher such a role would be to knock-down this factor through the intra-hippocampal injection of a specific shRNA within the astrocytes in adult mice, a strat-

egy that should prevent the positive effects of chronic fluoxetine treatment. It thus appears possible that under particular conditions, such as following the overexpression of astrocytic BDNF in adult mice [251], astrocytes could be a source of BDNF or can re-uptake BDNF as peripheral platelets do for 5-HT. To our knowledge, this "astrocytic BDNF synthesis" hypothesis has not been demonstrated *in vivo*, and we do not know whether these results can be observed under physiological conditions or in an animal model of depression.

CONCLUSION

This review illustrates the fact that astrocytes could respond to antidepressant drugs *in vivo* by sensing and regulating extracellular monoamine levels. In turn, these cells might provide instructive signals to neurons regulating the formation and development of synapses, cell fate in neurogenic niches and neurotransmission in glial-neuronal synaptic units. Given the emerging hypothesis of depression stipulating the existence of an impairment of glial network in this psychiatric disorder [27], antidepressant drugs could act on these cells to restore a normal dialogue between astrocytes and neurons. The Cxs proteins play a major role in this communication, and the observations that the expression and function of these proteins are decreased under stressful conditions [28] and are enhanced in response to antidepressant drugs [28] suggest that gap junctions are promising new targets for the treatment of MDD. This hypothesis is strengthened by the fact that astrocytic Cxs proteins are involved in the direct intercellular exchange of ions and small molecules (e.g., IP₃, ATP, glutamate, and energy metabolites) between neighboring cells [259] and in the exchange of neuroactive substances between the cell cytoplasm and the extracellular medium [260]. A current limitation in evaluating the role of the monoaminergic tripartite synapse in the mechanism of action of acute or sub-acute antidepressant drugs lies in the fact that most studies are based on *in vitro* experiments that do not necessarily mimic the complex events occurring in living animals or humans and do not allow for the extrapolation of behavioral consequences on the antidepressant-like activity. For this prospect, adapted tools, such as genetic and viral knock-down approaches, are required to identify the molecular partners recruited in response to these medications. Finally, one would also expect that the development of optogenetics in transgenic mice overexpressing excitatory channelrhodopsins in astrocytes [261] or the engraftment by glial progenitor cells [262] would be useful to elucidate the precise role of this cell type in higher brain function and the regulation of mood.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

ATP	=	Adenosine triphosphate
AC	=	Adenylate Cyclase

AMY	= Amygdala	OCT	= Organic Cation Transporter
BDNF	= Brain-derived neurotrophic factor	PLC	= Phospholipase C
bFGF	= Basic Fibroblast Growth Factor	K ⁺	= Potassium
Ca ²⁺	= Calcium	PKA	= Proteine Kinase A
Camk	= Calmodulin-Dependent Protein Kinase	PKC	= Proteine Kinase C
CREB	= cAMP Response Element-Binding protein	RT-PCR	= Reverse transcription polymerase chain reaction
COMT	= Catechol- <i>O</i> -methyltransferase	ROS	= Reactive oxygen species
CNS	= Central Nervous System	SSRI	= Selective Serotonin Reuptake Inhibitor
CNTF	= Ciliary Neurotrophic Factor	5-HT	= Serotonin
Cxs	= Connexins	SNRIs	= Serotonin/Norepinephrine Reuptake Inhibitor
cAMP	= Cyclic adenosine monophosphate	SERT	= Serotonin transporter
DA	= Dopamine	shRNA	= Short hairpin RNA
DAG	= Diacylglycerol	Na ⁺	= Sodium
DAT	= Dopamine transporter	SNARE	= Soluble N-ethylmaleimide-sensitive-factor Attachment protein Receptor
DR	= Dorsal Raphe	TH	= Thalamus
ECT	= Electroconvulsive therapy	TNF- α	= Tumor Necrosis Factor- α
EGF	= Epidermal Growth Factor	VEGF	= Vascular Endothelial Growth Factor
EAAT	= Excitatory Amino-Acid Transporter	VGF	= Vascular Growth Factor
Erk	= Extracellular signal-regulated kinase	VTA	= Ventral tegmental area
EMT	= Extraneuronal Monamine Transporter		
FC	= Frontal Cortex		
GDNF	= Glial cell line-neurotrophic factor		
GFAP	= Glial Fibrillary Acidic Protein		
HP	= Hippocampus		
HYP	= Hypothalamus		
iNOS	= Inducible Nitric Oxide Synthase		
IP3	= Inositol triphosphate		
IL	= Interleukin		
LPS	= Lipopolysaccharide		
LC	= Locus coeruleus		
MD	= Major Depression		
MAPK	= MAP kinase		
miR	= micro-RNA		
MAO	= Monoamine oxydase		
NMDA receptor	= N-methyl- D-aspartate receptor		
NO	= Nitric Oxyde		
NE	= Norepinephrine		
NRI	= Norepinephrine Reuptake Inhibitor		
NET	= Norepinephrine transporter		
NF κ B	= Nuclear Factor kappa-light-chain-enhancer of activated B cells		
NAcc	= Nucleus Accumbens		

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CONCLUSION :

Après avoir longtemps été considérés comme de simples cellules de soutien pour les neurones, les astrocytes apparaissent comme des acteurs majeurs de la fonction synaptique, de par leur structure, leur organisation en réseaux et leur relation neurochimique étroite avec les neurones pré- et post-synaptiques. Ces cellules expriment à leur surface une grande quantité de récepteurs, notamment monoaminergiques, leur permettant de répondre à des modifications de la neurotransmission sérotoninergique. Les astrocytes sont également capables d'influencer à leur tour l'activité neuronale via la production de BDNF et de métabolites nécessaires aux processus neuronaux. En accord avec ces propriétés, il est désormais clairement établi que des altérations du fonctionnement astrocytaire participent à l'émergence de nombreux troubles psychiatriques, dont les épisodes dépressifs majeurs, faisant des astrocytes une cible intéressante dans le développement de nouvelles thérapies antidépressives.

Dans cette optique, l'identification *in vitro* et *in vivo* du/des sous-type(s) de récepteur(s) sérotoninergique(s) impliqué(s) dans la synthèse/libération de facteurs neurotrophiques par les astrocytes, constitue un champ de recherche intéressant.

Dans la seconde partie de ce travail bibliographique, nous nous focalisons sur le lien entre le système sérotoninergique, le BDNF et la dépression ou le mécanisme d'action des ISRS. De manière plus spécifique nous apportons les arguments actuels en faveur d'un rôle des récepteurs 5-HT_{2A} dans la libération de ce facteur neurotrophique par les astrocytes.

Partie II : Interactions entre le BDNF et le système sérotoninergique : implication dans la dépression et le mécanisme d'action des antidépresseurs.

A. La famille des facteurs neurotrophiques :

Les facteurs neurotrophiques forment une superfamille de protéines impliquées dans de nombreux processus physiologiques telles que la prolifération, la maturation, la migration et la survie neuronale mais également dans la synaptogenèse. Il existe 6 neurotrophines différentes : le nerve growth factor (NGF), le Brain-derived neurotrophic factor (BDNF), la neurotrophine (NT) 3, la NT4/5, la NT6 et la NT7 (Nilsson et al. 1998). Les facteurs neurotrophiques possèdent des caractéristiques communes tels que leur homologie de structure primaire (environ 50%), leur poids moléculaire (Maisonpierre et al. 1990a; Maisonpierre et al. 1990b) et le fait qu'elles sont toutes synthétisées sous forme de précurseurs (pro-neurotrophine) clivés par différentes enzymes puis sécrétées dans le milieu extracellulaire. Les neurotrophines s'associent pour former des homodimères qui représentent alors la forme mature de la protéine. C'est sous cette forme que les facteurs neurotrophiques activent leurs récepteurs spécifiques (Lessman et al. 2003).

Les neurotrophines agissent par l'intermédiaire de l'activation de deux types de récepteurs : les récepteurs tyrosine kinase (TrK), possédant une forte affinité pour les neurotrophines matures, et les récepteurs p75, possédant à l'opposé une faible affinité pour les formes matures mais une forte affinité pour les pro-neurotrophines (Lu et al. 2005). Ainsi, il existe 3 types de récepteurs Trk (TrKA, TrKB et TrKC), chacun possédant une affinité préférentielle pour une neurotrophine donnée (Chao et al. 2003). Il est intéressant de noter que les récepteurs TrK existent sous deux formes, l'une dite longue et l'autre tronquée. La première possède des résidus tyrosine kinase permettant les réactions de phosphorylation nécessaires à l'activation de la signalisation intracellulaire. La seconde forme tronquée, est dépourvue de ces résidus tyrosine kinase, elle est donc incapable de phosphoryler les protéines de signalisation intracellulaire.

a) Exemple du BDNF.

1. Distribution et structure.

Le BDNF fut le second facteur neurotrophique identifié après le NGF (Barde et al. 1982). C'est un peptide homodimérisé de 120 acides aminés et de 27,2 KDa (Barde et al. 1994). Bien qu'il soit présent majoritairement dans le SNC, le BDNF est également exprimé dans d'autres tissus tels que le cœur, la rétine ou le rein (Ernfors et al. 1990; Hofer et al. 1990; Scarisbrick et al. 1993). Au niveau du cerveau, ce facteur est omniprésent mais sa plus forte densité a été retrouvée dans l'hippocampe, l'amygdale, le cortex, l'hypothalamus et le striatum (Conner et al. 1997).

Le gène codant pour le BDNF est localisé au niveau du chromosome 11 chez l'Homme, du chromosome 2 chez la souris et sur le chromosome 3 chez le rat (Ozcelik et al. 1991; Cunha et al. 2010). Ce n'est qu'en 2005 que la description complète du gène codant pour le BDNF a été réalisée. On a ainsi découvert qu'il comportait 9 exons et que la partie codante était localisée dans l'exon IX (Aid et al. 2007) (Figure 7). Il existe 9 promoteurs distincts à l'extrémité 5' qui régulent le processus de transcription de différents ARNm codant tous pour le BDNF (Aid et al. 2007; Greenberg et al. 2009). Ainsi, l'épissage alternatif et le processus de polyadénylation permet d'aboutir à la formation d'environ 18 transcrits chez l'Homme (Liu et al. 2006). Comme les autres neurotrophines, l'expression du gène codant pour le BDNF est sous le contrôle de processus complexes mobilisant des facteurs de transcription comme le C-AMP Response Element-binding protein, CREB (Finkbeiner et al. 2000; Tardito et al. 2006).

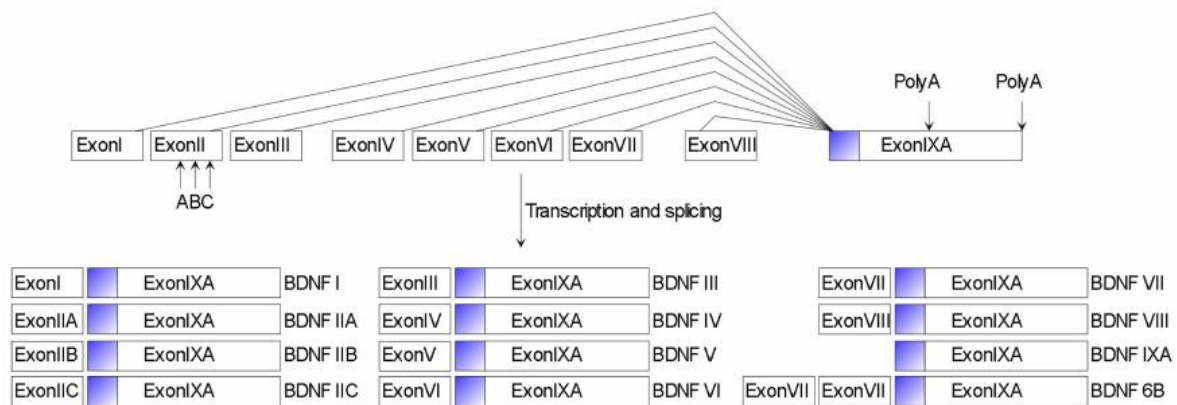


Figure 7 : Structure du gène murin codant pour le BDNF et de ses différents transcrits.

D'après Cunha et al. 2010

Les exons sont indiqués par des boîtes. La partie bleue dans l'exon IXA indique la région codante du gène du BDNF. Les lignes indiquent les variants d'épissage et les flèches, les sites d'épissage intra-exons et les sites de polyadénylation alternatifs. Le gène du BDNF est transcrit à partir de promoteurs différents de sorte que chaque transcrit pleine longueur contient un exon 5' unique et un exon 3' commun (exon IXA).

Il existe de nombreux polymorphismes du gène du BDNF, dont le plus décrit est le "Val66Met" qui correspond à la substitution de la méthionine par la valine dans le codon 66. La région du peptide affectée par ce SNP est impliquée dans l'adressage intracellulaire du BDNF et plus particulièrement dans sa libération régulée (Chen et al. 2004). Il est intéressant de noter que le polymorphisme "Val66Met" est impliqué dans l'anxiété et la dépression (Egan et al. 2003; Jiang et al. 2005; Schumacher et al. 2005; Chen et al. 2006; Hwang et al. 2006; Terracciano et al. 2010).

Le BDNF est synthétisé sous forme de précurseur appelé pré-pro-BDNF, puis clivé en pro-BDNF au niveau du réticulum endoplasmique (Greenberg et al. 2009) (Figure 8). Le pro-BDNF est alors transporté dans l'appareil de Golgi au niveau du réseau trans-Golgi où il sera :

- soit clivé par des protéases (la furine ou la pro-convertase) en BDNF mature (mBDNF) puis libéré dans le milieu extracellulaire par des vésicules de sécrétion régulées ou constitutives,
- soit libéré dans le milieu extracellulaire par des vésicules de sécrétion régulées ou constitutives puis clivé par les plasmines en mBDNF.

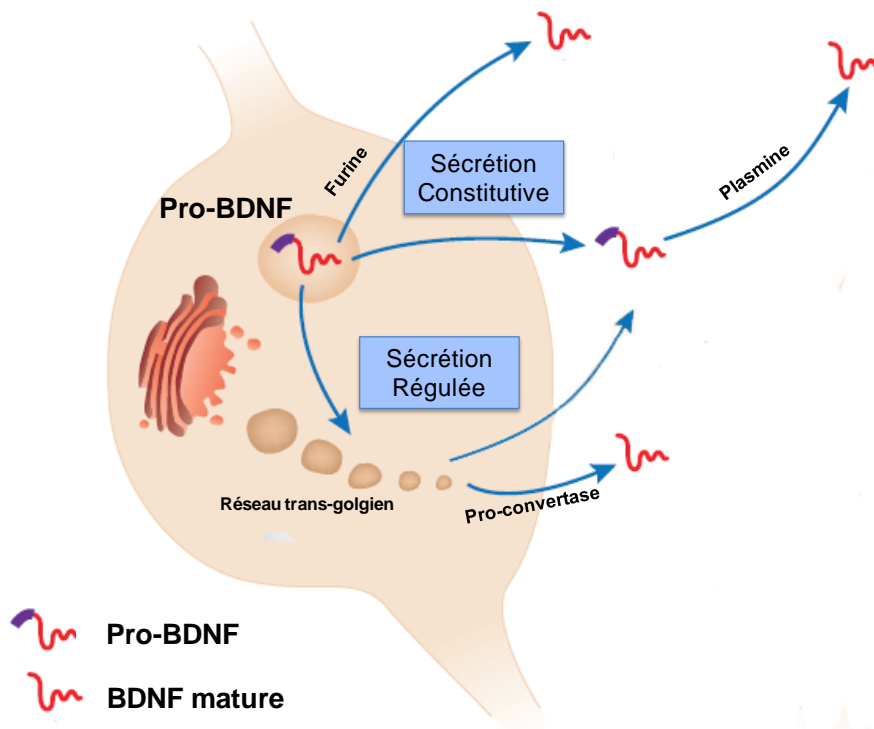


Figure 8 : Représentation schématique de la production et de la synthèse de BDNF.

Adapté à partir de Barker et al. 2009

Le pro-BDNF peut être clivé en BDNF par des furines au niveau du réticulum endoplasmique, puis sécrété dans le milieu extracellulaire. Le pro-BDNF peut également être libéré de manière régulée dans le milieu extracellulaire où il sera clivé en BDNF par les plasmines.

2. Rôles biologiques.

(i) Plasticité synaptique

Les synapses ont la capacité de moduler l'influx nerveux suite à des changements de l'activité neuronale, c'est ce que l'on appelle la plasticité synaptique. Ce processus très complexe comprend différents types de plasticité capables de réguler la force de la communication neuronale, tels que les mécanismes de désensibilisation de récepteurs mais aussi de potentialisation et de dépression à long terme (LTP, LTD). Les mécanismes sous-tendant la LTP et la LTD ont été

décrits pour la première fois dans l'hippocampe, bien que l'on sache désormais qu'ils peuvent être également observés dans d'autres régions cérébrales. La neurotransmission glutamatergique, et notamment les récepteurs NMDA et AMPA, sont à la base de ces deux types de plasticité. La LTP induit une augmentation durable de l'efficacité de la transmission synaptique facilitant ainsi la communication neuronale. Il est bien établi que le BDNF régule la plasticité synaptique en agissant sur les phases précoces et tardives de la LTP (Patterson et al. 1996; Xu et al. 2000; Gartner et al. 2006; Park et al. 2013), en stimulant l'amplitude des potentiels post-synaptiques excitateurs spontanés (PPSE spontanés) mais également la phosphorylation des récepteurs NMDA (Levine et al. 1995; Lin et al. 1999; Waterhouse et al. 2009). Ainsi, le BDNF synthétisé et libéré par les terminaisons neuronales pré- et post-synaptiques, induit une modulation paracrine ou autocrine de l'activité synaptique (Hartmann et al. 2001; Kojima et al. 2001; Lu et al. 2003; Park et al. 2013).

(ii) La neurogenèse hippocampique.

De nos jours, il est admis que le gyrus dentelé (DG) de l'hippocampe et la zone subventriculaire (ZSV) sont deux sous-régions cérébrales dans lesquelles se forment de nouveaux neurones et de nouvelles connections synaptiques chez différentes espèces depuis le rongeur jusqu'à l'Homme (Fuchs et Gould. 2000 ; Gage. 2002; Knoth et al. 2010). La naissance de ces nouveaux neurones porte le nom de neurogenèse. C'est un processus long qui se déroule en plusieurs étapes et qui donne naissance à environ 9.000 nouveaux neurones par jour chez les rongeurs (McDonald and Wojtowicz, 2005). Tout d'abord, les cellules souches neurales de type I, présentes dans la couche sous-granulaire (SGZ) du DG de l'hippocampe produisent, par divisions asymétriques, des cellules progénitrices de type II. Ces cellules progénitrices vont ensuite proliférer et se différencier en progéniteurs neuronaux (75%) ou astrocytaires (25%) (Steiner et al. 2004). Lors de cette différenciation les cellules progénitrices migrent dans la couche granulaire (GCL) du DG. Les progéniteurs vont alors mûrir et donner naissance à des cellules matures neuronales ou gliales (Zhao et al. 2008) ([Figure 9](#)).

La neurogenèse hippocampique chez l'adulte est régulée par de nombreux facteurs physiologiques tels que les systèmes de neurotransmission, les facteurs de croissance et neurotrophiques, les neuropeptides, les facteurs de transcription, les systèmes psychotropes endogènes, ou encore les hormones sexuelles (pour revue Balu and Lucki, 2009; McEwen, 2010). De nombreuses études rapportent que le BDNF joue un rôle majeur dans la neurogenèse hippocampique. Ainsi, une corrélation a été établie entre les facteurs environnementaux ou les situations qui augmentent l'expression du BDNF et ceux stimulant ce processus de neurogenèse adulte comme l'exercice physique ou l'apprentissage (Falkenberg et al. 1992 ; Nilsson et al. 1999 ; Ickes et al. 2000; Brown et al. 2003; Zhao et al. 2008). Au contraire, le stress est connu pour réduire le nombre de neurones dans le DG (Gould et al. 1997 ; Gould et al. 1998) mais également le taux d'ARNm codant pour le BDNF

(Smith et al. 1995 ; Marmigère et al. 2003). A l'heure actuelle, il est clairement établi que le BDNF joue un rôle direct sur le processus de neurogenèse dans l'hippocampe ventral chez l'adulte (Duman et al., 2001 ; Lee et al. 2002; Scharfman et al. 2005; Rossi et al. 2006; Wang et al. 2008; Taliaz et al. 2010). En accord avec cette hypothèse, une altération des étapes de prolifération et de survie de la neurogenèse hippocampique a été observée chez des souris hétérozygotes BDNF (BDNF +/-) (Lee et al. 2002), tandis que des injections intra-hippocampiques de BDNF induisent une augmentation du nombre de cellules positives à la fois au BrdU (analogue à la thymidine, intégrée dans l'ADN au moment de la phase S du cycle cellulaire) et la Neuréguline N (NeuN) utilisée comme marqueur neuronal (Scharfman et al., 2005).

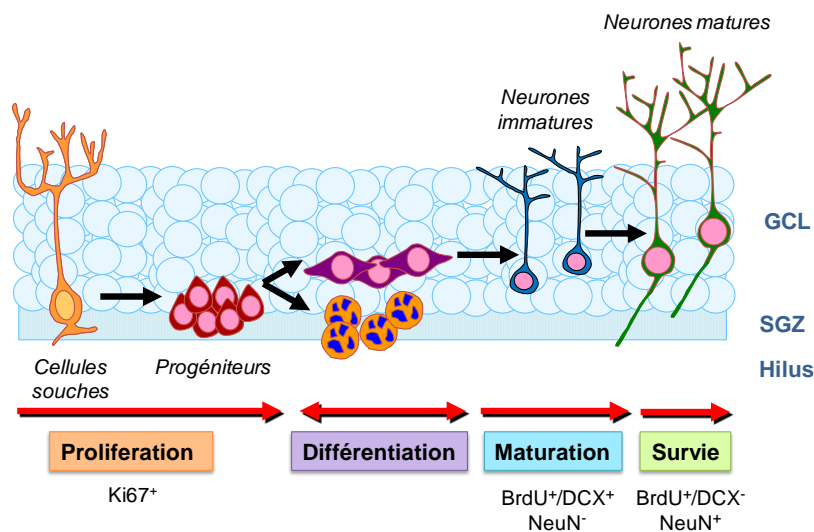


Figure 9 : Représentation du processus de neurogenèse hippocampique chez l'adulte.

Les cellules souches neurales de type I, présentes dans la couche sous-granulaire (SGZ) du DG de l'hippocampe, produisent, par divisions asymétriques, des cellules progénitrices de type II. Ces cellules progénitrices vont ensuite proliférer et se différencier en progéniteurs neuronaux ou astrocytaires. Lors de cette différenciation, les cellules progénitrices migrent dans la couche granulaire (GCL) du DG. Les progéniteurs vont alors mûrir et donner naissance à des cellules matures neuronales (75%) ou gliales (25%).

b) Le récepteur TrkB de haute affinité du BDNF.

1. Distribution et structure.

Dans le cerveau, le TrkB est présent sur les corps cellulaires, les axones et les dendrites des neurones (Fryer et al., 1996), mais également sur les cellules gliales (Wang et al. 1998; Rose et al. 2003; Ohira et al. 2007; Colombo et al. 2012; Grade et al. 2013). Ce récepteur existe sous deux formes : la forme longue (TrkB-FL) et la forme tronquée (TrkB-T) dont il existe 2 sous-types (T1 et T2). L'isoforme TrkB-FL est exclusivement exprimée par les neurones du SNC (Klein et al. 1990) tandis que les cellules gliales expriment le récepteur TrkB-T1. L'expression des récepteurs TrkB-FL et TrkB-T varie selon l'âge (Croll et al. 1998; Ohira et al. 1999) mais leur présence dans l'hippocampe, le cortex, les bulbes olfactifs, le thalamus, l'hypothalamus, le striatum, le septum, les

ganglions de la base, la substance noire, le cervelet et les noyaux monoaminergiques est clairement admise (Yan et al. 1997; Ohira et al. 1999; Ohira et al. 2003; Fenner, 2012). Dans l'hippocampe, plus précisément, un fort marquage a été détecté dans les couches des neurones pyramidaux des champs CA1-CA4 et dans les couches granulaires du DG (sur les corps cellulaires et les dendrites) (Yan et al. 1997). Le récepteur TrkB serait ainsi fortement exprimé par les cellules matures et, en revanche, peu exprimé par les cellules progénitrices (Donovan et al. 2008).

Le TrkB-FL et le TrkB-T sont des récepteurs actifs sous forme de dimère. Bien qu'ils soient tous deux codés par le même gène, ils possèdent des différences structurales (Stoilov et al. 2002). Ainsi, le TrkB-FL possède une activité tyrosine kinase lui permettant de recruter, lorsqu'il est activé par le BDNF, des protéines adaptatrices sur ses résidus tyrosines phosphorylés (Bertrand et al. 2012), déclenchant ainsi des cascades de signalisations intracellulaires, détaillées dans le prochain chapitre (Patapoutian and Reichardt, 2001). L'isoforme TrkB-T est dépourvue de domaines intracellulaires tyrosines kinases (Fenner, 2012). Cette différence est due à un épissage alternatif au niveau de l'exon 16 (Stoilov et al. 2002) (Figure 10).

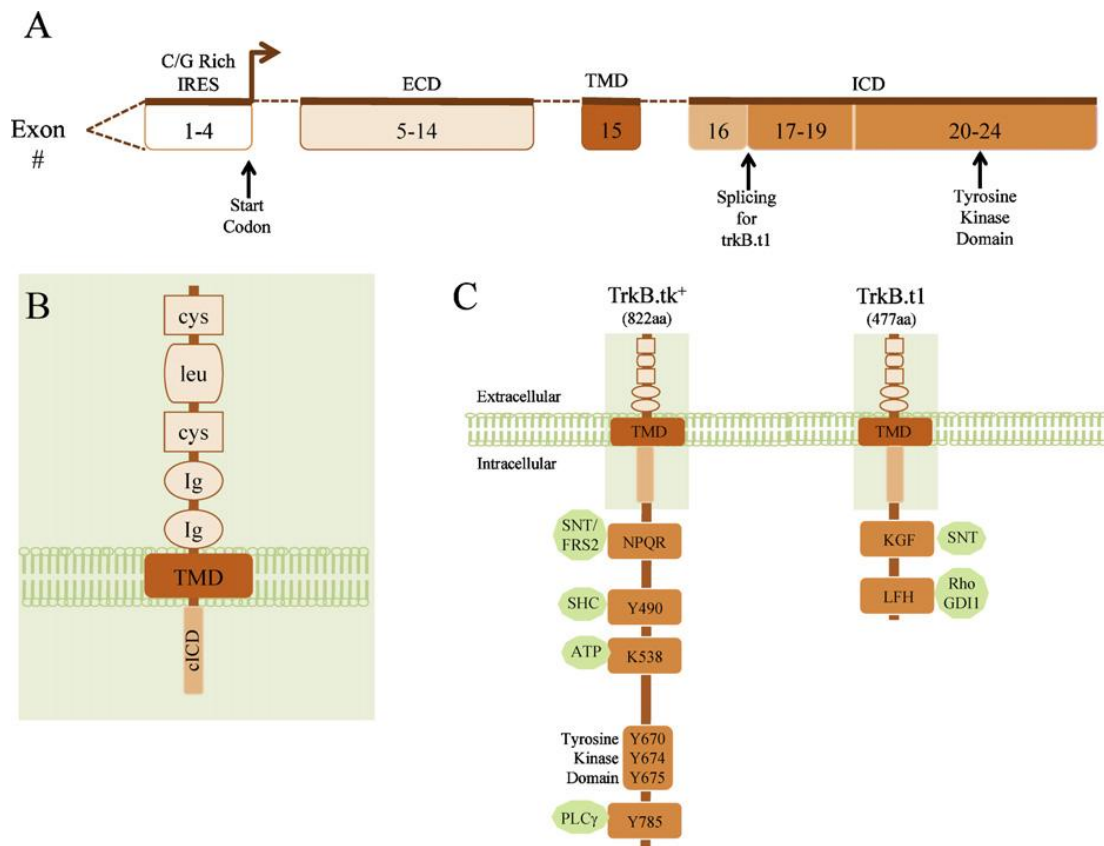


Figure 10 : Structure du gène et des deux isoformes du récepteur TrkB
D'après Fenner, 2012

A) Gène codant pour les récepteurs TrkB. B) Représentation du domaine protéique commun aux TrkB-FL et -T. C) Représentation schématique de la structure des récepteurs TrkB-FL et -T au niveau de la membrane cellulaire. Le TrkB-FL est une protéine de 822 acides aminés qui possède de nombreux domaines de liaisons intracellulaires. Le TrkB-T est une protéine de 477 acides aminés qui possède un domaine intracellulaire plus court et comporte moins de sites de liaison que le TrkB-FL.

2. Rôles biologiques.

(i) TrkB-FL

La forme homodimérisée du TrkB-FL possède une signalisation intracellulaire complexe, médiant entre autre les effets neurotrophiques du BDNF. L'activité kinase de ce récepteur est supportée par trois résidus tyrosines (Y670, Y674 et Y675). Lors de la fixation du BDNF, le TrkB-FL va s'autophosphoryler induisant ainsi un changement conformationnel, révélant des sites de liaison pour différentes protéines (Huang and Reichardt, 2001) (Figure 11). Les effets induits par le TrkB-FL passent ainsi par 3 voies de signalisation principales :

- la phospholipase C γ (PLC γ). L'activation de cette voie par le TrkB-FL va stimuler la survie neuronale et la transmission neuronale (Huang and Reichardt, 2001). En effet, la PLC γ va induire la production d'inositol-triphosphate (IP3) et diacylglycérol (DAG), qui à leur tour stimuleront la survie cellulaire et la plasticité synaptique (Minichiello et al. 2009).
- l'inositol-triphosphate kinase (PI3K). L'activation de cette voie va stimuler la croissance des neurites et la survie cellulaire (Minichiello et al. 2009).
- Erk/MAPK. La stimulation de cette voie va induire l'activation de facteurs de transcriptions tel que CREB qui joue un rôle clé dans la synthèse de nombreuses protéines (Numakawa et al. 2010). la voie Erk/MAPK régule notamment la survie neuronale.

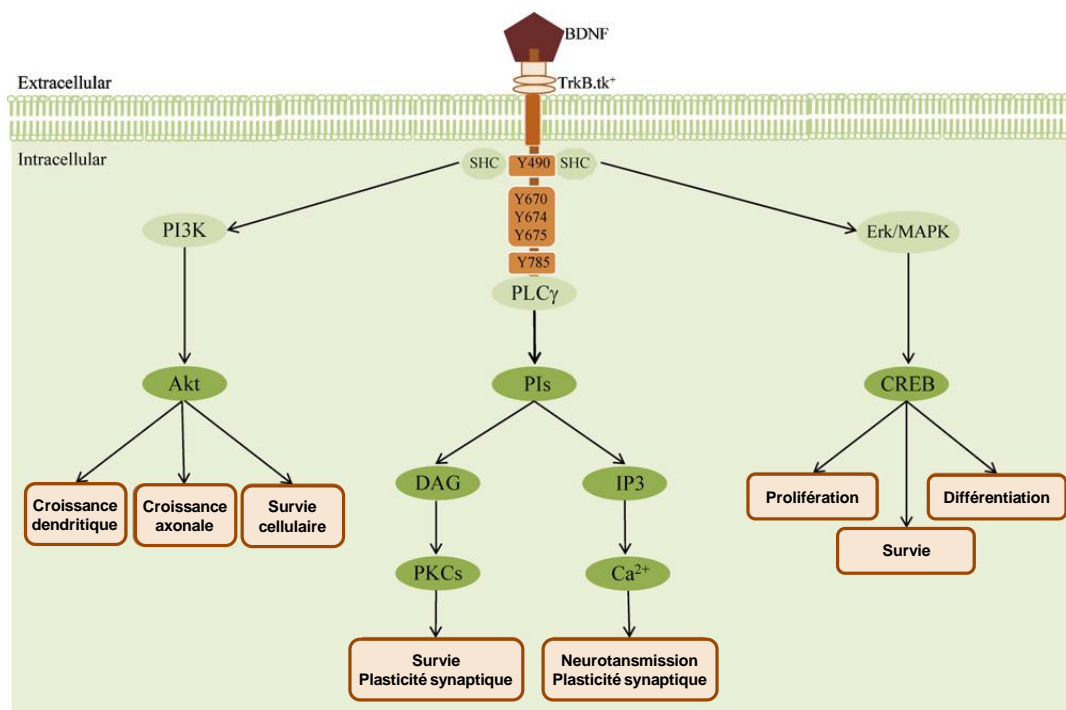


Figure 11 : Représentation des voies de signalisation intracellulaires induites par le TrkB-FL. D'après Fenner, 2012

Le TrkB-FL peut activer 3 voies de signalisation majeures : PLC γ , PI3K et MAPK. Ainsi, par ces voies de signalisation, le BDNF stimule la prolifération, la maturation et la survie neuronale.

(ii) TrKB-T1

Découvert en 1990, le TrKB-T1 a longtemps été considéré comme un "simple" dominant négatif de l'activité du TrKB-FL (Klein et al. 1990). En effet, des études montrent que le TrKB-T peut s'hétérodimériser avec le TrKB-FL, bloquant ainsi l'activité tyrosine kinase de ce dernier. Ceci a pour conséquence de bloquer la signalisation du BDNF (Eide et al. 1996; Fryer et al. 1997; De Wit et al. 2006). Dans le cerveau on trouve ainsi trois types de dimères : TrKB-FL/TrKB-FL, TrKB-T1/TrKB-T1 et TrKB-FL/TrKB-T1 (Ohira et al. 2001).

On sait désormais que le TrKB tronqué T1 possède d'autres propriétés indépendantes du TrKB-FL (Figure 12). Tout d'abord, des études révèlent que cette isoforme pourrait jouer un rôle dans la sécrétion du BDNF. En effet, lorsque le BDNF est en abondance dans le milieu extracellulaire, les cellules non-neuronales comme les astrocytes exprimant le TrKB-T1, internalisent et séquestrent le BDNF dans ces vésicules de transport, via sa liaison avec le TrKB-T1 (Biffo et al. 1995; Fryer et al. 1997; Alderson et al. 2000). Le TrKB-T1 semble également impliqué dans la croissance des neurites et filopodes. En effet, des études *in vitro* démontrent que l'activation de TrKB-T1 stimule la croissance des dendrites distaux et la croissance des filopodes gliaux (Yacoubian et al. 2000; Ohira et al. 2007). Ainsi, le BDNF à travers l'activation des TrKB-T1 pourrait changer la morphologie astrocytaire via la régulation de la stabilité du cytosquelette (Ohira et al. 2007). Enfin, il a été rapporté que l'activation de TrKB-T1 stimule des cascades de signalisation intracellulaires. Par exemple, les TrKB-T1 régulent l'activité de certaines kinases et le métabolisme cellulaire (Baxter et al. 1997). En 2000, Kryl et collaborateurs ont identifié une protéine capable d'interagir avec le domaine intracellulaire de l'isoforme tronquée de TrKB, appelée TTPI (pour truncated-trkb interacting protein) (Kryl and Barker, 2000). Bien que l'on ne connaisse pas les fonctions de cette protéine, ceci démontre que cette isoforme du TrKB est capable d'induire une signalisation intracellulaire en réponse à la fixation du BDNF, malgré l'absence de domaines tyrosines kinases. De nombreuses évidences révèlent que le BDNF, en se fixant sur le TrKB-T1, induit l'activation de protéines-G capables d'activer à leur tour la PKC et la PLC γ (Cheng et al. 2007). En retour, ces protéines modulent la signalisation calcique intracellulaire et le réarrangement du cytosquelette (Rose et al. 2003; Ohira et al. 2005) (Figure 12).

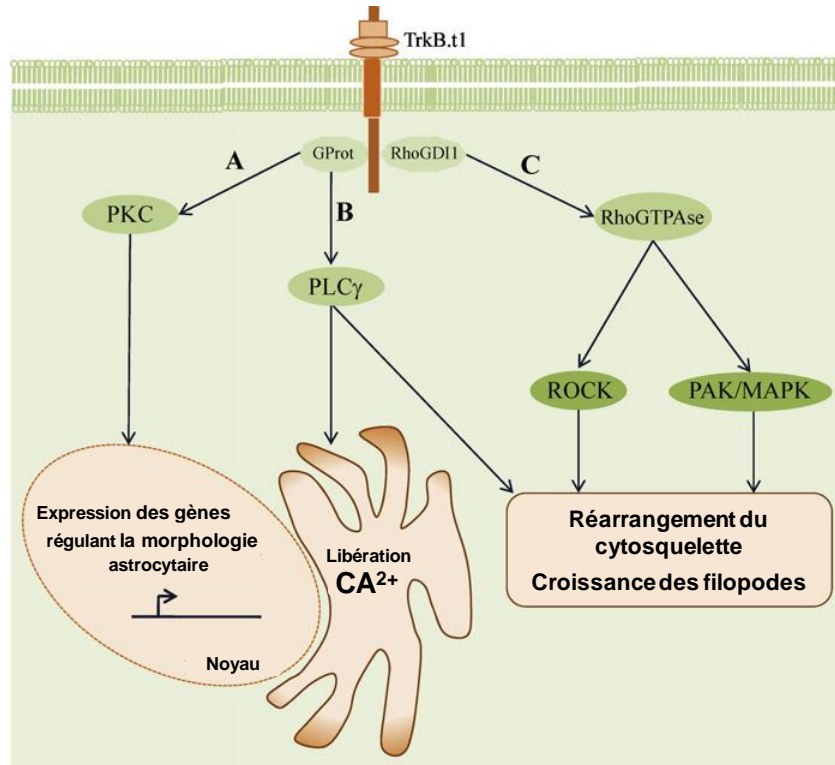


Figure 12 : Représentation des voies de signalisation intracellulaires induites par le TrkB-T1 dans les astrocytes.

D'après Fenner, 2012

A) Le TrkB-T1 active des protéines G qui stimulent la cascade de signalisations impliquant la PKC et aboutissant à des changements morphologiques astrocytaires. B) Les protéines G activent également la voie de signalisation de la PLC γ induisant la signalisation calcique intracellulaire mais également des réarrangements du cytosquelette. C) Le TrkB-T1 stimule une activité RhoGTPase nécessaire à l'organisation du cytosquelette et à la croissance des filopodes.

c) Source du BDNF.

Dans le SNC, la principale source BDNF sont les neurones (Rudge et al. 1995). Ainsi, au niveau des synapses, le BDNF peut être libéré par les terminaisons pré- et post-synaptiques en réponse à un stimulus (Hartmann et al. 2001; Kohara et al. 2001).

Des études *in vitro* suggèrent que les astrocytes joueraient également un rôle dans la synthèse et la libération de BDNF notamment en conditions pathologiques comme un état inflammatoire ou une lésion neuronale par exemple (Zafra et al. 1992; Miklic et al. 2004). Ces études *in vitro* révèlent que l'augmentation de la neurotransmission monoaminergique accroît les taux d'ARNm et de la protéine BDNF (Miklic et al. 2004; Juric et al. 2006; Juric et al. 2008; Laureys et al. 2010; Ohta et al. 2010). En effet, dans des cultures primaires d'astrocytes, différentes classes d'antidépresseurs mais également certains neurotransmetteurs tel que le glutamate, induisent la synthèse de ce facteur neurotrophique (Allaman et al. 2011; Kajitani et al. 2012; Kittel-Schneider et al. 2012; Takano et al. 2012). Comme nous l'avons vu précédemment, les astrocytes expriment à leur surface le récepteur TrkB-T1 qui a pour propriété de séquestrer le BDNF lorsque celui-ci est en excès

dans le milieu extracellulaire dévoilant ainsi un rôle potentiel de ces cellules gliales dans la synthèse et la libération de cette neurotrophine. Récemment, il a été rapporté que d'autres cellules gliales pouvaient libérer du BDNF. En effet, l'ARNm codant pour le BDNF a été détecté dans la microglie issue de cerveau de rat (Elkabes et al. 1996). D'ailleurs, l'activation des cellules microgliales par l'ATP, induit une libération de BDNF qui à son tour modulerait l'activité neuronale (Scholz and Woolf, 2007) soulignant un rôle du BDNF dans la communication neurone-glie.

Le BDNF, via l'activation de son récepteur de haute affinité TrKB-FL, joue donc un rôle crucial dans de nombreux mécanismes physiologiques du SNC tels que la plasticité synaptique et le processus de neurogenèse hippocampique au cours du développement mais aussi dans le cerveau mature. Le BDNF peut également se fixer sur la forme tronquée du récepteur TrKB (TrKB-T1) située sur les astrocytes et dont le couplage sert à réguler les concentrations extracellulaires de ce facteur neurotrophique par internalisation du complexe BDNF/TrKB-T1. Ces dernières observations renforcent l'idée que les astrocytes constituent une source potentielle de BDNF. Ce mécanisme pourrait expliquer, du moins en partie, le rôle de ces cellules gliales dans différentes fonctions cérébrales supérieures telle que la régulation des émotions.

B. Neuropsychopharmacologie comportementale du BDNF.

De nombreuses études génétiques, pharmacologiques et comportementales démontrent un lien entre le BDNF, ses récepteurs TrKB et la dépression (Duman et al. 1997; Sklar et al. 2002; Russo-Neustadt et al. 2003; Sen et al. 2003; Duman et al. 2006; Castren et al. 2007).

a) Effets de l'activation des voies du BDNF.

En accord avec ces données, d'autres analyses montrent que l'infusion centrale ou locale (intra-hippocampe) de BDNF induit une réponse comportementale similaire à celle des traitements antidépresseurs (Siuciak et al. 1997; Shirayama et al. 2002; Hoshaw et al. 2005). Différentes études rapportent que la stimulation de la signalisation BDNF/TrKB induit des effets de types antidépresseurs chez les rongeurs. Par exemple, Koponen et collaborateurs ont observé que la surexpression de TrKB chez la souris réduit le phénotype anxio-dépressif dans le test du labyrinthe en croix surélevé (EPM) et le test de Porsolt (Koponen et al. 2004; Koponen et al. 2005). De manière intéressante, il a également été observé que la phosphorylation de CREB, mais également du récepteur TrKB, est augmentée au niveau du cortex préfrontal suite à l'administration aigue d'antidépresseurs

(Saarelainen et al. 2003), démontrant ainsi que les effets thérapeutiques de ces molécules passe par l'activation des voies de signalisation du BDNF.

b) Effets de l'inactivation du BDNF.

Certaines études révèlent que les souris hétérozygotes pour le BDNF (BDNF^{+/-}) présentent un phénotype anxieux/dépressif (Rios et al. 2001; Chen et al. 2006; Monteggia et al. 2007) alors que d'autres analyses ne rapportent pas de modifications phénotypiques chez ces souris (MacQueen et al. 2001; Chourbaji et al. 2004; Ibarguen-Vargas et al. 2009). En revanche, les études s'accordent sur le fait que l'inactivation constitutive partielle du BDNF atténue les effets comportementaux et neurochimiques induis par un traitement aigu d'antidépresseurs chez le rat ou la souris (Guiard et al. 2008; Ibarguen-Vargas et al. 2009), suggérant que ce facteur serait nécessaire à l'activité thérapeutique des ISRS (Saarelainen et al. 2003). De manière intéressante, la neurogenèse dans le DG de l'hippocampe est réduite chez les souris BDNF^{+/-}, s'accompagnant également d'une réduction du volume du DG (Lee et al. 2002).

Chen et collaborateurs ont élaboré une lignée de souris transgéniques exprimant de manière constitutive le variant allélique "Met" du BDNF (souris homozygotes BDNF^{Met/Met}), associé à une diminution de sécrétion du BDNF dans le SNC. Ces souris présentent des caractéristiques phénotypiques similaires aux individus porteurs de l'allèle "Met", telles qu'une réduction de leur volume hippocampique et une altération des performances mnésiques dépendantes de l'hippocampe et de l'amygdale (Chen et al. 2006). De manière intéressante, les auteurs observent que l'intensité de ces déficits est proportionnelle au nombre d'allèles "Met" exprimés : les souris homozygotes BDNF^{Met/Met} présentent ainsi des troubles plus marqués que les animaux hétérozygotes BDNF^{Val/Met}. Le polymorphisme du gène du BDNF pourrait donc conférer une certaine vulnérabilité vis-à-vis d'une situation de stress, prédisposant les sujets porteurs de l'allèle "Met" à certaines formes d'anxiété. Bien que cette hypothèse soit en accord avec des études cliniques, les résultats des études d'associations génétiques chez l'Homme entre ces variants alléliques et l'anxiété et/ou la dépression semblent plus controversés (Sen et al. 2003; Jiang et al 2005; Lang et al. 2005; Surtees et al. 2007).

Par ailleurs, la fixation du BDNF sur son récepteur TrkB induit l'activation de cascades intracellulaires, faisant intervenir entre autre ERK. Ainsi, il a été observé que l'infusion d'inhibiteurs d'activité tyrosine kinase ou d'inhibiteurs sélectifs du ERK bloque l'apparition des effets comportementaux des antidépresseurs (Shirayama et al. 2002).

La régulation transcriptionnelle du BDNF est sous le contrôle de nombreux facteurs de transcription, incluant CREB. De manière intéressante, Nakagawa et collaborateurs ont observé que la surexpression transgénique du dominant négatif de CREB entraîne une diminution de la

neurogenèse dans le DG de l'hippocampe (Nakagawa et al. 2002), un mécanisme qui pourrait contribuer à l'atténuation de l'activité thérapeutique des antidépresseurs (Santarelli et al. 2003).

Des observations précliniques, chez le rat, révèlent que le stress et la corticostérone, des facteurs connus pour leur implication dans l'apparition des épisodes dépressifs majeurs, diminuent l'ARNm et la protéine du BDNF dans l'hippocampe, et plus particulièrement dans les régions CA1, CA3 et le DG (Barbany et al. 1992; Nibuya et al. 1995; Smith et al. 1995; Ueyama et al. 1997; Russo-Neustadt et al. 2001; Marakami et al. 2005; Duman et Monteggia, 2006) mais également celle du récepteur TrKB (Nibuya et al. 1999). Or, une neurogenèse active a été associée à une rémission du phénotype de type dépressif chez le rat (Mateus-Pinheiro et al. 2013).

c) Études cliniques.

Les études cliniques sur le BDNF révèlent l'existence d'un lien entre l'expression de ce facteur et la gravité des épisodes dépressifs majeurs (Karege et al. 2002; Lang et al. 2004; Shimizu et al. 2003). Ainsi, il a été observé dans le sérum de patients dépressifs, une diminution des concentrations en BDNF (Karege et al. 2002; Karege et al. 2005a), corrigée par un traitement chronique d'antidépresseurs tels que les ISRS et les TCA (Chen et al. 2001; Shimizu et al. 2003; Gervasoni et al. 2005; Gonul et al. 2005; Karege et al. 2005b). Des données récentes montrent aussi une réduction du volume de certaines aires cérébrales et notamment de l'hippocampe et du cortex préfrontal chez des patients dépressifs (Sheline et al. 2000; Pezawas et al. 2004, Chepenik et al. 2009). Comme l'hippocampe est l'un des sièges du processus de neurogenèse chez l'adulte, la diminution de l'expression de BDNF dans cette région pourrait induire un effet délétère sur la production de nouveaux neurones et participer à la diminution du volume hippocampique, prédisposant alors à l'apparition des troubles psychiatriques (Duman et al. 1997; Gould et al. 1998). Ces données sont en accord avec d'autres études cliniques démontrant une augmentation des concentrations de BDNF hippocampique (Chen et al. 2001; Sheline et al. 2003) mais également une augmentation de la neurogenèse et du volume hippocampique, suite à un traitement chronique d'antidépresseurs (Sahay et al. 2007; Boldrini et al. 2009; Boldrini et al. 2012; Kheirbek et al. 2013; Spalding et al. 2013).

En plus du stress environnemental, certains polymorphismes génétiques influent sur la physiopathologie de la dépression mais également sur l'activité des antidépresseurs (Sullivan et al. 2000). Des techniques d'imagerie médicale montrent que les individus porteurs de l'allèle muté du polymorphisme "Val66Met", présentent une réduction du volume de l'hippocampe et du cortex préfrontal (Pezawas et al. 2004) ce qui prédisposerait à l'apparition d'épisodes dépressifs majeurs (Chen et al. 2004; Pezawas et al. 2004). Or, d'autres observations ne sont pas parvenues à démontrer un tel lien (Tsai et al. 2003; Kunugi et al. 2004; Surtees et al. 2007). L'implication de ce SNP dans la réponse aux antidépresseurs a été proposée mais demeure controversée (Tsai et al. 2010) (Tableau 1).

Récemment, 83 nouveaux polymorphismes du BDNF ont été identifiés dont certains semblent impliqués dans la qualité de réponse aux antidépresseurs (Licinio et al. 2009). L'analyse individuelle de ces SNP permettra de préciser le lien éventuel entre le BDNF et la réponse aux antidépresseurs chez les patients déprimés.

Polymorphisme	genotype	Ethnie	ISRS	Corrélation	Référence
196 G/A	GG/AG/AA	Caucasien	ISRS	0	Bukh et al., 2010
	GG/AG/AA	Asiatique	fluoxetine	0	Chi et al., 2010
	GG/AG/AA	Caucasien	ISRS	0	Wilkie et al., 2007
	GG/AG/AA	Asiatique	fluvoxamine	0	Yoshida et al., 2007
	GA	Caucasien	ISRS	↗	Taylor et al., 2011
	GA	Asiatique	fluoxetine	↗	Zou et al., 2010
	GA	Asiatique	citalopram	↗	Choi et al., 2006
	GA	Asiatique	fluoxetine	↗	Tsai et al., 2003
	AA	Caucasien	ISRS	↗	Domschke et al., 2010
	AA	Caucasien	escitalopram	↗	Alexopoulos et al., 2010
	GG	Asiatique / Caucasien	citalopram	↗	Lanctôt et al., 2010

Tableau 1 : Tableau récapitulatif des principales données bibliographiques sur l'impact du polymorphisme "Val66Met" du BDNF sur l'activité des antidépresseurs.

Le genotype GG correspond à l'acide aminé valine et le génotype AA correspond à l'acide aminé méthionine. Le signe 0 représente l'absence de corrélation tandis que la flèche représente une corrélation positive.

d) Intéactions entre le BDNF et le système sérotoninergique.

1. Impact du BDNF sur le système sérotoninergique.

Le récepteur TrkB est exprimé au niveau des noyaux du raphé dorsal et médian (Madhav et al. 2001), et il existe un transport rétrograde du BDNF des terminaisons synaptiques sérotoninergiques situées dans le cortex vers les corps cellulaires des noyaux du raphé (Anderson et al. 1995; Sobreviela et al. 1996). Ainsi, des études *in vitro* et *in vivo* démontrent que le BDNF stimule la survie et la différenciation morphologique des neurones 5-HT au cours du développement (Zhou et al. 2000; Rumajogee et al. 2002; Djalali et al. 2005). De même, il a été observé que l'infusion de BDNF stimule la croissance des axones sérotoninergiques (Mamounas et al. 2000) mais également l'expression du transporteur SERT et de la tryptophane hydroxylase, l'enzyme responsable de la synthèse de 5-HT (Siuciak et al. 1996; Zhou et al. 2000; Goggi et al. 2002; Guiard et al. 2008). De

plus, l'administration intra-cérébroventriculaire (ventricule latéral) de BDNF induit des effets de type antidépresseurs accompagnés d'une augmentation de l'expression des gènes codant pour les récepteurs 5-HT_{1A} et 5-HT_{2A} au niveau de l'hippocampe (Naumenko et al. 2012) tandis que l'infusion de BDNF dans l'hippocampe ventral potentialise les effets de la fluoxétine (Deltheil et al. 2009) suggérant que les effets comportementaux et neurochimiques induits par l'infusion de BDNF passeraient, entre autre, par la stimulation de la neurotransmission sérotoninergique (Figure 13). En accord avec ces données, les souris hétérozygotes BDNF^{+/-} présentent au contraire une diminution du nombre et de la densité des neurones 5-HT au niveau du tronc cérébral, mais également une réduction de la clairance synaptique de sérotonine, en rapport avec une diminution de l'expression et de l'activité du SERT (Lyons et al. 1999; Szapacs et al. 2004; Daws et al. 2007; Deltheil et al. 2008; Guiard et al. 2008). Enfin, il a été observé une altération de l'activité du récepteur 5-HT_{1A} (Hensler et al. 2007) ainsi que des récepteurs 5-HT_{2A} et 5-HT_{2C} dans l'hippocampe des souris BDNF^{+/-} (Lyons et al. 1999; Rios et al. 2006).

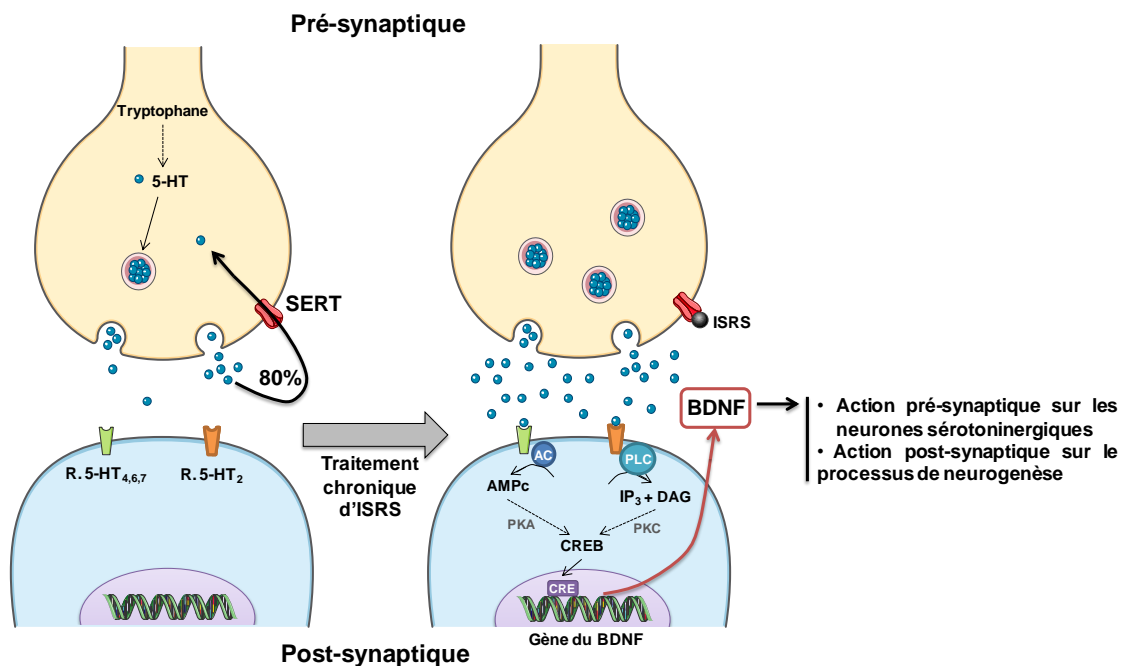


Figure 13 : Représentation schématique du mécanisme d'action des antidépresseurs du type ISRS

La sérotonine (5-HT) est synthétisée à partir du tryptophane. En conditions physiologiques, 80% de la 5-HT libérée est recaptée par le transporteur de haute affinité SERT, situé au niveau des terminaisons pré-synaptiques. Lors d'un traitement chronique d'ISRS, le blocage du SERT, associé à une désensibilisation du frein inhibiteur exercé par l'autorécepteur 5-HT_{1A}, induit une forte élévation des concentrations extracellulaires de 5-HT conduisant à la stimulation des récepteurs sérotoninergiques présents sur les cellules post-synaptiques. Ceci va provoquer l'activation des voies de signalisation intracellulaires mettant en jeu notamment l'adénylate cyclase (AC), la phospholipase C (PLC) et les protéines kinases A et C (PKA et PKC). L'activation de ces effecteurs et enzymes aboutirait à l'augmentation du facteur de transcription CREB, responsable du contrôle de l'expression du gène codant pour le BDNF. Une fois synthétisé et libéré, le BDNF exercerait des effets pré-synaptiques sur les neurones sérotoninergiques mais également post-synaptiques sur le processus de neurogenèse hippocampique via l'activation de son récepteur de plus haute affinité TrkB.

2. Impact du système sérotoninergique sur le BDNF.

Les stress aigus et chroniques ont des effets délétères sur l'expression du BDNF (Nibuya et al. 1999; Karege et al. 2005) et il a été proposé que le système sérotoninergique pourrait jouer un rôle dans ces effets. D'ailleurs, des études révèlent que le stress perturbe le système sérotoninergique et plus particulièrement l'expression de ses récepteurs dans l'hippocampe (Flugge et al. 1995; Lanfumey et al. 1999; Bambico et al. 2009). Ainsi, il a été proposé que la diminution de BDNF induite par le stress pourrait être due, en partie, à une diminution de la neurotransmission sérotoninergique (Figure 14). Au contraire, de nombreuses études laissent entrevoir que la transmission sérotoninergique exerce un puissant contrôle positif sur l'expression du BDNF, à l'origine des effets thérapeutiques des antidépresseurs (Nibuya et al. 1995) (Figure 14). Ces données supposent que l'élévation des concentrations extracellulaires de 5-HT induite par le blocage du SERT, augmenterait la synthèse de BDNF via l'activation des récepteurs sérotoninergiques post-synaptiques excitateurs tels que les récepteurs 5-HT_{4,6,7} couplés à des protéines G_s ou les récepteurs 5-HT₂ couplés à des protéines G_q, associés respectivement à l'adénylate cyclase et à la phospholipase C. Ainsi, la stimulation de ces récepteurs pourrait induire l'activation de cascades de signalisation intracellulaire aboutissant à la phosphorylation CREB, et par voie de conséquence à l'augmentation de l'expression du BDNF (Nibuya et al. 1996; Shaywitz et Greenberg, 1999; Conti et al. 2002).

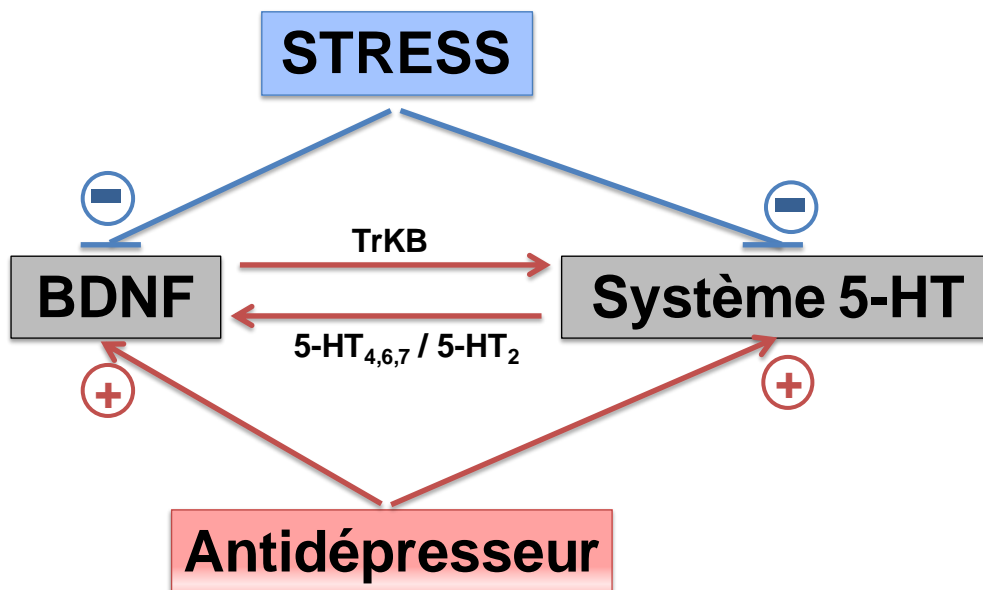


Figure 14 : Représentation schématique des interactions réciproques entre le BDNF et le système sérotoninergique en réponse à un stress ou à l'administration d'ISRS.

Bien que d'autres études cliniques soient nécessaires pour confirmer ces observations, l'ensemble de ces données cliniques et précliniques mettent en exergue le rôle du BDNF dans les troubles dépressifs, et notamment dans la vulnérabilité au stress, mais également

dans la réponse aux antidépresseurs. L'hypothèse concernant l'implication du BDNF en lien avec le système sérotoninergique dans cette réponse est apparue au milieu des années 90 mais doit encore être confirmée. En particulier le(s) sous-type(s) de récepteur(s) post-synaptique(s) responsable(s) de l'augmentation de l'expression de BDNF n'ont pas été identifiés. Ce travail de thèse nous a conduit à explorer le rôle éventuel du récepteur 5-HT_{2A}.

C. Le récepteur 5-HT_{2A} : nouvelle cible post-synaptique dans le traitement de la dépression.

a) Nouvelle cible dans le traitement de la dépression.

Les récepteurs de la famille 5-HT₂ comme la plupart des récepteurs sérotoninergiques appartiennent à la grande classe des récepteurs couplés aux protéines G (GPCR), possédant 7 domaines transmembranaires. Les récepteurs 5-HT₂ sont couplés à la phospholipase C (PLC), par l'intermédiaire de protéines G_{q/11} (Raymond et al. 2001; Masson et al. 2012). Il existe trois sous-types de récepteurs 5-HT₂ : les récepteurs 5-HT_{2A}, 5-HT_{2B} et 5-HT_{2C}.

La stimulation de ces récepteurs induit l'activation de la voie de signalisation de la PLC, provoquant l'hydrolyse du phosphatidyl-inositol biphosphate (PIP₂) en inositol triphosphate (IP₃) et diacylglycérol (DAG), agissant tous deux comme des seconds messagers. Ainsi l'IP₃, en se liant aux récepteurs présents sur le réticulum endoplasmique, va induire une élévation des [Ca²⁺]_i (Millan et al. 2008). Par la suite, le Ca²⁺ se fixe sur la calmoduline, ce qui va avoir pour conséquence d'activer la protéine kinase C (PKC) (Landolt et al. 2009). L'activation de cette enzyme stimule la voie de signalisation de ERK_{1/2} (Millan et al. 2008) conduisant à l'induction de différents gènes dont celui du BDNF. En accord avec ces données, une étude *in vitro* démontre que l'application d'un agoniste préférentiel des récepteurs 5-HT_{2A}, le DOI, induit une élévation de la signalisation de CREB et de l'expression de l'ARNm du BDNF dans des cultures de neurones corticaux (Vaidya et al. 1997; Chalecka-Franaszek et al. 1999; Vaidya et al. 1999). En revanche, le DOI induit une diminution de l'activation CREB et de l'expression de l'ARNm du BDNF dans des neurones hippocampiques. De manière intéressante, le BDNF joue en retour un rôle important dans le maintien de l'activité des récepteurs 5-HT_{2A} post-synaptiques au niveau cortical (Rios et al. 2006) et hippocampique (Klein et al. 2010), mettant ainsi en lumière un éventuel "cross-talk" dans les liens entre le BDNF et le système sérotoninergique mais dont les modalités restent encore peu connues. Étant donné l'impact du BDNF dans la neurogenèse hippocampique, ces observations suggèrent un rôle des récepteurs 5-HT_{2A} dans la

régulation de ce processus, ce qui en fait donc une cible intéressante dans le développement de nouvelles thérapies antidépressives.

De nombreuses études démontrent que le récepteur 5-HT_{2A} est exprimé chez l'Homme dans la plupart des régions riches en terminaisons sérotoninergiques (Nomura et al. 2006). L'utilisation des techniques d'immunohistochimie ont permis de démontrer que les récepteurs 5-HT_{2A} étaient principalement localisés sur les inter-neurones GABAergiques situés dans les régions dopaminergiques (aire tegmentale ventrale, striatum et noyau accumbens), noradrénergiques (locus coeruleus) et probablement sérotoninergique (Burnet et al. 1995; Rodriguez et al. 2000; Bombardi et al. 2011; Bombardi et al. 2012; Celada et al. 2013). Toutefois, la lésion des neurones 5-HT par la 5,7-dihydroxytryptamine (5,7-DHT) ne modifie pas la densité des sites de liaison de l'antagoniste 5-HT_{2A} la kétansérine, démontrant que ce récepteur ne serait pas directement présent sur les neurones 5-HT (Fischette et al. 1987). Dans le cortex, les récepteurs 5-HT_{2A} sont situés sur des neurones glutamatergiques pyramidaux qui se projettent dans le DRN (Celada et al. 2001; Martin-Ruiz et al. 2001). Par ce mécanisme les récepteurs 5-HT_{2A} semblent jouer un rôle important dans l'activité des neurones sérotoninergiques pré-synaptiques dans l'hippocampe.

Le détail plus précis de la distribution de ce récepteur et de son rôle dans la dépression, le mécanisme d'action des ISRS et le contrôle de la neurotransmission monoaminergique pré-synaptique, sont présentés dans la revue 2 de ce mémoire bibliographique. Les prochains chapitres de cette thèse apportent les éléments en faveur d'un rôle potentiel des récepteurs 5-HT_{2A} dans la synthèse de BDNF par les astrocytes.

b) Astrocytes et récepteur 5-HT_{2A}.

La première démonstration de la présence de récepteurs sérotoninergiques sur les astrocytes a été faite par Hertz et collaborateurs en 1979. Ces données ont ensuite été confirmées par l'identification des récepteurs 5-HT_{2A}, 5-HT_{2B} et 5-HT_{2C} et de leur ARNm dans des cultures primaires d'astrocytes issus de différentes régions cérébrales de rat dont l'hippocampe (Hansson et al. 1987; Decher et al. 1993; Chen et al. 1995; Hirst et al. 1998).

1. Récepteur 5-HT_{2A} et métabolisme astrocytaire.

L'application de 5-HT sur des cultures d'astrocytes, mais également d'agonistes du récepteur 5-HT_{2A}, stimule le métabolisme astrocytaire. Ainsi la 5-HT augmente la glycolyse dans le cerveau (Quach, 1978; Quach et al. 1982). A l'inverse des neurones dépourvus de réserve en glycogène, les astrocytes possèdent toutes les enzymes capables de synthétiser et métaboliser le

glycogène, lui permettant d'être la principale source de ce métabolite dans le cerveau (Ignacio et al. 1990; Pfeiffer et al. 1990). Le métabolisme du glycogène astrocytaire assure la production de métabolites énergétiques, comme le lactate et le glucose, qui sont nécessaires à l'activité et à la survie neuronale (Figure 15). De manière intéressante, des études *in vitro* révèlent que l'application de 5-HT et d'agonistes du récepteur 5-HT_{2A} stimule l'activité de la glycogène phosphorylase, l'une des enzymes impliquée dans la glycogénolyse (Poblete et al. 1995). Ainsi, la neurotransmission sérotoninergique et plus particulièrement l'activation du récepteur 5-HT_{2A} astrocytaire, permet de stimuler la glycogénolyse responsable entre autre la production de lactate astrocytaire nécessaire à l'activité neuronale.

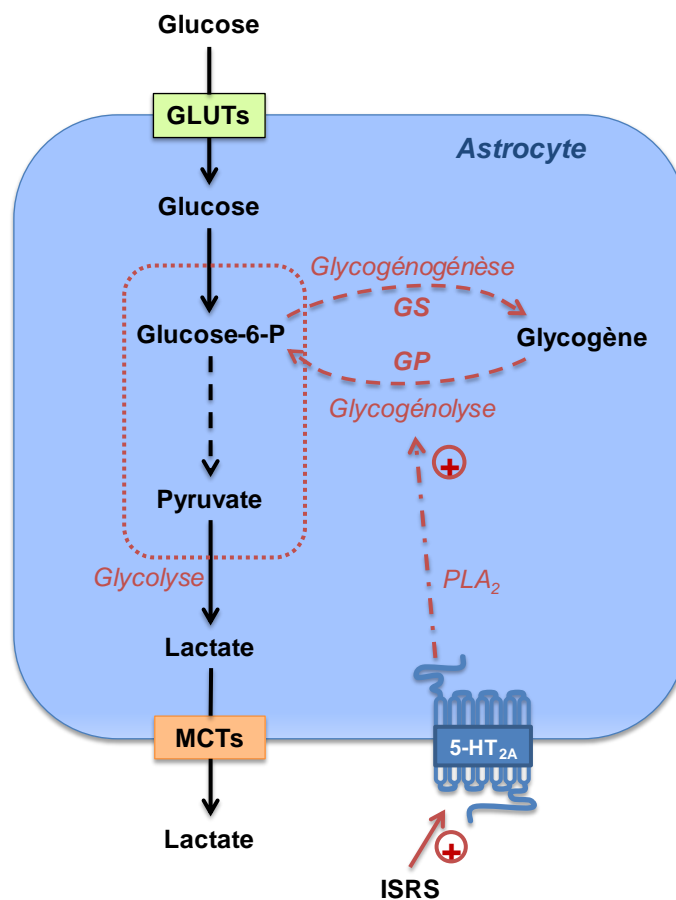


Figure 15 : Représentation schématique du métabolisme astrocytaire.

Le glucose est capté par les transporteurs spécifiques (GLUTs) puis métabolisé en glucose-6-phosphate (glucose-6-P). Le glucose-6-P, en poursuivant la voie de la glycolyse, permet la production de pyruvate et de lactate, un métabolite énergétique utilisé par les neurones. Le glucose-6-P peut également suivre la voie de la glycogénogénèse afin de permettre la formation de glycogène. Lors d'un besoin énergétique important le glycogène peut être métabolisé en glucose-6-P, via le processus de glycogénolyse, afin d'alimenter le processus de glycolyse permettant la production de lactate nécessaire à l'activité neuronale. GS : glycogène synthase ; GP : glycogène phosphorylase.

2. Récepteur 5-HT_{2A} et propriétés neurotrophiques astrocytaires.

In vitro, les astrocytes sont capables de synthétiser et de libérer de nombreux facteurs neurotrophiques, tels que le GDNF et le BDNF (Martin et al. 2013). Des observations

récentes sur des cultures d'astrocytes démontrent que l'application de 5-HT induit l'expression (ARNm) et la libération du GDNF par l'intermédiaire de l'activation des récepteurs 5-HT₂, et notamment du sous-type 5-HT_{2A} (Hisaoka et al. 2005; Tsuchioka et al. 2008) (Figure 16). Dans ce travail, les auteurs démontrent que la fixation de la 5-HT sur le récepteur 5-HT_{2A} induit la transactivation des récepteurs au FGF (pour fibroblast growth factor) aboutissant à la phosphorylation de ERK_{1/2} et par conséquent l'activation de CREB nécessaire à la synthèse du GDNF. De plus, d'autres travaux menés sur des cultures cellulaires de type C6 glioma démontrent que le GDNF n'est pas le seul facteur libéré en réponse à une stimulation sérotoninergique (Meller et al. 2002). En effet, l'application de 5-HT stimule la synthèse de novo de BDNF via des voies de signalisation Ca²⁺-dépendant, impliquant l'activation des récepteurs 5-HT_{2A}. Ces données sont en accord avec une analyse démontrant que la réduction de l'expression de l'ARNm codant pour le BDNF induite par un stress, peut être prévenue par l'administration d'un antagoniste du récepteur 5-HT_{2A} (Vaidya et al. 1997; Vaidya et al. 1999). Bien que ces données doivent être confirmées par des études *in vivo*, l'ensemble de ces observations suggèrent que la neurotransmission sérotoninergique, via la stimulation des récepteurs 5-HT_{2A}, pourrait participer à l'activation des propriétés neurotrophiques des astrocytes.

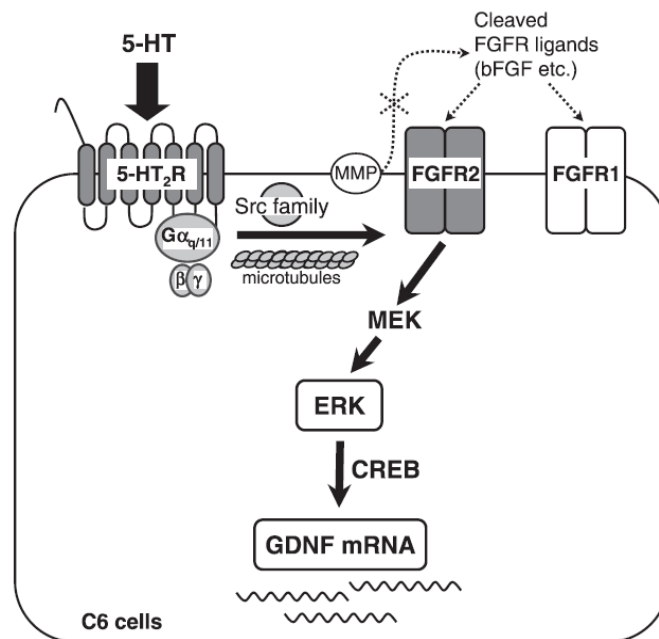


Figure 16 : Représentation schématique de la cascade de signalisation du récepteur 5-HT₂ induisant l'expression de l'ARNm du GDNF dans les cellules C6.

D'après Tsuchioka et al. 2008

Sur les cellules C6 glioma, la fixation de la 5-HT sur le récepteur 5-HT_{2A} induit la transactivation des récepteurs FGF par l'intermédiaire de processus de signalisation intra et extracellulaires impliquant les protéines de la famille SRC. La stimulation du récepteur FGF2 provoque l'activation de ERK induisant ainsi l'expression de l'ARNm codant pour le GDNF.

3. Récepteurs 5-HT_{2A} astrocytaires et antidépresseurs.

Dans les chapitres précédents nous avons vu que la stimulation des astrocytes résultant de l'augmentation de la neurotransmission sérotoninergique entraîne

l'activation/phosphorylation de ERK_{1/2}. Or, une diminution de l'activation et/ou de l'expression de ERK_{1/2} a été observée dans l'hippocampe de patients dépressifs et dans différents modèles animaux atteints de la pathologie (Gourley et al. 2008; Yuan et al. 2010). D'autres observations *in vitro* démontrent que la stimulation de ERK_{1/2} par les ISRS conduit à l'augmentation de l'expression de BDNF et GDNF dans des cultures primaires d'astrocytes et dans des cellules de type C6 glioma (Meller et al. 2002b; Mercier et al. 2004; Hisaoka et al. 2007; Tsuchioka et al. 2008; Allaman et al. 2011; Martin et al. 2013). Cette activation passerait par une transactivation des récepteurs FGF induisant la phosphorylation de ERK_{1/2} et l'activation de CREB. Ainsi, les ISRS, via la restauration de la voie de signalisation de ERK_{1/2}, permettraient de stimuler l'activité neurotrophique des astrocytes. De manière intéressante il a été montré que l'administration d'ISRS, et notamment la fluoxétine, entraîne la phosphorylation de ERK_{1/2} chez des souris ayant un phénotype dépressif via l'activation des récepteurs 5-HT_{2A} (Qi et al. 2008; Li et al. 2009; Musazzi et al. 2009) (Figure 17).

En accord avec ces résultats, d'autres données révèlent que la stimulation de ERK_{1/2} par les antidépresseurs augmente le métabolisme glucidique et la glycogénolyse (Poblete et al. 1995; Li et al. 2009), via la production d'acide arachidonique, connu pour stimuler l'absorption du glucose et la glycogénolyse cérébrale (Yu et al. 1993; Sorg et al. 1995) (Figure 17). Ces données suggèrent qu'une partie des effets thérapeutiques induit par les ISRS pourrait aussi passer par l'activation de la voie de signalisation de ERK_{1/2} astrocytaire, permettant de stimuler et/ou de restaurer les propriétés neurotrophiques et métaboliques des cellules gliales.

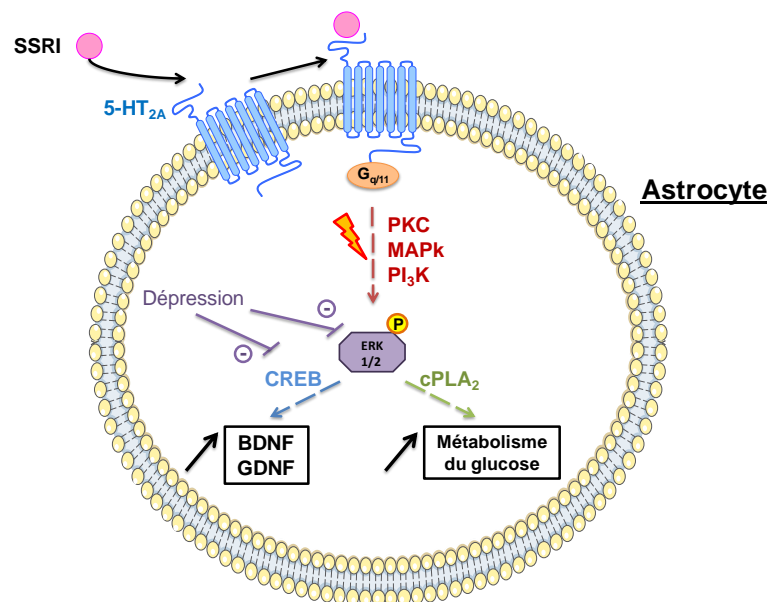


Figure 17 : Rôle des récepteurs 5-HT_{2A} astrocytaires *in vitro* dans la modulation des effets neurotrophiques et métaboliques d'un traitement d'ISRS

Les ISRS, en se fixant sur les récepteurs 5-HT_{2A} astrocytaires, induisent l'activation de cascades de signalisation intracellulaire et notamment la stimulation des protéines kinase PKC, MAPK et PI₃K, aboutissant à la phosphorylation de ERK_{1/2}. A son tour ERK_{1/2} active l'expression de BDNF et le métabolisme du glucose via la stimulation de CREB et de la cPLA₂. A l'inverse, l'exposition à un stress chronique, notamment lors d'épisodes dépressifs, réduit l'activation de ERK_{1/2} et CREB conduisant à une diminution de l'expression des facteurs neurotrophiques astrocytaires.

La neurotransmission sérotoninergique joue un rôle important dans la communication neurone/astrocyte selon différentes modalités : métaboliques et neurotrophiques. Bien qu'ils semblent avoir des effets négatifs sur l'activité des neurones 5-HT pré-synaptiques lorsqu'ils sont localisés sur les interneurones GABAergiques, les récepteurs 5-HT_{2A} situés sur les astrocytes pourraient constituer des éléments cruciaux de la communication entre ces types cellulaires. Il est donc possible qu'au niveau post-synaptique, l'activation des récepteurs 5-HT_{2A} soit nécessaire à l'activité des ISRS, notamment via la synthèse et la libération de BDNF par les astrocytes. Or, cette hypothèse repose principalement sur des observations in vitro, ce qui ouvre la voie à de nombreuses études in vivo ayant pour but de confirmer que les récepteurs 5-HT_{2A} identifiés sur ces cellules gliales représentent une des cibles des ISRS. En retour, le BDNF pourrait induire des effets bénéfiques sur la survie et la maturation des neurones sérotoninergiques mais également sur l'ensemble des étapes de la neurogenèse hippocampique. A l'instar du glutamate, la 5-HT jouerait donc un rôle de couplage entre le fonctionnement astrocytaire et neuronal.

Revue 2

5-HT₂ ligands in the treatment of anxiety and depression

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Expert Opin Investig Drugs. 2012 Nov;21(11):1701-25

RÉSUMÉ :

Bien qu'il existe actuellement une grande variété d'antidépresseurs efficaces, de nombreux patients dépressifs ne répondent pas de manière optimale à ces thérapies. Il a ainsi été observé qu'environ 30% des patients souffrant de troubles anxio-dépressifs sont résistants aux traitements conventionnels, dont les inhibiteurs sélectifs de recapture de la sérotonine (ISRS). Afin de lutter contre ce phénomène, de nouvelles stratégies thérapeutiques reposant sur l'utilisation d'agents pharmacologiques ayant différentes cibles monoaminergiques (récepteurs/transporteurs) ont vu le jour.

Les mécanismes d'action ISRS reposent sur l'inhibition sélective du transporteur assurant la recapture de la sérotonine (5-HT) permettant ainsi une augmentation des concentrations extracellulaires dans la fente synaptique au niveau des terminaisons neuronales. Une des stratégies de potentialisation consiste à bloquer le frein biologique exercé par les autorécepteurs 5-HT_{1A} situé sur les corps cellulaires des neurones 5-HT dans les noyaux du raphé, longtemps considérés comme le principal mécanisme responsable du long délai d'action de cette classe d'antidépresseurs. Or de nouvelles preuves scientifiques montrent que d'autres récepteurs sérotoninergiques, jouent également un rôle inhibiteur sur le tonus 5-HT. En particulier, des travaux soulignent le fait que les récepteurs 5-HT_{2A} et 5-HT_{2C} présents dans de nombreuses structures cérébrales modulent le tonus sérotoninergique. En effet, l'inactivation des récepteurs 5-HT_{2A} et 5-HT_{2C} potentialisent les neurotransmissions sérotoninergiques et noradrénergiques centrales en réponse à l'administration chronique d'ISRS. Pourtant au niveau post-synaptique, l'inactivation de ces récepteurs pourrait avoir des conséquences délétères sur la manifestation des effets thérapeutiques des ISRS.

Cette revue bibliographique illustre le fait que les ligands des récepteurs 5-HT₂ ont de multiples effets sur les systèmes monoaminergiques, la neurogenèse hippocampique et certaines fonctions astrocytaires, ce qui complique leur utilisation clinique.

EXPERT OPINION

1. Introduction
2. Distribution of 5-HT₂ receptor subtypes in rodent brain regions related to mood disorders
3. The Role of 5-HT₂ receptors in anxiety and depression
4. Role of 5-HT₂ receptors in adult hippocampal neurogenesis
5. Expert opinion and conclusion

5-HT₂ ligands in the treatment of anxiety and depression

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Introduction: One third of depressed patients do not respond adequately to conventional antidepressants including the selective serotonin reuptake inhibitors (SSRIs). Therefore, multi-target drugs or augmentation strategies have been developed for the management of SSRIs-resistant patients. In this context, the 5-HT₂ receptor subtypes represent promising targets but their precise roles have yet to be determined.

Areas covered: The aim of this review is to shed some light on the preclinical evidence supporting the use of 5-HT_{2A} and/or 5-HT_{2C} receptor antagonists such as antipsychotics, as potential effective adjuncts in SSRIs-resistant depression. This review synthesizes the current literature about the behavioral, electrophysiological and neurochemical effects of 5-HT₂ receptors ligands on the monoaminergic systems but also on adult hippocampal neurogenesis.

Expert opinion: Although studies support the hypothesis that the inactivation of 5-HT_{2A} and/or 5-HT_{2C} receptors might be of interest to reinforce different facets of the therapeutic activity of SSRIs, this pharmacological strategy remains debatable notably because of the lack of chronic data in relevant animal models. Conversely, emerging evidence suggests that the activation of 5-HT_{2B} receptor is required for antidepressant-like activity, opening the way to new therapeutic approaches. However, the potential risks related to the enhancement of monoaminergic neurotransmissions could represent a major concern.

Keywords: 5-HT₂ receptors, antidepressant, anxiety, atypical antipsychotics, depression, monoamines, neurogenesis, selective serotonin reuptake inhibitors.

Expert Opin. Investig. Drugs [Early Online]

1. Introduction

Anxiety and depression are major burdens on society. They affect 7% of the world's population, while severe forms of depression impact 2 – 5% of the US population [1]. Furthermore, approximately 32 – 35 million adults in the United States (16%) experience an episode of major depression in their lifetime [2]. While many classes of drugs with antidepressant activity such as selective serotonin reuptake inhibitors (SSRIs) have been developed and approved [3], one third of patients do not respond to these medications [4]. Moreover, for patients who respond, 2 – 4 weeks of treatment are required to achieve a clinically meaningful effect [5]. This delayed action can be somewhat puzzling since SSRIs produce a rapid blockade of the serotonin (5-HT) transporter (SERT) and a concomitant increase in extracellular levels of 5-HT *in vivo* [6]. This gap in timing between SSRIs near immediate effect on neurotransmitter system and the slow symptomatic recovery has been explained, at least in part, from electrophysiological and microdialysis studies. Indeed, at presynaptic level, SSRIs-induced inhibition of SERT results in the activation of 5-HT_{1A} and 5-HT_{1B} autoreceptors, which produces a rapid suppression of the firing activity of 5-HT neurons and limits 5-HT release; respectively. When the treatment is prolonged, inhibitory 5-HT_{1A} autoreceptor progressively

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Article highlights.

- The pathophysiology of anxiety and depression results, at least in part, from a dysregulation of brain monoaminergic (serotonin, 5-HT; norepinephrine, NE; and dopaminergic, DA) neurotransmission. Monoaminergic neurons have complex functional and reciprocal interactions intimately linked to 5-HT₂ receptors and any action on one system may reverberate in the other systems.
- Preclinical studies indicate that the 5-HT_{2A} and 5-HT_{2C} receptor subtypes represent promising targets in selective serotonin reuptake inhibitors (SSRIs)-resistant depression. In particular, their pharmacological inactivation with antipsychotics produces antidepressant-like activities *per se* and potentiates the behavioral effects of SSRIs. Conversely, growing evidence suggests that the stimulation of 5-HT_{2B} receptor subtype is required for SSRIs-induced antidepressant-like activity.
- Because anxiety is a comorbid illness usually observed in depressed patients after acute administration of SSRIs, the beneficial effects of 5-HT_{2A} and 5-HT_{2C} receptor antagonists or 5-HT_{2B} receptor agonists on the antidepressant response could involve anxiolytic activities.
- The enhancement of monoaminergic neurotransmission constitutes another property underpinning the putative beneficial effect of 5-HT_{2A} and 5-HT_{2C} receptor antagonists in SSRIs-resistant depression.
- 5-HT_{2A} and 5-HT_{2C} receptors are expressed in the dentate gyrus of the hippocampus but their putative involvement in the enhancement of adult neurogenesis has yet to be confirmed.
- Lastly, the lack of selective 5-HT₂ receptor ligands prompts fundamental research to combine pharmacological and genetic approaches to highlight the respective contribution of 5-HT₂ receptor subtypes in the modulation of anxiety/depression and related neuronal pathways.

This box summarizes key points contained in the article.

desensitizes thereby producing an enhancement of 5-HT neurotransmission at nerve terminal [6,7]. By using a new strategy to manipulate 5-HT_{1A} receptor, it has been shown recently that mice with a low expression of 5-HT_{1A} autoreceptor in the dorsal raphe (DR) display a more rapid increase in extracellular levels of 5-HT associated with antidepressant-like phenotype in response to repeated administration of fluoxetine compared to wild-types. These results establish a causal relationship between the delay of antidepressant activity and 5-HT_{1A} receptor levels [8]. Among the strategies developed for producing faster acting antidepressants, the inactivation of 5-HT_{1A} autoreceptor with pindolol proved to be effective presumably by preventing the initial decrease in firing activity of 5-HT neurons [5]. However, several studies suggest that stimulation of 5-HT_{1A} receptor with gepirone or buspirone may also represent a valuable approach to accelerate the therapeutic activity of SSRIs [5]. Although the mechanism of action by which these 5-HT_{1A} receptor agonists would

produce beneficial effects has not been completely solved yet, it is possible that the stimulation of post-synaptic 5-HT_{1A} heteroreceptor positively affects mood [9,10]. Alternatively, one would expect a more rapid desensitization of 5-HT_{1A} autoreceptors by combining 5-HT_{1A} receptor agonist and an SSRI [7,11]. Evidence has also linked the onset of action of SSRIs with the stimulation of adult neurogenesis in the hippocampus, supporting the notion that this event is necessary for antidepressant activity. Increasing serotonergic neurotransmission in response to the chronic administration of SSRIs is thus believed to produce post-synaptic effects including the synthesis of neurotrophic substances such as brain-derived neurotrophic factor (BDNF) and the proliferation/differentiation of stem cells into neurons [12]. The identification of the post-synaptic 5-HT receptors involved in this mechanism is of particular interest.

Certain postsynaptic 5-HT heteroreceptors including 5-HT₂ receptor subtypes have warranted consideration in anxiety, depression and the mechanism of action of related treatments. 5-HT₂ receptors are members of the 7 transmembrane-spanning receptor superfamily frequently referred to as G protein coupled receptors (GPCRs). 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors couple to multiple cellular signaling pathways and are involved in the regulation of a variety of physiological brain functions [13] thereby constituting therapeutic targets for obesity, sleep, memory, addiction or psychiatric disorders such as schizophrenia, anxiety and depression [14-16]. The connection between mood disorders, antidepressant responses and 5-HT₂ receptors (mainly 5-HT_{2A} and 5-HT_{2C} subtypes) is based on several preclinical and clinical observations. First, pharmacogenetic studies in human have reported that one of the most investigated polymorphism within the *5-HT2A* gene A-1438 (rs6311) is associated with antidepressant response [17]. Regarding the 5-HT_{2C} receptor, although Cys23ser polymorphism-associated changes in 5-HT_{2C} receptor function were found to influence the vulnerability to affective disorders [18,19], there are, however, no unequivocal results for such association with antidepressant response [20]. Second, an hypersensitivity/upregulation of 5-HT_{2A} receptors [21,22] as well as an increased RNA editing and/or functional activity of 5-HT_{2C} receptor has been reported in depressed patients or in animal models of depression. Conversely, the progressive therapeutic improvement achieved with some SSRIs in rodents is accompanied by a downregulation of 5-HT_{2A} and 5-HT_{2C} receptors in rodents [23]. Accordingly, studies performed in 5-HT transporter knock-out (KO) mice, indicated that a lifelong elevation of the synaptic 5-HT concentration resulted in a downregulation of 5-HT_{2A} and 5-HT_{2C} receptor-mediated phospholipase A2 (PLA2) signaling [24] and in a reduced expression of these receptors [25]. Although the extrapolation of these findings to humans remains indeterminate, a decrease in platelet functional response mediated by 5-HT_{2A} receptors following imipramine treatment was reported in depressed patients. Hence, the desensitization or downregulation of

this receptor could be linked to the therapeutic effects of some antidepressants [26]. Imaging studies also supported this hypothesis since a downregulation of 5-HT_{2A} receptor was detected in the brain of depressed patients in response to SSRI treatment [27]. It is noteworthy that citalopram, does not produce 5-HT_{2A} or 5-HT_{2C} receptor downregulation [28] while being as effective as other SSRIs in the treatment of depression and depressive-like states, thus suggesting that such adaptive change is not sufficient to account for the therapeutic activity of this class of antidepressants. Third, the intrinsic antidepressant activity of compounds displaying 5-HT_{2A} or 5-HT_{2C} receptor antagonistic activity such as atypical antipsychotics in placebo-controlled trials [29] and the reports of the augmenting action of these pharmacological agents in SSRIs-resistant patients [30,31], further support the interest of inactivating both receptors in the treatment of depression. In an attempt to determine the mechanism of action of atypical antipsychotics, particularly on monoaminergic neurotransmission, it has been demonstrated that the sustained inactivation of the SERT induced by SSRIs progressively dampens the firing activity of noradrenergic and dopaminergic neurons via activation of 5-HT_{2A} and 5-HT_{2C} receptors in the locus coeruleus (LC) and ventral tegmental area (VTA), respectively [32-34]. The blockade of these electrophysiological responses with aripiprazole, olanzapine, paliperidone or risperidone [35] increases extracellular levels of catecholamines [36-38] which exert excitatory influence upon the 5-HT system. In addition, since the enhancement of NE or DA neurotransmission achieved with NE and/or DA reuptake inhibitors can lead to an antidepressant activity, it is conceivable that these actions account, at least in part, for the beneficial therapeutic effects of antipsychotics in SSRIs-resistant depression. Finally, there is a co-localization between 5-HT_{2A} and 5-HT_{2C} receptors within brain monoaminergic areas such as the DR nucleus, the LC, VTA and their respective nerve terminal regions eg the frontal cortex (FCx), the nucleus accumbens (NAc), the amygdala and the hippocampus that might add to our comprehension of the mechanism of action of 5-HT_{2A} and 5-HT_{2C} receptor agonists and antagonists.

The present review describes the distribution and the behavioral properties of 5-HT₂ receptors in relation with anxiety and depression in rodents supporting the idea that 5-HT_{2A}, 5-HT_{2C}, but also 5-HT_{2B} receptor agonists/antagonists, might be useful in the treatment of these pathologies given either alone or in combination with conventional antidepressants. The impact of 5-HT₂ receptors and related pharmacological agents in the modulation of monoaminergic neurotransmission is also addressed particularly from electrophysiological and neurochemical studies. Lastly, since growing evidence suggests that part of the antidepressant activity of SSRIs is mediated by the stimulation of adult neurogenesis in response to increased monoaminergic tone in the hippocampus [12], this review raises the possibility that 5-HT₂ receptor ligands might significantly influence cell fate.

Importantly, current research is facing the challenge of the absence of selective 5-HT₂ receptor subtype ligands (Table 1). As an example, after cloning the mouse 5-HT_{2C} receptor cDNAs, the respective affinity for 5-HT_{2B} and 5-HT_{2C} receptors of commercially available compounds was recently determined. The study revealed that, among these compounds, many of the so-called selective 5-HT_{2C} compounds have similar (if not higher) affinity for 5-HT_{2B} receptors in mice [39]. This point is important since it considerably limits the understanding of the precise role of the 5-HT₂ receptor subtypes in the control of mood and monoaminergic neurotransmission and might have directed researches in wrong directions. Similar remarks can be made with the lack of selective 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor antibodies that force us to consider immunohistochemistry data with caution. In this prospect, combination of pharmacological and genetic approaches will be extremely helpful and could even contradict some obvious demonstrations.

2. Distribution of 5-HT₂ receptor subtypes in rodent brain regions related to mood disorders

In the rat brain, 5-HT_{2A} and 5-HT_{2C} receptors have a widespread distribution particularly in monoaminergic regions (Figure 1 and Table 2). Their first mapping comes from an *in situ* hybridization study consisting in visualizing specific mRNA probes for 5-HT₂ receptors. In this initial study, high levels of 5-HT_{2A} receptor mRNA were detected in cortical areas including the frontal, cingulate and piriform cortices, but also in the amygdala [40]. In the hippocampus, 5-HT_{2A} receptor mRNA levels were low in the pyramidal cell layer of the CA2 and CA1 but they were very high in the ventral part of CA3 and the dentate gyrus (DG) [40]. Intermediate levels of 5-HT_{2A} receptor were seen in the NAc. Finally, in brainstem nuclei, low levels of 5-HT_{2A} receptor mRNA were found in the raphe nuclei [40,41], while no signals were detected in the LC and the VTA [40]. Advances in the field of autoradiography have also yielded considerable information on the regional distribution of 5-HT₂ binding sites. However, autoradiography, as well as *in situ* hybridization approaches, lacks high resolution prompting research to develop immunohistochemistry at the light and electron microscopic levels. Using this approach, Descarries' group confirmed the localization of 5-HT_{2A} receptor obtained from *in situ* hybridization studies. However, differences in both the number of labeled cells and the intensity of immunolabeling between anatomical regions were reported [42]. For example, in the cingulate, frontal and parietal cortices, a high number of large layer V pyramidal neurons exhibit intense 5-HT_{2A} receptor labeling of their soma and dendrites [42,43]. In the amygdala, most nuclei displayed somatodendritic labeling of weak to strong intensity but relatively low abundance, except in the basolateral nucleus [42]. The hippocampal formation showed some of the most intense and abundant 5-HT_{2A} immunostaining in the rat brain,

Table 1. Affinity constants for various 5-HT₂ receptor agonists and antagonists.

Agonists	Affinity constants (pKi ± SEM)		
	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}
5-HT	6.50 ± 0.13	6.73 ± 0.09	6.69 ± 0.12
mCPP	7.26 ± 0.02	7.39 ± 0.02	7.85 ± 0.07
DOI	9.03 ± 0.11	7.55 ± 0.13	8.08 ± 0.11
BW723C6	ND	7.89 ± 0.01	6.90 ± 0.01
Ro600175	7.44 ± 0.04	8.27 ± 0.06	8.22 ± 0.28
MK212	5.99 ± 0.06	6.21 ± 0.09	7.01 ± 0.09
WAY161503	ND	7.28 ± 0.19	7.46 ± 0.05
Antagonists	Affinity constants (pKi ± SEM)		
	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}
Ritanserin	8.34 ± 0.09	8.67 ± 0.09	8.18 ± 0.15
MDL100907	8.73 ± 0.20	5.99 ± 0.06	7.52 ± 0.13
RS127445	6.03 ± 0.13	8.97 ± 0.09	6.33 ± 0.10
Ketanserin	8.09 ± 0.08	6.13 ± 0.07	7.21 ± 0.12
Mianserin	7.74 ± 0.13	7.92 ± 0.03	8.26 ± 0.07
S32006	6.00 ± 0.07	8.03 ± 0.05	8.43 ± 0.06
SB206553	5.64 ± 0.09	7.65 ± 0.07	7.79 ± 0.07
SB242084	6.07 ± 0.13	6.84 ± 0.28	8.15 ± 0.10
SDZ SER082	6.29 ± 0.03	6.69 ± 0.05	8.12 ± 0.07

Higher the pKi value higher is the affinity for the corresponding human recombinant 5-HT₂ receptor subtype. These values have been obtained from binding studies into transfected HEK-293 or CHO cells.

Adapted from [39,245].

particularly in the DG and throughout the pyramidal neurons in CA1, CA2 and CA3 [42]. Interestingly, in contrast to *in situ* hybridization, immunoreactive soma/dendrites and axons were visible at all monoaminergic brainstem levels. Abundant, but less intense, immunoreactivity was observed in the median raphe nucleus. There were no labeled soma/dendrites within the dorsal raphe nucleus. In the pons, only few weakly labeled dendrites, but no labeled soma, were visible in the LC. In contrast, moderate to strong somatodendritic labeling was found in the VTA [42]. Recently, it has been reported that cells within the rat VTA displayed a strong immunoreactivity for 5-HT_{2A} receptor co-localized with tyrosine hydroxylase suggesting the presence of this receptor subtype on DA neurons cell bodies [44,45]. Although more rare, non-DA neurons would also express 5-HT_{2A} receptor immunoreactivity in the VTA [45].

Regardless 5-HT_{2C} receptor, immunohistochemical analysis of the rat brain unveiled the presence of this receptor subtype in the cerebral cortex with highest levels in the frontal, parietal and cingulate cortices [46,47]. Most of the nuclei in the amygdala contained dense 5-HT_{2C} receptor labeling. Abundant immunopositive neurons were found in the pyramidal cell layer of the CA1, CA2 and CA3 regions of the hippocampus [46,47]. In the brainstem, the raphe nucleus is endowed with a rich population of 5-HT_{2C} receptor suggesting the presence of this protein on 5-HT neurons. Using double *in situ* hybridization to examine the cellular localization of 5-HT_{2C} receptor mRNA in relation to serotonergic and

GABAergic neurons, it was reported that in the dorsal and median raphe nuclei, 5-HT_{2C} receptor mRNA was not detected in serotonergic cells identified as those expressing 5-HT transporter mRNA. In contrast, 5-HT_{2C} receptor mRNA was found in most GABAergic cells, identified by the presence of glutamic acid decarboxylase (GAD) mRNA [48]. Although data are lacking regarding the putative localization of 5-HT_{2C} receptor in the LC, using a double double-label immunofluorescence techniques in the VTA, it has been recently reported that the 5-HT_{2C} receptor was present on GABA neurons in rats [49] but also present in cells that contained immunoreactivity for tyrosine hydroxylase, validating the localization of this receptor type directly on DA neurons which project to the NAC [49,50].

Finally, in the rat brain 5-HT_{2B} receptor is present in discrete nuclei and evidence for its presence in the central nervous system (CNS) mainly comes from pharmacological studies. The first characterization of the 5-HT_{2B} receptor in the adult brain was obtained from the mouse brain using a subtype-specific antiserum directed against this receptor. Data showed that the expression of this receptor was mainly found in cerebellar nuclei [51].

3. The Role of 5-HT₂ receptors in anxiety and depression

Pharmacological evidence supports a role of 5-HT₂ receptors in the modulation of anxiety and depression. As an example, the non-selective 5-HT₂ receptor agonist mCPP precipitates anxiogenic responses in naïve rodents [52-56], whereas numerous examples unveiled an anxiolytic-like effect of non-selective 5-HT₂ receptor antagonists including ritanserin, mesulergine and ketanserin [56-61]. Despite these data, little is known about the specific contribution of each 5-HT₂ receptor subtype in the modulation of these behavioral responses because of the lack of highly selective receptor ligands. This chapter synthesizes the current knowledge about the effects of the preferential 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor agonists/antagonists in animal paradigms used to screen anxiolytic-/antidepressant-like activities (Table 3). It also provides an overview of the electrophysiological and neurochemical effects of these ligands on the monoaminergic systems that might explain, at least in part, their behavioral effects.

3.1 The 5-HT_{2A} receptor

3.1.1 Behavioral studies

In the mouse elevated plus maze (EPM), the preferential 5-HT_{2A} receptor agonist DOI produced anxiolytic-like activity that was attenuated by mianserin, ketanserin or the 5-HT_{2A} receptor antagonist SR46949B [62]. The observation that the 5-HT_{2C} or 5-HT_{2B/2C} receptor antagonists RS10-2221 and SB206553, respectively, failed to block DOI-induced anxiolysis demonstrated the selective involvement of 5-HT_{2A} receptor in this activity [61,63]. This response is apparently mediated by post-synaptic 5-HT_{2A} receptor since

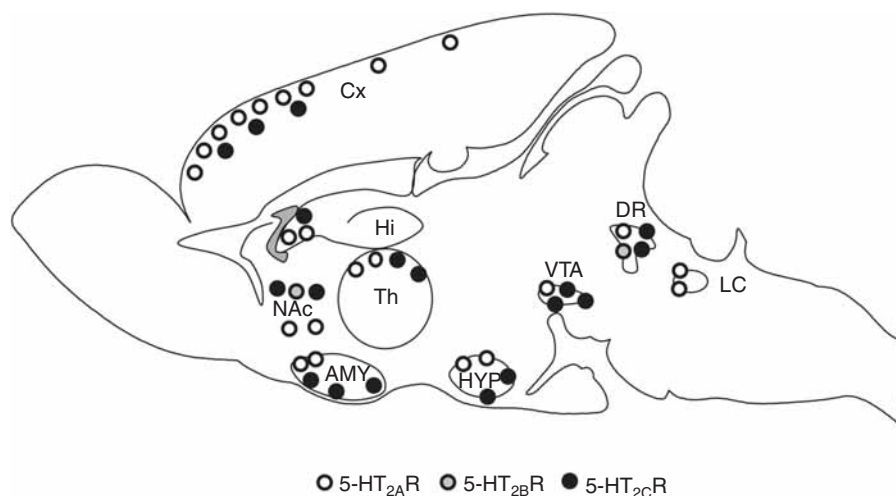


Figure 1. Distribution of the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in the brain monoaminergic regions involved in anxiety and depression.

AMY: Amygdala; Cx: Cortex; DR: Dorsal raphe; Hi: Hippocampus; HYP: Hypothalamus; LC: Locus coeruleus; NAc: Nucleus accumbens; Th: Thalamus; VTA: Ventral tegmental area.

the lesion of the serotonergic or the noradrenergic system did not affect the anxiolytic-like effect of DOI evaluated, for example, in the four plate test (FPT) [64]. In order to determine the brain region involved in this behavior, the possibility that the anxiolytic-like effects of DOI resulted from the activation of 5-HT_{2A} receptors in the hippocampus [65] or the periaqueductal grey (PAG) [66] has been proposed notably after its local microinjection in these brain regions. Interestingly, studies indicate that the 5-HT_{2A} receptor antagonist MDL100907 alone lacked consistent activity in selected rodent models of anxiety [60,67], thus suggesting that 5-HT_{2A} receptor is not tonically activated to regulate this behavior or that distinct neuro-anatomical pathways are involved with major regional differences. To illustrate this latter hypothesis, a reduced level of anxiety has been reported in constitutive 5-HT_{2A} receptor KO mice evaluated in the EPM, the open field (OF) and the light-dark [68]. The selective re-introduction of the 5-HT_{2A} receptor in the cortex normalized anxiety-like behavior in these 5-HT_{2A} KO mice. Consequently, although data emphasized that 5-HT_{2A} receptor activation in the hippocampus or PAG produced anxiolysis, a specific anxiogenic role of this receptor in the cortex is also possible [68].

Numerous studies have associated 5-HT_{2A} receptor activation with a depressive-like behavior. In behavioral paradigms relevant to depression, DOI significantly increased immobility time in the mouse forced swim test (FST) and this effect was abolished by a pretreatment with MDL100907 [69]. This result strongly suggested that 5-HT_{2A} receptor antagonists might produce antidepressant-like activity. Accordingly, in the rat FST, the 5-HT_{2A} receptors antagonists EMD281014 or MDL100907 decreased the immobility time [70,71]. Similarly, the novel 5-HT_{2A} receptor antagonist BIP-1 has been shown to produce antidepressant-like activities in the mouse FST

and TST [72]. Although, these findings are consistent with the observation that antisense-mediated downregulation of the 5-HT_{2A} receptor decreased immobility in the mouse FST [73], the role and mechanism of action of 5-HT_{2A} in other animal paradigms relevant to depression or after chronic administration should inspire us for future investigations. In this prospect, it has been demonstrated recently that the sustained administration of BIP-1 attenuated behavioral anomalies detected in bulbectomized rats [72]. Finally, the blockade of 5-HT_{2A} receptor by MDL100907 augmented the antidepressant-like effects of fluoxetine in rats [74] underlying the possible synergic effects between these pharmacological agents.

3.1.2 Electrophysiological studies

In vitro recordings in the DR showed that the local application of 5-HT produced hyperpolarization of tryptophan hydroxylase (Tph) positive neurons [75]. Similarly, in rat brain slices, DOI induced a concentration-dependent increase in the frequency of inhibitory postsynaptic currents (IPSCs). Together with the observation that these effects were blocked by bicuculline or MDL100907, a local interaction between 5-HT_{2A} receptor and GABAergic neurons in the DR has been proposed [76]. Indeed, although low levels of 5-HT_{2A} receptor mRNA have been detected in the DR [40,41], endogenous 5-HT would act on excitatory 5-HT_{2A} receptor located on GABA neurons that would ultimately suppress the firing of DR 5-HT neurons. This mechanism is consistent with the hypothesis that the 5-HT_{2A} receptor antagonists could prevent the inhibitory effects of SSRIs on 5-HT neurons by preventing the local release of GABA release within the DR. *In vivo* studies performed in rodents confirmed these *in vitro* data since the systemic or local administration of DOI in the DR reduced the discharge of 5-HT neurons [77-82]

Table 2. Relative distribution of 5-HT₂ receptors in monoaminergic region in rat.

Monoaminergic terminal regions	Density of 5-HT ₂ receptors	
	5-HT _{2A}	5-HT _{2C}
Cortex		
Frontal	+++	++
Parietal	++	+++
Cingulate	+++	++
Amygdala		
Basolateral nucleus	++	+++
Medial nucleus	+	+++
Hippocampus		
CA1/CA2	+++	+++
CA3	+++	+++
DG	+++	+++
Nucleus accumbens	++	++
Monoaminergic somatodendritic region		
Dorsal raphe nucleus	+	+++
Ventral tegmental area	+	++
Locus coeruleus	+	ND

The intensity of immunostaining is graded as weak (+), moderate (++) or strong (+++).

Adapted from [42,47].

ND: Not Determined.

and these effects were reversed by ritanserin or MDL100907 [81]. Importantly, the systemic administration of DOI increased c-Fos immunoreactivity in the DR specifically in GABAergic interneurons [83]. The role of 5-HT_{2A} receptor in the regulation of DR 5-HT neuronal activity might also involve indirect mechanisms. As an example, the LC that sends noradrenergic projections to the DR [84,85], expresses 5-HT_{2A} receptor. It is well accepted that sustained SSRIs administration enhanced 5-HT transmission to NE neurons in the LC [32,86,87] thereby producing a marked suppression of the firing activity of NE neurons through activation of excitatory 5-HT_{2A} receptors located on GABAergic interneurons [32,86,87]. Given the excitatory influence of noradrenergic terminals on 5-HT neurons in the DR (Figure 2) [88,89], activation of 5-HT_{2A} receptor in the LC seemingly favors the inhibition of the serotonergic system and might account for the depressive-like behavior induced by acute administration of DOI.

Another modality of interaction between 5-HT_{2A} heteroreceptor and DR 5-HT neurons concerns the recruitment of the medial prefrontal cortex (mPFCx) [42]. Several studies from Dr Artigas' group demonstrated that the local application of DOI in this cortical area increased the firing rate of DR 5-HT neurons [80,82]. This might result from activation of excitatory cortical glutamatergic pyramidal neurons projecting to the DR in response to DOI administration (Figure 2) and might explain, at least in part, that the systemic administration of 5-HT_{2A} receptor antagonists such as risperidone or

clozapine, dose-dependently decreased DR 5-HT neuronal activity [90,91]. In addition, evidence also demonstrated that 5-HT_{2A} receptor located in the mPFCx modulated the neuronal activity of VTA DA neurons [92] which have strong anatomical and functional interactions with the DR [85]. Electrophysiological studies showed that the systemic or local injection of DOI in the mPFCx increased VTA DA firing rate [93], an effect that might contribute to facilitate the activation of 5-HT neurons since several studies demonstrated the excitatory impact of DA in the DR [85,89,94]. Therefore, it appears that 5-HT_{2A} receptor activation elicits inhibitory or excitatory influences on DR 5-HT neuronal activity depending on the brain region where this receptor type is recruited.

3.1.3 Neurochemical studies

In agreement with the fact that activation of 5-HT_{2A} receptor can reduce the firing activity of DR 5-HT neurons, it has been demonstrated that the systemic administration of DOI to chloral hydrate-anesthetized rats reduced the extracellular 5-HT concentration in the mPFCx, an effect antagonized by MDL100907 [80]. Despite this result, the local application of DOI, through reverse dialysis, in the medial rat or mouse PFCx dose-dependently increased 5-HT local outflow which is blocked by the application of MDL100907, but not of SB242084 [80,82]. Subsequent studies demonstrated that the neurochemical effects of DOI in the mPFCx involved the activation of 5-HT_{2A} receptor expressed on glutamatergic neurons. This activation would enhance the activity of excitatory pyramidal neurons projecting to the DR, thus increasing serotonergic activity and 5-HT release in projection areas. Additionally, glutamate might also activate terminal glutamatergic receptors located on 5-HT terminals and increase 5-HT release locally in the mPFCx (Figure 3) [80,95,96]. These examples concur with electrophysiological studies demonstrating the existence of distinct pathways leading to inhibitory or excitatory effects on the serotonergic system. Basically, the net effect of 5-HT_{2A} receptor activation after systemic administration of selective agonists decreases 5-HT tone whereas the recruitment of 5-HT_{2A} receptor specifically in the cortex can produce opposite responses.

Interactions between 5-HT_{2A} and DA neurons in the PFCx may also be important in the modulation of DA release. In rat, the systemic or local injection of DOI in the mPFCx increased cortical DA release [97-102] probably through the recruitment of a specific population of excitatory cortical pyramidal neurons projecting to the VTA [93,103]. Alone, the 5-HT_{2A} receptor antagonists MDL100907, MDL11939 and SR46349B injected systemically or directly in the rat mPFCx through reverse dialysis had little or no effect on basal DA efflux [98,100]. In the NAc, the systemic administration of DOI also resulted in a robust increase in DA outflow [104,105]. These results agreed with the observation that stimulated DA release was blocked by MDL100907, thus strengthening the hypothesis that 5-HT_{2A} receptor activation favors DA neurotransmission in the NAc [106]. Interestingly, the electrical

Table 3. Behavioral effects of 5-HT₂ receptor ligands given alone in animal paradigms relevant to anxiety/depression.

5-HT ₂ receptor ligand	Route	Test	Specie	Behavioral property		Ref.
				Anxiety	Depression	
mCPP	Syst	Light/Dark	Mouse	↗		[54]
mCPP	Syst	SIT	Mouse	↗		[55]
mCPP	Syst	EPM	Mouse	↗		[119]
mCPP	Syst	EPM	Mouse	↗		[120]
mCPP	Intra-AMY	EPM	Mouse	↗		[121]
mCPP	Syst	SIT	Rat	↗		[53]
mCPP	Syst	SIT	Rat	↗		[138]
mCPP	Syst	Tmaze	Rat	↗		[56]
mCPP	Syst	EPM	Rat	↗		[118]
DOI	Syst	EPM	Mouse	↘		[62]
DOI	Syst	FPT	Mouse	↘		[64]
DOI	Syst	EPM/FPT	Mouse	↘		[61]
DOI	Intra-PAG	EPM	Mouse	↘		[65]
DOI	CA2 Hipp	FPT	Mouse	↘		[66]
DOI	Syst	FST	Mouse		↗	[69]
BW723C86	Syst	SIT	Rat	↘		[108]
BW723C86	Syst	Vogel Conflict Test	Rat	↘		[109]
BW723C86	Syst	EPM/FPT	Mouse	↘		[61]
BW723C86	Intra-AMY	SIT	Rat	↘		[110]
BW723C86	Syst	FST	Mouse		↗	[69]
Ro60175	Syst	EPM	Mouse	↘		[61]
Ro60175	Syst	SIT	Mouse	↘		[55]
Ro60175	Syst	FST	Rat		↘	[134]
Ro60175	Syst	FST	Rat		↘	[69]
MK212	Syst	Air Pet	Rat	↘		[124]
MK212	Syst	EPM	Rat	↘		[123]
MK212	Intra-BLA	ETM	Rat	↘		[125]
MK212	Ventral-Hipp	EPM	Rat	↘		[127]
WAY163909	Syst	FST	Rat		↘	[71]
WAY163909	Syst	FST	Rat		↘	[132]
WAY163909	Syst	FST	Rat		↘	[133]
WAY163909	Syst	FST	Wistar-Kyoto Rat		↘	[133]
WAY163909	Syst	FST	Bulbectomy		↘	[133]
WAY161503	Syst	FST	Rat		↘	[134]
WAY161503	Syst	FST	Mouse		↘	[69]
Ritanserine	Syst	OF	?	↘		[59]
Ritanserine	Syst	Tmaze	Rat	↘		[56]
Ritanserine	Syst	Xmaze	Rat	↘		[57]
Ketanserine	Syst	EPM	Rat	↘		[58]
Ketanserine	Syst	Light/Dark	Rat	↘		[63]
Ketanserine	Syst	Xmaze	Mouse	↘		[57,63]
Mianserine	Syst	Vogel Drinking Test	Mouse	↘		[60]
MDL100907	Syst	Light/Dark	Mouse	0		[60]
MDL100907	Syst	?	?	0		[67]
MDL100907	Syst	FST	Rat		↘	[71]
EMD281014	Syst	FST	Rat		↘	[70]
SR46949B	Syst	FST	Mouse		↘	[56]
SB242084	Syst	ETM	Rat	↘		[128]
SB242084	Intra-BLA	ETM	Rat	↘		[125]
SB242084	Syst	FST	Rat		↘	[71]
SB200646A	Syst	ETM	Rat	↘		[56]
S32006	Syst	FST	Rat		↘	[131]

Signs ↗ indicate an anxiogenic- or depressive-like activity. Signs ↘ indicate an anxiolytic or antidepressant-like activity. Syst: systemic administration.

stimulation of the DR eliciting the release of endogenous 5-HT has been reported to increase the DA neurotransmission in the NAc, and this effect was attenuated by the 5-HT_{2A} receptor antagonist SR46349B, but not the

5-HT_{2B/2C} receptor antagonist SB206553 (Figure 3) [107]. Regarding the modulation of NE neurotransmission by 5-HT_{2A} receptor, DOI has been reported to exert an excitatory effect on the release of NE in the FCx of freely-

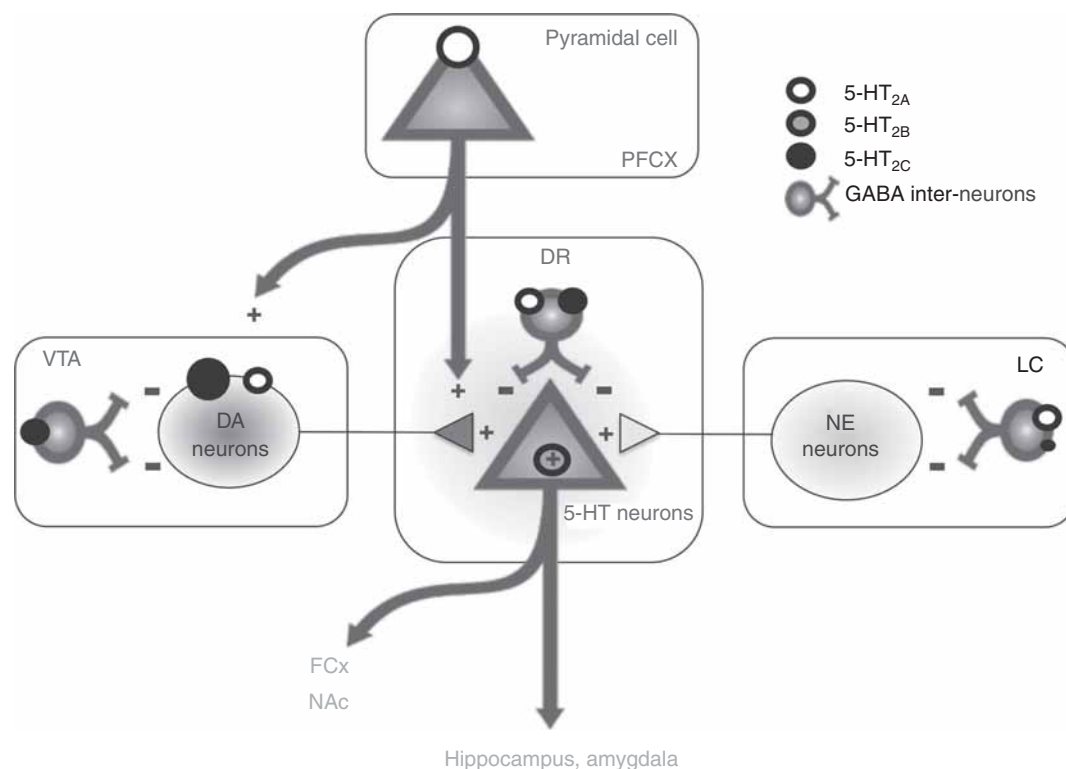


Figure 2. Neuro-anatomical substrates underlying the putative role of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor in the modulation of dorsal raphe 5-HT neuronal activity. In the dorsal raphe (DR), 5-HT_{2A/2C} receptors stimulate GABAergic interneurons thus triggering the inhibition of firing of neighboring 5-HT neurons. The presence of 5-HT_{2A} receptor in the DR mainly comes from functional studies. A recent hypothesis assumes that 5-HT_{2B} receptor would be directly express on DR 5-HT neurons and their stimulation would exert excitatory influence on this neuronal population. 5-HT_{2A/2C} receptor are also present in the ventral tegmental area (VTA) and the locus coeruleus (LC). 5-HT_{2A} and 5-HT_{2C} receptors are believed to play a prominent inhibitory effect on noradrenergic and dopaminergic neurons respectively through the stimulation of local GABA release. Remarkably, in the VTA 5-HT_{2C} receptor is also express on DA neurons where it could exert excitatory effect. Because VTA DA and LC NE neurons have strong anatomical and functional (excitatory) interaction with DR 5-HT neurons, the inhibition of catecholaminergic neurons in response to 5-HT_{2A} and/or 5-HT_{2C} receptor would favor the inhibition of serotonergic neurons through an attenuation of excitatory tone. Finally, there is a high density of 5-HT_{2A} receptor in the prefrontal cortex (PFCX), particularly on pyramidal neurons. The activation of these cells in response to 5-HT_{2A} receptor would enhance the activity of DR 5-HT by a direct or indirect pathway involving VTA DA neurons. Size of the symbols represent the relative density of 5-HT₂ receptor subtypes.

moving rats [98] but further studies are required to precise the nature of interactions between 5-HT_{2A} receptor and NE outflow at nerve terminals.

3.2 The 5-HT_{2B} receptor

3.2.1 Behavioral studies

The involvement of 5-HT_{2B} receptor in mood disorders has been poorly studied notably due to the absence of highly selective compounds. Despite such a deficiency, the recent demonstration that the 5-HT_{2B} receptor is located in brain regions relevant to anxiety and depression, strongly suggests their role in psychiatric disorders. For example, it has been proposed, that the stimulation of 5-HT_{2B} receptor produces anxiolysis [108]. As observed with benzodiazepines, the 5-HT_{2B} receptor agonist BW723C86 increased the number

of punishments accepted in a rat Vogel drinking conflict test indicative of an anxiolytic-like effect whereas this response was prevented by a 5-HT_{2B}, but not a 5-HT_{2C}, receptor antagonist [109]. The anxiolytic-like effect of BW723C86 would involve the amygdala since its bilateral microinjection in this brain region increased the total interaction in the rat social interaction test, whereas this effect was prevented by the 5-HT_{2B/2C} receptor antagonist SB200646A [110]. Consistent with these results, BW723C86 also provoked an anxiolytic-like response in the mouse FPT and the EPM [61,63].

In the mouse FST, recent findings have shown that BW723C86 significantly reduced immobility time in wild-types but not in 5-HT_{2B} KO mice [69]. Interestingly, although the genetic inactivation of 5-HT_{2B} receptor failed to affect the spontaneous behavior of mice in the FST, 5-HT_{2B} KO

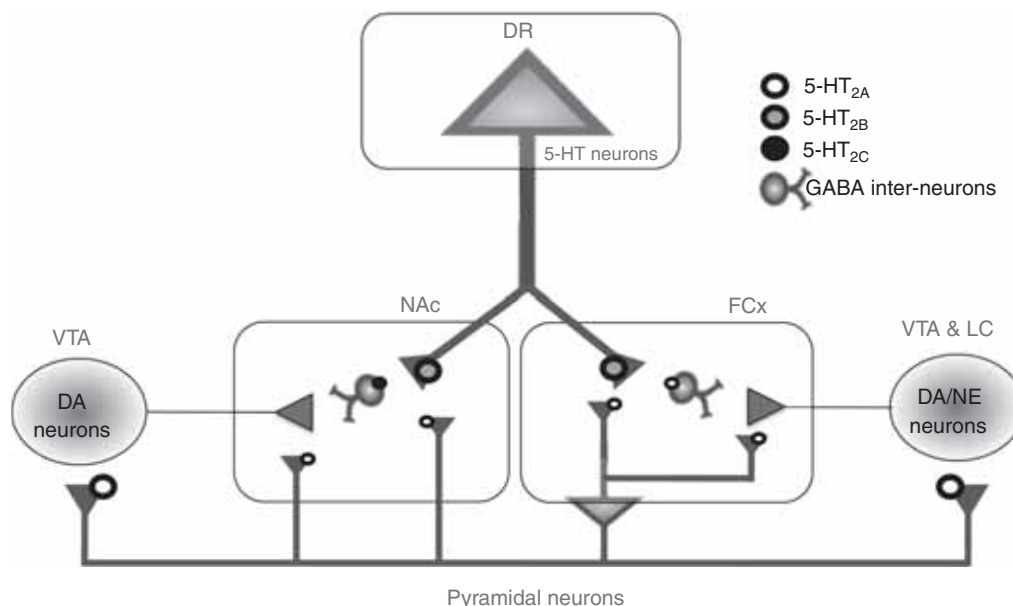


Figure 3. Neuro-anatomical substrates underlying the putative role of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor in the modulation of 5-HT release at terminal level. The dorsal raphe (DR) sends serotonergic projection in the nucleus accumbens (NAc) and the frontal cortex (FCx). The release in 5-HT in the NAc and the FCx would be positively regulated by 5-HT_{2B} autoreceptors located presynaptically on 5-HT terminals. Increase in 5-HT release stimulates 5-HT_{2A} and 5-HT_{2B} heteroreceptors located postsynaptically on GABAergic interneurons thus inhibiting the local release of dopamine (DA) and/or norepinephrine (NE). In addition 5-HT_{2A} receptors have been identified on cortical pyramidal neurons and its activation on pyramidal neurons produces release of glutamate which in turn activate the meso- or cortico-limbic pathway at distinct levels i.e., the VTA, the FCx or the NAc.

mutants did not respond to the doses of SSRIs that were efficacious in reducing immobility time in wild-type littermates [69]. These results indicate that 5-HT_{2B} receptor is required for acute SSRIs effects. Accordingly, it has been recently reported that chronic administration of paroxetine or fluoxetine failed to elicit antidepressant-like effects in 5-HT_{2B} KO mice or in 5-HT_{2B} wild-types treated with the 5-HT_{2B} receptor RS127445 in the novelty suppressed feeding paradigm [111] confirming that this receptor is required for SSRIs-mediated antidepressant-like activity [111].

3.2.2 Electrophysiological studies

The contribution of central 5-HT_{2B} receptors in the modulation of monoaminergic neuronal activities has not been documented, likely due to its modest expression in the rodent brain [51]. Nevertheless, the presence of 5-HT_{2B} receptor has been reported in a large majority Tph2 expressing cells suggesting for the first time a possible existence of an excitatory autoreceptor regulating the 5-HT system [111]. Such a property would explain the fact that the 5-HT_{2B} receptor is required for the antidepressant-like activity of SSRIs. In a near future, electrophysiological studies will likely address this possibility, notably by studying the putative excitatory effect of 5-HT_{2B} receptor agonists on DR 5-HT neuronal activity. In particular such approach would be relevant under

conditions that produce a marked attenuation of 5-HT synthesis and/or release.

In the VTA, it has been shown that the non-selective 5-HT_{2B/2C} receptor antagonist SB206553 caused a dose-dependent increase in the basal firing rate of DA neurons associated with an increase in bursting activity [112,113]. These results suggest that 5-HT_{2B} receptor exerts an inhibitory influence on DA neurons, but once again, the lack of selectivity of SB206553 do not allow identifying the respective contribution of 5-HT_{2B} and/or 5-HT_{2C} receptors. In the LC, excitatory effects of SB206553 were reported on the firing activity of NE neurons but the observation that these compound reversed the 5-HT_{2C} receptor agonist Ro 600175-induced complete inhibition of LC NE neuronal activity strongly suggested the involvement of 5-HT_{2C} receptor in these electrophysiological response [113].

3.2.3 Neurochemical studies

A recent microdialysis study in mice reported that the local injection of the 5-HT_{2B} receptor agonist BW723C86 in the DR increased the local extracellular levels of 5-HT whereas pretreatment with the selective 5-HT_{2B} receptor antagonist RS127445 completely blocked this effect [114]. This is compatible with the existence of a positive feedback. However, the 5-HT_{2B} receptor antagonist RS127445 or the genetic inactivation of this receptor type in 5-HT_{2B} KO mice had

no effect on basal 5-HT extracellular concentrations in the DR, the hippocampus or the NAc [111,114] suggesting that this receptor is recruited under specific conditions to stimulate the release of 5-HT. Accordingly, it has been shown that the increase in extracellular concentrations of 5-HT in the hippocampus induced by the SSRI paroxetine in WT mice was attenuated in mice pre-treated with the 5-HT_{2B} receptor antagonist RS127445 or in 5-HT_{2B} KO mice [111]. Although the mechanism by which activation of 5-HT_{2B} receptor may stimulate the release of 5-HT is still not known, the observation that MDMA induced 5-HT release from superfused midbrain synaptosome preparation is blunted in 5-HT_{1B} KO mice, reinforced the hypothesis that 5-HT_{2B} receptor would act presynaptically in DR 5-HT neurons to enhance 5-HT release [114].

Regarding the other monoaminergic systems, recent studies suggest that 5-HT_{2B} receptor agonists or antagonists had no influence on basal DA outflow in the rat NAc [115]. However, in the NAc, it has been shown that the selective 5-HT_{2B} receptor antagonist LY266097 significantly reduced basal or stimulated DA release by haloperidol supporting a facilitatory control on mesoaccumbens DA pathway activity [115,116]. Finally, RS127445 completely blocked MDMA-induced increase in DA release in the VTA [114] and this effect could be attributed to a decreased serotonergic tone in the VTA and/or NAc, two brain regions where activation of postsynaptic 5-HT receptors such as 5-HT_{2A} receptor are known to exert stimulatory effects [117]. In the mPFCx, RS127445 has been shown to increase basal DA levels or potentiate haloperidol-induced increase in cortical DA levels [116]. These results demonstrate that the 5-HT_{2B} receptor exerts a region-dependent modulation of DA ascending pathways by producing opposite facilitatory and inhibitory control of NAc and mPFCx DA release.

3.3 The 5-HT_{2C} receptor

3.3.1 Behavioral studies

Evidence also suggests the involvement of 5-HT_{2C} receptor in anxiety. The observation that the anxiogenic-like effects of the non selective 5-HT₂ receptor agonist mCPP in rats [118-120], were blocked by various 5-HT_{2C} receptor antagonists given either systemically or directly injected in the amygdala, supported this hypothesis [121]. The role of amygdala has been strengthened by the observation that the microinjection of the 5-HT_{2C} receptor agonists mCPP or CP809101 into the amygdala induced anxiogenic-like effects [121] or mimicked the effect of stress in rats [122]. These results also agree with findings reporting that the systemic injection of the preferential 5-HT_{2C} receptor agonists MK-212 or Ro600175 produced anxiogenic-like effects in rodents [55,123-125] whereas such responses were abolished by the microinjection of ritanserin into the basolateral nucleus of the amygdala (BLA) [123]. Given the lack of high selectivity of these agonists, part of their anxiogenic-like activity might have resulted from the activation of 5-HT_{2B} receptor. This is, however, unlikely

since we have detailed above the anxiolytic effect of 5-HT_{2B} receptor activation. A recent study aimed at increasing 5-HT_{2C} receptor expression in the mouse amygdala using recombinant viral approach reported a robust correlation between anxiogenic-like effects in the EPM and OF and the density of the receptor [126]. In addition, the observation that the microinjection of MK-212 in the rat ventral, but not dorsal, hippocampus also resulted in anxiogenic-like effects in the EPM [127] indicated that other brain regions of the limbic system are involved in the anxiogenic effect of 5-HT_{2C} receptor activation. Conversely, the genetic or pharmacological inactivation of 5-HT_{2C} receptor with SB200646A, SB242084 or S32006 reduced anxiety levels [56,125,128,129] but evidence suggests that stressful or anxiogenic conditions are a prerequisite for such behavioral activity [23,130,131].

With respect to depression, initial studies reported that the 5-HT_{2C} receptor agonist WAY-163909 decreased immobility time in the rat FST with a significant effect on swimming behavior, but not on climbing. The reduction in immobility produced by WAY163909 were fully reversed by the 5-HT_{2C/2B} receptor antagonist SB206553 underlying the role of 5-HT_{2C} and/or 5-HT_{2B} receptors in mediating these behavioral effects [71,132,133]. Accordingly, antidepressant-like effects of the 5-HT_{2C} receptor agonists were confirmed with WAY161503 and Ro600175. It is noteworthy that in these latter studies 5-HT_{2C} receptor agonists were injected three times before the test session [71,134], a procedure that might have induced a desensitization of 5-HT_{2C} receptor. However, this possibility is unlikely because co-administration of a 5-HT_{2C} receptor antagonist prevented the antidepressant-like effect of 5-HT_{2C} agonists in the FST. Remarkably, 5-HT_{2C} receptor agonists were also shown to produce antidepressant-like effects in animal models of depression including in Wistar-Kyoto or bulbectomized rats [133,135] or in the anhedonia model of depression in rat [136]. Despite these clear evidence for a beneficial effect of 5-HT_{2C} receptor activation in mediating antidepressant properties, other studies showed that the 5-HT_{2C} receptor agonists WAY161503 or CP809101 significantly increased immobility time in the mouse FST [69] or produced learned helplessness-behaviors [137] whereas the novel 5-HT_{2C} receptor antagonist S32006 displayed a broad spectrum of antidepressant-like effects notably in the rat FST [71,131] and suppressed anhedonia in a chronic mild stress paradigm after two weeks of treatment [131]. These latter data with S32006 are somewhat puzzling since genetic (i.e., in constitutive 5-HT_{2C} KO mice) or acute pharmacological inactivation of this receptor did not affect behavior in the FST or in the tail suspension test (TST) [69,134,138-142], thus reflecting a lack of tonic control of this specific behavior by 5-HT_{2C} receptor. However, they concur with the potentiating activity of 5-HT_{2C} receptor antagonist on various SSRIs-induced antidepressant-like effects in the FST or TST [141-145]. In light of the conflicting data reporting similar antidepressant-like effects of 5-HT_{2C} receptor agonists or

antagonists, further analyses are needed to define their precise role in the regulation of depressive-like symptoms. One reason of such discrepancies might come from the weak selectivity of the ligands tested [39]. For example, S32006 display a non-negligible 5-HT_{2B} antagonistic activity as demonstrated from *in vitro* data [131]. However, as long as the relative *in vivo* occupancy at both 5-HT_{2C} and 5-HT_{2B} receptors will remain indeterminate, it will be difficult to anticipate the real target of S32006. Alternatively, the experimental conditions and by analogy with anxiety, the levels of stress before the tests, might affect the behavioral response of 5-HT_{2C} ligands. Strain differences might be considered as well since this parameter has a significant impact on stress sensitivity [146]. It has also been proposed that such contradictions could be related to the defense reaction of the animals submitted to stressful situations particularly in the FST and TST [147]. This implicates that a battery of behavioral assays known to recapitulate diverse anomalies observed in depression will help probably refining the role of 5-HT_{2C} receptor in this disorder. Finally, psychostimulant effects or signs of serotonergic syndrome resulting from 5-HT_{2C} receptor agonists [69,145] might had represented confounding parameters towards the resignation evaluated in these paradigms. Despite all these considerations, further investigations evaluating the impact of long-term administration of 5-HT_{2C} receptor agonists are required. These receptors can be submitted to adaptive changes such as a functional desensitization and this process could provide a cellular explanation for the antidepressant effect of 5-HT_{2C} receptor agonists and antagonists.

3.3.2 Electrophysiological studies

In the DR, *in vitro* recordings of 5-HT neurons showed that 5-HT application evoked inhibitory GABA-mediated postsynaptic currents which were partially attenuated by the 5-HT_{2C} receptor antagonist SB242084 [76]. More recently, *in vivo* electrophysiological studies reported that the systemic administration of the preferential 5-HT_{2C} receptor agonists WAY161503 and Ro 600175 inhibited DR 5-HT neuronal activity in a SB24204-reversible manner [81,148,149]. Immunohistochemical experiments demonstrated that WAY161503 and Ro 600175 increased Fos expression specifically in GAD-positive neurons located in the DR, whereas SB 242082 antagonized this effect [81,83,148]. These data seemingly confirm that 5-HT_{2C} receptor activation in the DR stimulated neighboring GABA neurons. Substantial evidence demonstrated that the 5-HT_{2C} receptor also modulates the neuronal activities of LC NE and VTA DA neurons. Indeed, the 5-HT_{2C} receptor agonists Ro 600175, MK-212 and WAY163909 decreased the firing activity and/or the number of spontaneously active LC NE [113] and VTA DA neurons [34,150-154] whereas these effects were blocked by SB 242084. Interestingly, in the VTA SB242084 caused a dose-dependent increase in the basal firing rate of DA neurons [154] suggesting that the 5-HT_{2C} receptor exerts a tonic inhibitory control of mesolimbic dopaminergic pathway

(Figure 2) [112,150]. All these inhibitory actions of 5-HT_{2C} receptor agonists after acute administration on monoaminergic neuronal activities seem in accordance with a depressive-like effect but the it is noteworthy that chronic treatments might produce opposite effects.

3.3.3 Neurochemical studies

The role of 5-HT_{2C} receptor in the regulation of 5-HT extracellular levels at nerve terminals has been poorly documented. A recent study reported however that an acute administration of the 5-HT_{2C} receptor agonist Ro 600175 reduced extracellular 5-HT levels while increasing GABA levels in the DR of DBA/2 mice [155]. Similar inhibitory properties of Ro 600175 were reported on cortical 5-HT outflow in response to increased serotonergic tone in mice [23]. These results concur with electrophysiological studies reporting an indirect inhibitory effect of 5-HT_{2C} receptor activation on 5-HT neuronal activity. Remarkably, the acute systemic and/or intradrug infusion of 5-HT_{2C} receptor antagonist had no effect alone on extracellular 5-HT level in the DR and mPFCx in rats [113,156] or mice [143,155] but potentiated escitalopram induced increase in 5-HT outflow in these brain regions in relation with a decrease in GABA concentrations [155]. These results suggest the existence of a low level of constitutive activation of 5-HT_{2C} receptor involved in the regulation of the serotonergic system. In contrast, in response to an elevation of endogenous 5-HT induced by SSRIs, 5-HT_{2C} receptor is likely recruited to exert a negative feedback. In this specific condition of elevated 5-HT tone, 5-HT_{2C} receptor antagonists represent a classical augmentation strategy for antidepressants (see expert opinion section).

The role of this receptor type on DA neurotransmission attracted more attention, particularly in the PFCx and the NAc. In the PFCx, the systemic or intra-VTA administration of Ro 600175 blocked stress-induced increase in DA extracellular levels without altering basal levels [157]. Alone, SB242084 increased basal extracellular DA and completely prevented the effects of Ro 600175 in anaesthetized rats [157] confirming that endogenous 5-HT acting on 5-HT_{2C} receptor tonically inhibits basal or stimulated DA release in the PFCx. A particularly intriguing hypothesis is that fluoxetine may modulate DA outflow in cortical areas by interaction with 5-HT_{2C} receptor. Fluoxetine has moderate antagonistic activity towards 5-HT_{2C} receptors in rats brain [158] and this pharmacological property may favor the release of DA in the PFCx [156], possibly by blocking tonic and phasic 5-HT inhibitory control of VTA DA neurons (Figure 3) [150]. However, this hypothesis should be tempered by the fact that the affinity of fluoxetine for the SERT is 20 fold higher than for the 5-HT_{2C} receptor and consequently a marginal effect at this receptor could be expected. In the NAc, systemic administration of 5-HT_{2C} receptor agonists significantly decreased basal or stimulated DA release in rats [150,159-161] and these effects were blocked by SB 242084 [151,161-163]. These effects were mediated by 5-HT_{2C} receptor located in both VTA and

NAC because intra-VTA or- NAC microinjection of SB242084 and/or SB243213 prevented the decrease in accumbal DA outflow induced by Ro 600175 [164]. Conversely, basal DA release was significantly enhanced in the NAC following the systemic administration of selective or non selective 5-HT_{2C} receptor antagonists such as SB242084 and SB206553, respectively [161,165], indicating that 5-HT_{2C} receptor also exerts a tonic and phasic inhibitory control on mesolimbic DA neuron activity [112]. The observation that SB206553-stimulated DA release was insensitive to reduction of 5-HT neuronal function induced by the 5-HT_{1A} agonist 8-OHDPAT or intra-raphé injections of the neurotoxin 5,7-dihydroxytryptamine provided the first *in vivo* evidence that constitutive activity of the 5-HT_{2C} receptor tonically inhibits mesencephalic DA neurons [161]. Finally, several studies have shown that NE release is increased following systemic administration of 5-HT_{2C} receptor antagonists [15,113,156].

4. Role of 5-HT₂ receptors in adult hippocampal neurogenesis

Preclinical studies emphasized the role of neurogenesis in the pathophysiology and the mechanism of action of SSRIs. Although careful examination of the literature suggests that the complex biological and psychological changes associated with depression cannot be attributed to disturbance in hippocampal neurogenesis alone [166], fundamental research remains of interest to anticipate the putative therapeutic activity of new drugs on this process. 5-HT is a potent regulator of adult hippocampal neurogenesis [12] and an increase in 5-HT tone resulting from prolonged SSRI treatment favors this process [167]. In contrast, decrease in 5-HT is believed to attenuate neurogenesis as suggested by the observation that 5-HT depletion in rats significantly reduced the number of newborn cells in the subgranular zone (SGZ) of the hippocampus [168]. Interestingly, ablation of neurogenesis by X-rays attenuated the antidepressant-like effects of SSRI in mice [169]. All these data suggested that part of the antidepressant-like activity of SSRIs would be mediated through neurogenesis-dependent mechanism in rodents [170,171]. Despite the extensive literature concerning the impact of 5-HT in adult neurogenesis, its precise mechanism of action is not fully understood and various post-synaptic 5-HT receptors might have diverse, possibly opposing effects on different stages of neuronal development in the adult dentate gyrus (DG) of the hippocampus. The first step in improving the knowledge on the effect of 5-HT on the various stages of the neurogenesis phenomenon (proliferation, differentiation, maturation and survival) is to identify the cell types in the course of adult neurogenesis on which 5-HT receptors are expressed.

4.1 Direct stimulation of neurogenesis by 5-HT₂ ligands

A dense staining of 5-HT_{2A} receptors in the hilus and of 5-HT_{2C} receptors in the granule cell layer of the DG has

been yielded (Table 2) [172] suggesting a role of these receptors in the modulation of adult neurogenesis in the hippocampus. An *ex vivo* study in rats, pointed out that acute treatment with either the 5-HT_{2A} and 5-HT_{2C} receptor agonists DOI or Ro 600175 respectively, had no effect on cell proliferation in the SGZ of the dorsal hippocampus in rats [173,174]. However, these results have been challenged by a more recent study showing that an acute administration of the non-selective 5-HT₂ receptor agonist alpha-methyl-5-HT and the selective 5-HT_{2C} receptor agonist WAY161503 decreased cell proliferation in this brain region [172]. In this study, the fact that the acute administration of the 5-HT₂ receptor antagonist cinanserin increased cell proliferation while reducing the percentage of BrdU⁺/DCX⁺ (immature neurons) strongly suggest that the inactivation of 5-HT₂ receptor stimulate the proliferation of cells displaying an undermined phenotype [172]. The hypothesis that some modifications could occur following chronic administration as observed with SSRIs has been also examined. It has been observed that the sustained administration of the non selective 5-HT₂ receptor antagonist ketanserin resulted in a robust increase in progenitor proliferation [173] without commensurate change in doublecortin (DCX)-positive immature neurons within this duration and dendritic maturation of DCX-positive newborn neurons [173,174]. Similarly repeated administration of the 5-HT_{2C} receptor antagonists such as cinanserin, SB243213 or S32006 increased cell proliferation [131,172,175] thus suggesting that the specific blockade of 5-HT_{2C} receptor is involved in hippocampal plasticity. Despite these data, the mechanism by which 5HT_{2A} and/or 5HT_{2C} receptor(s) might interfere on neurogenesis remains poorly documented. It was, however, reported that the systemic administration of DOI led to a significantly decrease in brain-derived neurotrophic factor (BDNF) mRNA levels within the DG of the hippocampus [176]. These data suggest that the inactivation of 5-HT_{2A} and/or 5-HT_{2C} receptor might stimulate the production of neurotrophins. To our knowledge, no evidence has been reported that 5-HT_{2C} receptor antagonists stimulate the production of neurotrophins in the hippocampus. Although these receptors may regulate neurogenesis directly, there are some indicators that they might exert their neurotrophic activity through the regulation of NE and/or DA release in the hippocampus. These data are of particular interest since it was reported that inactivation of D2-like receptor decreased neurogenesis [177] and the production of CNTF [178]. With respect to the role of 5-HT_{2B} receptor in the modulation of hippocampal neurogenesis, there is only once recent study showing that sustained administration of the 5-HT_{2B} receptor agonist BW723C86 stimulates cell proliferation whereas SSRI-induced neurogenesis (i.e., cell proliferation and survival) is impaired in 5-HT_{2B} receptor KO [111]. These latter results may be explained by the fact that the enhancement of hippocampal serotonergic transmission induced by SSRI is attenuated in mutant mice.

4.2 Indirect stimulation of neurogenesis by 5-HT₂ ligands through modulation of astrocytes function

The role of astrocytes in depression and the therapeutic effects of SSRIs is gaining growing interest [179]. For example, it has been reported that the density of astrocytes was dramatically reduced in the brain of depressed patients [180] and in animal models of depression [181] while lesioning astrocytes, notably in the cortex by the local injection of the gliotoxin L-alpha-aminodipate, produced behavioral changes indicative of depressed-like symptoms [182]. Conversely, the administration of antidepressants would normalize this cellular alteration [179]. 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor subtypes were identified in primary cultures of astroglial cells and their activation by SSRIs enhance the metabolic activity of astrocytes [183-185]. In particular, it is well accepted that SSRIs activate 5-HT_{2A} receptor and stimulate signaling intracellular cascades leading to the phosphorylation/activation of extracellular signal-regulated kinases (ERK1/2) [186-188]. Hence, antidepressants would exert their therapeutic activity, at least in part, by stimulating this pathway (Figure 4). In the hippocampus, ERK1/2 have been implicated in mood [189] as suggested by their blunted activation and/or expression in both depressed patient [190] and animal models of depression [191]. Moreover, a downregulation of ERK1/2 has been associated with an impairment of adult hippocampal neurogenesis [192]. All together, these results suggest that astrocytes may constitute a microenvironment permissive for neurogenesis [193]. Recent arguments also suggest that astrocytes could promote the synthesis and release of growth and neurotrophic factors [194,195], a mechanism required for the neurogenesis-dependant activity of SSRIs. Accordingly, *in vitro* studies reported that SSRIs stimulate the expression of BDNF, Glial-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF and VEGF) in primary culture of astrocytes and C6 glioma cells [195-198]. *In vivo* preliminary data showed that the specific over-expression of BDNF in hippocampal astrocytes produced antidepressant-like effect in relation with an increase in cell proliferation, maturation and survival of new generated cells in the DG of the hippocampus [199].

5. Expert opinion and conclusion

Most antidepressants currently approved act by enhancing 5-HT neurotransmission. SSRIs increase the synaptic availability of 5-HT by inhibiting the 5-HT transporter SERT. This class of antidepressant is well tolerated and safer than the first generation agents. Despite their therapeutic action, residual symptoms remain and may explain the fact that approximately 50% of depressive individuals do not respond adequately to these agents [200]. Moreover, for patients who respond, 2-4 weeks of treatment are required to achieve a clinically meaningful effect [5]. It has been proposed that antidepressants with more than one mechanism of action would be more effective than agents with a single

pharmacological target [201] [202]. A network meta-analysis reveals that the dual serotonin/norepinephrine reuptake inhibitors (SNRI) venlafaxine is more efficacious than the SSRIs fluoxetine, fluvoxamine and paroxetine [203] but less than citalopram, escitalopram [204-206] and sertraline [203]. Extending this observation to the fact the SNRI duloxetine failed to display a clinical superiority over SSRIs [203], there is no clear evidence that the simultaneous blockade of the 5-HT and NE transporters offers a beneficial therapeutic alternative in non-responders to SSRIs. Importantly, the superiority of a pharmacological compound lies on both its ability to reverse depressive symptoms (therapeutic efficacy) and its acceptability. Hence, regarding SNRIs, it clearly appears that their « acceptability » is lower than that of SSRIs [203] thereby challenging their superiority. In addition, the current evaluations of antidepressants are mainly focusing on mood, whereas other types of symptoms regulated by 5-HT and NE such as pain [207] or cognition [208] should be considered as well. For example, growing evidence suggests that changes in emotional memory are particularly relevant to antidepressant response [209]. In addition, because both 5-HT and NE are involved in mood, emotion, cognition while symptoms such as vigilance, arousal, interest and energy are most closely associated with NE neurotransmission, SNRIs could be used to trigger residual symptoms that may impede full remission or favor relapse within brief delay [210]. Converging lines of evidence also indicates that drugs enhancing dopaminergic neurotransmission such as bupropion can diminish anhedonia and produce antidepressant activities by itself or in combination with SSRIs in treatment-resistant patients [211]. These clinical considerations, have given rise to a new class of antidepressants, named the Triple Reuptake Inhibitors (TRIs), that simultaneously inhibit 5-HT, NE and DA reuptake, with the hope to offer a clinically relevant advantage over single- or dual-acting agents [84]. However, most of them have been discontinued in part due to the risk of addiction resulting from DA increases in the NAc pointing out the necessity to develop compounds that would regionally elevate DA in brain areas outside the NAc. The 5-HT_{2B} receptor antagonists decreasing DA release in this brain region [114-116] can be interesting in this perspective. An other reason that might explain the disappointment based on TRIs concerns the functional interactions between 5-HT, NE and DA neurons [84] occurring at both somatodendritic and nerve terminals levels. These interactions are strongly regulated by 5-HT₂ receptors and an increase in 5-HT levels in the brain induced by SSRIs may produce counter-productive effects on NE or DA neuronal activities but also on the 5-HT systems itself. The first example is provided by the activation of 5-HT_{2A} known to decrease the activity of 5-HT and NE systems. These effects might explain the depressive-like effects produced by the acute systemic administration of selective 5-HT_{2A} receptor agonists. Hence, pharmacological compounds blocking these heteroreceptors might be of interest for preventing SSRI-induced inhibition of NE neurons

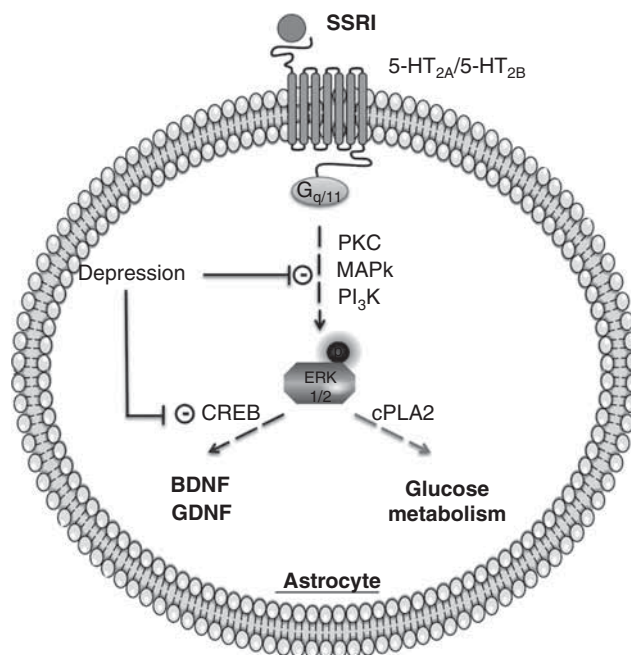


Figure 4. Putative role of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor located on astrocytes in the modulation of adult neurogenesis in the dentate gyrus of the hippocampus. 5-HT₂ receptors are Gq/11 protein-coupled receptors and their activation stimulates phospholipase C (PLC), which generate diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP₃), by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂). The ultimate step of this intracellular cascade lead to an increase in cytosolic calcium concentration ($[Ca^{2+}]_i$). Increase in $[Ca^{2+}]_i$ was detected in primary cultures of cortical astrocytes after the stimulation of the 5-HT₂ receptor induced by the SSRIs fluoxetine and citalopram. Then, in this signaling cascade, increase in $[Ca^{2+}]_i$ and DAG results in the activation of protein kinase C which in turn, stimulates phosphorylation of extracellular signal-regulated kinases (ERK1/2). On one hand, ERK1/2 may favor the production of neurotrophic factors such as BDNF and GDNF and consequently may play a critical role in the neurogenesis. On the other hand, ERK1/2 may activate PLA2, which is involved in glucose metabolism. Importantly, correlation has been observed between severity of depression and reduction of glucose metabolism in depressed patients whereas chronic treatment with SSRIs normalizes this alteration by activating PLA2. This effect of SSRIs has been attributed to the activation of 5-HT_{2B} located on astrocytes.

[29,33,91] and consequently for improving antidepressant activity. In agreement with this assumption, 5-HT_{2A} receptor antagonists were recently reported to potentiate the neurochemical effects of SSRIs, particularly their ability to increase the extracellular levels of NE (Table 4) [36-38,74,149,212-214]. It is conceivable that the advantages of this combination therapy may bear a relationship with the extracellular concentration of NE rather than 5-HT. In line with this argument, it has been shown that the blockade of 5-HT_{2A} receptor augmented the antidepressant-like effects of fluoxetine in rats without a concurrent increase in extracellular levels of 5-HT [74]. Further preclinical investigations are required to confirm the putative relevance of inactivating 5-HT_{2A} receptor [72] and determine the precise mechanism by which 5-HT_{2A} receptor antagonists (i.e., antipsychotic drugs) such as aripiprazole, clozapine, olanzapine, risperidone, quetiapine, and ziprasidone have been reported to be effective in SSRI-resistant depression [35,215-219] either alone or as adjuncts to SSRIs.

Several reports, also pointed out a clear inhibitory action of 5-HT_{2C} receptor agonists on 5-HT and DA systems [117] and these effects may hamper the antidepressant activity of SSRIs. It can thus be anticipated that the pharmacological inactivation of 5-HT_{2C} receptor might also facilitate 5-HT and DA neurotransmissions. Accordingly, the selective 5-HT_{2C} receptor antagonist SB242084 or the non selective 5-HT_{2C/2A} receptor antagonist ketanserin prevented the inhibitory effect of citalopram on 5-HT cell firing in the DR [220] and potentiated the neurochemical effect of this SSRI on 5-HT extracellular levels in both the hippocampus and frontal cortex (Table 4) [139,220,221]. Additionally, SB-242084 was found to restore the antidepressant-like effect of citalopram in DBA/2N mice that do not respond to SSRIs alone [143] or more generally to enhance the behavioral responses of serotonergic antidepressants in the FST and TST [141,142,144,145]. Regarding the dopaminergic system, as detailed before, it is well known that SSRIs induced decrease in the activity of the dopaminergic system is blocked by 5-HT_{2C} receptor

Table 4. Potentiation of SSRI-induced increase in extracellular levels of monoamines at nerve terminal by (A) antipsychotics displaying 5-HT_{2A} receptor antagonistic activity or (B) by 5-HT_{2C} receptor antagonist.

SSRI	5-HT _{2A} antagonist	Potentiation of SSRI-induced increase in extracellular levels of monoamines				
		Region	5-HT	NE	DA	Ref.
Citalopram	Risperidone	mPFCx	0	+	+	[38]
	MDL100907	HP	+	ND	ND	[149]
	MDL100907	HP	0	ND	ND	[139]
Fluoxetine	Olanzapine	PFCx	0	+	+	[36]
	Clozapine	PFCx	0	0	+	[36]
	Risperidone	PFCx	0	0	+	[36]
	MDL100907	PFCx	0	0	+	[36]
	Olanzapine	PFCx	0	+	+	[37]
	Perospirone	mPFCx	ND	ND	+	[214]
	Risperidone	mPFCx	ND	ND	+	[214]
	Olanzapine	mPFCx	0	0	0	[213]
	MDL100907	PFCx	0	ND	ND	[74]
Fluvoxamine	Quetiapine	PFCx	0	ND	+	[212]

SSRI	5-HT _{2C} antagonist	Region	5-HT	NE	DA	Ref.
Citalopram	SB242084	HP	+	ND	ND	[139]
	SB242084	HP	+	ND	ND	[221]
	SB242084	PFCx	+	ND	ND	[220]
	SB242084	HP	+	ND	ND	[149]

Signs + indicate a potentiating effect of the combination over SSRI alone. 0 indicate a lack of potentiating effect.

antagonists. Moreover, after a few weeks, SSRIs treatments lead to a downregulation of the 5-HT₂ receptors that allows for increased dopaminergic firing, which is proposed to be decisive for the antidepressant effect [222]. Despite these encouraging results, whether 5-HT_{2C} activation or inactivation is required to produce antidepressant-like response in pre-clinical studies remains controversial as emphasized in this review. Future investigations using other paradigms than FST and TST will undoubtedly help determine the real impact of 5-HT_{2C} ligands on depressive-like symptoms. Indeed, the multiplication of behavioral tests known to recapitulate diverse anomalies and symptoms encountered in psychiatric disorders as well as the evaluation of anxiolytic/antidepressant-like activities of pharmacological compounds in relevant animal models [171,223] is a prerequisite to anticipate their putative activity in human. Despite these considerations the use of complementary tests may lead to discrepancies. Hence, to overcome these problems the determination of an integrated “emotionality score” in rodents has been created to facilitate the comparison between animal and human studies [224]. It thus appears important to put emphasis on modeling symptoms rather than disorder per se [225]. Taking into consideration all these aspects, it is not clear yet which strategy would be the most appropriate. It is however interesting to note that 5-HT_{2C} receptor antagonists may be useful tools to prevent comorbidities, most notably anxiety that can be enhanced in the first day of treatments with SSRIs [130,226,227].

The observation that the inactivation of both 5-HT_{2A} and 5-HT_{2C} might potentiate 5-HT and NE neurotransmissions

and prevent anxiety in response to SSRI respectively, support the interest of associating this class of antidepressant with non-selective 5-HT_{2A/2C} receptor antagonists. This is not a novel idea and it is likely that most of the second-generation of antipsychotics such as clozapine, olanzapine and asenapine exerts antidepressant effects alone or in combination with SSRIs due to their high affinity for these receptors [228]. Nevertheless, the pathophysiology of anxiety and depression are largely unknown and some symptoms remain difficult to quantify in animals making the validation of animal models particularly challenging. As emphasized earlier, the consideration of other markers of depression and antidepressant response such as pain and cognition could reinforce the potential utility of 5-HT_{2A} and/or 5-HT_{2C} receptor antagonists in psychiatric disorders. Both receptors are involved in the regulation of nociception and memory but if these drugs are evaluated clinically on the basis of conventional scales, they will not necessarily demonstrate a higher efficacy than SSRIs.

Using the clinicaltrials.gov website, the Boolean research for the following keywords “antipsychotic” and “major depression” provide 151 results. The detail of the completed studies in patients suffering from unipolar depression without psychotic features and treated with antipsychotics and/or antidepressants/SSRIs (Table 5) shows that 70% of the clinical trials focus on combination studies mainly using aripiprazole, olanzapine, quetiapine and risperidone. None of these studies included a translational approach coupling genetic, serum dosages, imagery techniques and behavioral/clinical

Table 5. Clinical trials evaluating the antidepressant effect of antipsychotics given either alone or in combination with antidepressants in major depressive disorder (MDD).

Antipsychotic	Antidepressant	Detail of the study		
		Comparator(s)	Phase	Condition
Quetiapine		Placebo	3	MDD (x6)
Quetiapine		Placebo or duloxetine	3	MDD (x1)
Ziprasidone		Placebo	3	MDD (x3)
Aripiprazole	Sertraline	Placebo/sertraline	3	MDD (x1)
Aripiprazole	Escitalopram	Aripiprazole/placebo placebo/escitalopram	3	MDD (x2)
Aripiprazole	ADs	Placebo/placebo placebo/ADs	3	MDD (x4)
Aripiprazole	ADs	Placebo/placebo placebo/ADs	2/3/4	Augmentation agent in treatment-resistant MDD (x4)
Iloperidone	SSRIs	Placebo/SSRIs	4	Augmentation agent in MDD (x1)
Olanzapine	-	5-HT type ADs	1/2	Augmentation agent in treatment-resistant MDD (x1)
Olanzapine	Fluoxetine	Olanzapine/placebo placebo/fluoxetine	3	Augmentation agent in treatment-resistant MDD (x1)
Pipamperone	Citalopram	Placebo/citalopram	2	MDD (x1)
Quetiapine	ADs	ADs alone	3	MDD (x1)
Quetiapine	SSRIs	Placebo/placebo quetiapine/placebo	2/3/4	Augmentation agent in treatment-resistant MDD (x3)
Risperidone	ADs	Placebo/ADs	3	Augmentation agent in treatment-resistant MDD (x1)
Risperidone	Citalopram	Placebo/citalopram	3	Augmentation agent in treatment-resistant MDD (x1)
Risperidone	SSRIs	Bupropion/SSRIs	3	Augmentation agent in treatment-resistant MDD (x1)

characterization. Interestingly, some compounds with more than one mode of action, notably with the ability to block simultaneously the 5-HT transporter SERT and 5-HT_{2A} and/or 5-HT_{2C} receptors including the atypical antipsychotics trazodone, nefazodone, arylpiperazine [229-231] or YM992 [232] have been developed. Preclinical studies are now required to determine whether these compounds produced greater antidepressant-like, electrophysiological and neurochemical effects than SSRIs. In particular, since adaptive changes occur after sustained administration of pharmacological compounds, it appears inevitable to test the effect of chronic administration of 5-HT₂ ligands in relevant animal paradigms making the distinction between anxiety and/or depression. In this section, we willingly omitted to mention the putative therapeutic interest of central 5-HT_{2B} receptor since its role in the field of neuropsychopharmacology remains poorly documented.

Lastly, it is also important to emphasize the potential central and peripheral risks related to these pharmacological strategies which can be mild, moderate or even life-threatening [233]. Risks of excessive weight loss [234] have been observed in response to the stimulation of 5-HT_{2A} or 5-HT_{2C} receptors. Consequently, it is important to watch over putative weight gain in response to strategies inactivating these receptors. Drug–drug interactions should also be considered when combining antidepressants and antipsychotics since toxicity might be more severe due to the high levels of monoamines [235].

The serotonin syndrome is a serious disorder reported in humans and that most commonly appears after antidepressant overdose or after combining several psychotropic medications [236]. Acute signs of 5-HT syndrome are possibly related to an excess of extracellular 5-HT activating 5-HT_{2A} and 5-HT_{2C} receptors [69,237]. Then, it can be anticipated that the pharmacological blockade of these receptors could prevent this syndrome. However, various atypical antipsychotic drugs, such as olanzapine, have been reported as causing the serotonin syndrome [238]. Finally, suicide is one of the most often risk encountered after overdose [239] and the fact that associations of polymorphisms in HTR2A and HTR2C gene with suicide attempts or suicidal ideation in depressive patients have been described, should draw our attention [240,241]. Regarding 5-HT_{2B} receptor, benfluorex (Mediator), a 5-HT releasing agent and a potent 5-HT_{2B} receptor agonist, has been recently suspected to cause deaths due to valvular insufficiency [242]. The activation of this receptor in the lung and cerebral vasculature has also been incriminated in pulmonary hypertension and migraine attacks, respectively [243,244]. In the CNS, 5-HT_{2B} sites are poorly expressed but the possibility that it acts as an excitatory autorceptor on the serotonergic system [111] prompt us to be vigilant. Indeed, although preliminary data suggest that activation of this receptor would produce antidepressant-like effects or potentiate the therapeutic activity of SSRIs, the implication of 5-HT_{2B} receptor in the serotonin syndrome is strongly suspected [69]

Declaration of interest

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Objectif de la thèse :

L'ensemble des données de la littérature laisse entrevoir un rôle des astrocytes dans la physiopathologie de la dépression et le mécanisme d'action des antidépresseurs tels que les inhibiteurs sélectifs de recapture de la sérotonine (ISRS). Il est désormais bien établi qu'une partie des effets thérapeutiques de ces traitements passe par la stimulation de la synthèse de BDNF, un facteur neurotrophique régulant positivement la prolifération, la maturation et la survie neuronale mais également la plasticité synaptique dans une région essentielle du contrôle de l'humeur : l'hippocampe. Bien que les neurones représentent la source majeure de BDNF dans cette région du cerveau, les astrocytes pourraient constituer un microenvironnement cellulaire contribuant, via la synthèse de facteurs neurotrophiques, à maintenir une activité neuronale normale. Toutefois, les évidences scientifiques suggérant que ces cellules synthétisent du BDNF émanent d'études *in vitro*, loin de mimer les conditions physiologiques ou physiopathologiques chez l'animal vivant.

Au cours de ce travail de thèse, nous avons mené en parallèle deux études portant d'une part sur le rôle du BDNF astrocytaire et d'autre part sur le récepteur 5-HT_{2A} de la 5-HT dans les troubles anxio-dépressifs et la réponse aux antidépresseurs. Ces études n'avaient pas de liens directs mais nous avons tenté de réconcilier nos résultats *in vivo* de manière à fournir un modèle d'action original d'un ISRS, la fluoxétine, à travers la mobilisation éventuelle de ces deux acteurs au sein d'un même mécanisme.

Ainsi, dans un premier temps, nous nous sommes intéressés à l'étude des effets de la surexpression de BDNF astrocytaire dans l'hippocampe, seule ou associée à un traitement chronique de fluoxétine 1) chez des souris naïves ou 2) chez celles exposées à la corticostérone. Pour cela nous avons réalisé deux types d'approches :

- Une approche post-synaptique évaluant le phénotype des souris et le lien d'une part avec la neurogenèse hippocampique adulte et d'autre part avec le réseau astrocytaire reposant sur les protéines de jonction telles que les connexines 30 et 43,
- Une approche pré-synaptique à travers des analyses électrophysiologiques et neurochimiques (microdialyse intracérébrale) visant à caractériser l'activité du système sérotoninergique.

Dans un second temps, nous nous sommes intéressés à apporter des éléments en faveur du rôle éventuel du récepteur 5-HT_{2A} dans la physiopathologie des troubles anxio-dépressifs et la réponse à un traitement chronique de fluoxétine. Pour cela nous avons réalisé :

- des études comportementales précliniques et cliniques caractérisant les effets de la délétion constitutive du récepteur 5-HT_{2A} sur la sévérité des symptômes anxio-dépressifs chez des souris exposées à la corticostérone et chez 480 patients dépressifs issus de la cohorte METADAP,
- des études combinant des approches pré- et post-synaptiques afin de déterminer l'impact de l'inactivation des récepteurs 5-HT_{2A} dans la réponse à un traitement chronique de fluoxétine.

L'objectif final de ce travail était de confronter l'ensemble nos résultats dans le but de mettre en évidence un lien éventuel entre le récepteur 5-HT_{2A} et le BDNF astrocytaire dans la communication entre astrocytes et neurones au sein de la synapse tripartite, un lien qui pourrait finalement s'avérer important dans la réponse aux ISRS.

Résultats

Expérimentaux

Article 1

BDNF overexpression in mouse hippocampal astrocytes promotes local neurogenesis and elicits anxiolytic-like activities

Quesseveur G, David DJ, Gaillard MC, Pla P, Wu MV, Nguyen HT, Nicolas V, Auregan G, David I, Dranovsky A, Hantraye P, Hen R, Gardier AM, Déglon N, Guiard BP.

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RÉSUMÉ :

L'activité thérapeutique des inhibiteurs sélectifs de recapture de la sérotonine (ISRS) repose sur leur capacité à induire des modifications à long terme aux niveaux pré- et post-synaptiques. L'administration chronique d'ISRS stimule la neurotransmission sérotoninergique dans différentes régions cérébrales impliquées dans la régulation de l'humeur. Dans l'hippocampe, l'élévation des concentrations extracellulaires de sérotonine stimule la synthèse et la libération de BDNF, un mécanisme majeur favorable au processus de neurogenèse chez l'adulte (Duman et al. 2001). Bien que cette neurotrophine soit majoritairement synthétisée par les neurones, des études *in vitro* révèlent que les ISRS induisent également une augmentation de l'expression de l'ARNm codant pour le BDNF dans des cultures primaires d'astrocytes. Les astrocytes, acteurs majeurs de la synapse tripartite, pourraient ainsi jouer un rôle significatif dans la manifestation des effets thérapeutiques des ISRS.

Afin de tester cette hypothèse, nous avons utilisé une stratégie innovante de transfection de gène codant pour le BDNF à l'aide d'un lentivirus muni d'une enveloppe ciblant les astrocytes. De plus, le transgène possède une séquence cible des microARN-124, en amont du promoteur codant pour le BDNF, permettant d'éliminer l'expression résiduelle du BDNF dans les neurones. A l'aide de cet outil injecté de façon bilatérale dans l'hippocampe de souris adultes, nous avons ainsi pu étudier l'impact de la surexpression de BDNF astrocytaire seule ou associée à un ISRS, la fluoxétine, sur le comportement anxio-dépressif des souris.

Tout d'abord nous avons observé une augmentation de l'expression du BDNF mais également des voies de signalisation induite par la fixation de ce facteur neurotrophique sur son récepteur de plus haute affinité TrKB, chez les souris ayant reçu l'injection du lentivirus codant pour le BDNF. Nos résultats montrent que dans le test d'hypophagie induite par la nouveauté (Novelty Suppressed Feeding : NSF), la surexpression de BDNF provoque des effets de type anxiolytique/antidépresseur associés à une stimulation de la neurogenèse hippocampique. En accord avec la littérature, la

fluoxétine présente les mêmes propriétés chez les souris contrôles (Lenti-GFP), tandis que ses effets ne sont pas potentialisés chez les souris lenti-BDNF dans le NSF. En revanche, la surexpression de BDNF permet de révéler un effet anxiolytique de la fluoxétine dans le test du labyrinthe en croix surlévé (Elevated Plus Maze : EPM). De manière intéressante l'irradiation de l'hippocampe, par rayon X, bloque les effets neurogéniques et comportementaux de la surexpression de BDNF astrocytaire observé dans le NSF sans toutefois affecté ces effets dans l'EPM.

Nos résultats montrent également que le lenti-BDNF atténue la capacité de la fluoxétine à augmenter les concentrations extracellulaires de sérotonine dans l'hippocampe.

Le rôle original des astrocytes hippocampiques dans la synthèse de BDNF, modulant différentes facettes de la réponse aux ISRS par des mécanismes neurogène-dépendants et -indépendants, a ainsi été proposé dans cette étude.

Contribution personnelle :

Au cours de ce travail :

- j'ai participé aux injections de lentivirus,
- j'ai réalisé le suivi des animaux incluant la préparation et l'administration des différents traitements pharmacologiques,
- j'ai réalisé l'ensemble des tests comportementaux sous la responsabilité du Dr Denis David,
- j'ai participé aux analyses immunohistochimiques des étapes de la neurogenèse hippocampique depuis la perfusion, le prélèvement et la coupe des cerveaux jusqu'à l'incubation des lames avec les anticorps et leurs analyses (comptage). Ces études ont été effectuées sous la supervision du Dr Denis David,
- j'ai participé aux études électrophysiologiques (enregistrement des neurones sérotoninergiques) et également aux études de microdialyse intracérébrale (implantation des sondes et analyses chromatographiques par HPLC) sous la supervision du Dr Bruno Guiard.

BDNF overexpression in mouse hippocampal astrocytes promotes local neurogenesis and elicits anxiolytic-like activities

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The therapeutic activity of selective serotonin (5-HT) reuptake inhibitors (SSRIs) relies on long-term adaptation at pre- and post-synaptic levels. The sustained administration of SSRIs increases the serotonergic neurotransmission in response to a functional desensitization of the inhibitory 5-HT_{1A} autoreceptor in the dorsal raphe. At nerve terminal such as the hippocampus, the enhancement of 5-HT availability increases brain-derived neurotrophic factor (BDNF) synthesis and signaling, a major event in the stimulation of adult neurogenesis. In physiological conditions, BDNF would be expressed at functionally relevant levels in neurons. However, the recent observation that SSRIs upregulate BDNF mRNA in primary cultures of astrocytes strongly suggest that the therapeutic activity of antidepressant drugs might result from an increase in BDNF synthesis in this cell type. In this study, by overexpressing BDNF in astrocytes, we balanced the ratio between astrocytic and neuronal BDNF raising the possibility that such manipulation could positively reverberate on anxiolytic-/antidepressant-like activities in transfected mice. Our results indicate that BDNF overexpression in hippocampal astrocytes produced anxiolytic-/antidepressant-like activity in the novelty suppressed feeding in relation with the stimulation of hippocampal neurogenesis whereas it did not potentiate the effects of the SSRI fluoxetine on these parameters. Moreover, overexpressing BDNF revealed the anxiolytic-like activity of fluoxetine in the elevated plus maze while attenuating 5-HT neurotransmission in response to a blunted downregulation of the 5-HT_{1A} autoreceptor. These results emphasize an original role of hippocampal astrocytes in the synthesis of BDNF, which can act through neurogenesis-dependent and -independent mechanisms to regulate different facets of anxiolytic-like responses.

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Introduction

The therapeutic activity of selective serotonin (5-HT) reuptake inhibitors (SSRIs) relies on long-term adaptation at pre- and post-synaptic levels. At the post-synaptic level, compelling evidence has demonstrated that the sustained, but not acute, administration of SSRIs stimulates adult hippocampal neurogenesis, whereas the disruption of this phenomenon prevents the behavioral effects of these antidepressants in mice.¹ Although the complex biological and psychobiological changes associated with depression and related treatments cannot be attributed to neurogenesis alone,² this particular property of SSRIs has been reported in humans³ and in non-human primates.⁴ We recently demonstrated that the disruption of adult hippocampal neurogenesis compromises the anxiolytic-/antidepressant-like activity of the SSRI fluoxetine in some, but not all, behavioral paradigms, thereby suggesting the existence of both neurogenesis-dependent and neurogenesis-independent mechanisms of action.⁵ A number of

factors have been proposed to participate in SSRI-induced adult hippocampal neurogenesis. Among the many long-term targets of SSRI treatments, neurotrophins, particularly brain-derived neurotrophic factor (BDNF), have been well studied. BDNF is the most abundant neurotrophin in the brain and is predominantly synthesized by neurons, where the normal physiological levels are rapidly and potently regulated through ongoing neuronal activity.⁶ Evidence demonstrates that chronic SSRI treatments increase BDNF expression in the dentate gyrus (DG) of the hippocampus,⁷ which in turn promotes the *in vivo* proliferation and differentiation of neuronal progenitor cells,^{8–11} accelerates the maturation of newborn neurons and facilitates their survival.^{10–12} Taken together, these data establish a relationship between antidepressant activity, BDNF synthesis and the stimulation of hippocampal neurogenesis.

There is increasing evidence that SSRIs also activate glial cells, particularly astrocytes.¹³ Consistent with this hypothesis,

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fluoxetine has been shown to reverse the stress-induced reduction of hippocampal glial fibrillary acidic protein (GFAP) expression in rats.¹⁴ Therefore, an increased activity or density of astrocytes could represent a common cellular mechanism underlying the effects of pharmacological¹⁵ and non-pharmacological antidepressive strategies.¹⁶ *In vitro* studies suggest that astrocytes express very low levels of BDNF,¹⁷ and whether this neurotrophin would be recruited in response to antidepressant treatment to modulate their therapeutic activity remains unknown. The presence of SERT (serotonin transporter) on astrocytes^{18,19} along with the recent observation that fluoxetine upregulates BDNF in primary astrocyte cultures²⁰ support this hypothesis but has never been examined *in vivo*.

In the present study by selectively overexpressing BDNF in hippocampal astrocytes by the mean of an original and efficient gene transfer strategy,²¹ we balanced the ratio between neuronal vs astrocytic BDNF thereby modeling the putative post-synaptic activity of SSRIs. We anticipated that such manipulation either alone or in combination with the sustained administration of fluoxetine could positively reverberate on the anxiolytic-/antidepressant-like behavior of transfected mice. In addition, we tested the hypothesis that changes in behavioral responses might result from the finely tuned regulation of hippocampal neurogenesis and/or serotonergic neurotransmission, which are two major elements in the therapeutic activity of SSRIs. Depending on the paradigm, the overexpression of BDNF in hippocampal astrocytes elicited anxiolytic-/antidepressant-like effects either alone or in combination with fluoxetine thereby providing the first *in vivo* evidence that this cell type might be a key partner of neurons during antidepressant treatment.

Materials and methods

Animals. Male SvEv129 mice (7- to 9-week-old), weighing 25–35 g (Taconic, Ejby, Denmark) were used in the present study according to the protocol described in Supplementary Figure S1. They were initially divided into two groups pre-injected with lentiviral vector containing the green fluorescent protein (GFP) (lenti-GFP control mice) or the BDNF (lenti-BDNF mice). One month after stereotaxic injection of lentivirus, lenti-GFP and lenti-BDNF mice were administered the vehicle or fluoxetine for 4 weeks.

Stereotaxic injection of lentiviral vector. For the stereotaxic injections, mice were anesthetized with a mixture of ketamine (75 mg kg⁻¹) and xylazine (10 mg kg⁻¹), administered intraperitoneally (i.p.). Lentiviral vectors (see Supplementary Materials and Methods) were injected using 34-gauge blunt-tip needle linked to a Hamilton syringe (Hamilton, Reno, NV, USA) by a polyethylene catheter. The viruses were diluted in PBS-1% BSA to reach a final concentration of 100 ng p24 μl⁻¹. Mice received a total volume of 1 μl at a rate of 0.2 μl min⁻¹. The stereotaxic coordinates for the bilateral injections in the hippocampus were (in mm from bregma): anteroposterior (AP) -2.2, lateral (L) ±2.0 and ventral (V) -2.0 (according to Paxinos and Franklin²²). At the end of the injection, the needle was left in place for 5 min before being slowly removed.

The labeling of the site of injection by GFP is depicted in Figure 1e and Supplementary Figure S2A.

ELISA and western blots. BDNF levels in the hippocampus and the striatum of lenti-GFP and lenti-BDNF were measured by anti-BDNF sandwich-ELISA using BDNF Emax Immunoassay system (Promega, Charbonnières, France) according to a protocol described previously. Additionally, western blots were used to detect extracellular signal-regulated kinase (ERK) and protein kinase B (Akt) phosphorylation (see Supplementary Materials and Methods).

Behavioral testing. The originality of our protocol lies in the fact that the same cohort of animal was tested in different behavioral paradigms of anxiety and depression according to a procedure described previously.⁵ Behavioral procedure starts with the less stressful paradigm and finishes with the most stressful one. So each animal, over 1 week, was successively tested in the elevated plus maze (EPM), the novelty suppressed feeding (NSF), the tail suspension test (TST) and the splash test (ST) paradigm. A 2-day period between each test was respected (Supplementary Figure S1). Detail of these tests as well as x-irradiation procedure can be retrieve in Supplementary Materials and Methods.

Immunohistochemistry

Ki67 labeling for proliferation study. We first looked at proliferation using Ki-67 as a marker for cell division. Ki-67, a nuclear protein expressed in all phases of the cell cycle except for G0, can be used as an endogenous marker alternative to bromodeoxyuridine (BrdU) incorporation.²³

BrdU labeling for survival study. Mice were administered with BrdU (150 mg kg⁻¹, i.p. dissolved in saline), twice a day during 3 days before the start of the treatment. After anesthesia with ketamine and xylazine (75 mg kg⁻¹ ketamine; 10 mg kg⁻¹ xylazine; i.p.), mice were perfused transcardially (cold saline, followed by 4% cold paraformaldehyde at 4 °C). The identification of survival cells was performed by BrdU labeling (see Supplementary Materials and Methods).

Doublecortin labeling for maturation index and dendritic morphology studies. The identification of survival cells was performed by doublecortin (DCX) labeling (see Supplementary Materials and Methods). Once performed, DCX+ cells were subcategorized according to their dendritic morphology: DCX+ cells with no tertiary dendritic processes and DCX+ cells with complex, tertiary dendrites. The maturation index was defined as the ratio of DCX+ cells possessing tertiary dendrites over the total DCX+ cells.

Intracerebral electrophysiology. Mice were anesthetized with chloral hydrate (400 mg kg⁻¹; i.p.) and placed in a stereotaxic frame (using the David Kopf mouse adaptor) with the skull positioned horizontally. Micropipettes were positioned on the lambda and lowered into the dorsal raphe (DR), usually attained at a depth of between 2.5 and 3.5 mm from the brain surface.²² The presumed DR 5-HT neurons were then identified accordingly using the following criteria: a slow

(0.5–2.5 Hz) and regular firing rate and a long duration, positive action potential.²⁴ Changes in the firing activity were expressed as percentages of baseline firing rate.

Intracerebral microdialysis. Mice were anesthetized with chloral hydrate (400 mg kg⁻¹; i.p.) and placed in a stereotaxic frame (using the David Kopf mouse adaptor) with the skull positioned horizontally. Anesthetized mice were implanted with probes located in the hippocampus and DR. The stereotaxic coordinates for the hippocampus were (in mm from bregma): AP - 2.5, L ± 2.5, V - 2.5 and for the DR (in mm from lambda): AP 0, L 0, V - 3.5 (according to Paxinos and Franklin²²). Active lengths of the microdialysis probes in the hippocampus and DR were 1.5 and 1 mm, respectively. Microdialysis experiments were conducted following a protocol previously described in our laboratory.²⁵ These basal values were calculated for each mouse before the challenge of fluoxetine or vehicle. Dialysate samples were analyzed for 5-HT by high-performance liquid chromatography method (limit of sensitivity ≈ 0.5 fmol per sample; signal-to-noise ratio = 2). At the end of the experiments, localization of microdialysis probes was verified histologically.

8-Hydroxy-*N,N*-dipropyl-2-aminotetralin-induced hypothermia. Body temperature was assessed intrarectally, using a lubricated probe inserted ~2 cm and a TH-5 Thermalert Monitoring Thermometer (Physitemp, Clifton, NJ, USA). Mice were singly housed in clean cages for 10 min, and three baseline body temperature measurements were taken. After the third baseline measurement, animals received 8-Hydroxy-*N,N*-dipropyl-2-aminotetralin (8-OH-DPAT) (100 µg kg⁻¹; subcutaneous (s.c.)) and body temperature was monitored every 10 min for a total of 60 min. Decreases in body temperatures were represented as a change from the mean baseline measurement.

Drugs and administration. Fluoxetine (18 mg kg⁻¹ per day) was purchased from ANAWA (Wangen, Switzerland) and administered in the drinking water in opaque bottles. For electrophysiological and microdialysis studies, 8-OH-DPAT (100–400 µg kg⁻¹; s.c.), WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide) (300 µg kg⁻¹; s.c.) and were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Statistical analysis. For all experiments, statistical analysis (StatView 5.0 Abacus Concepts, Berkeley, CA, USA) used mean ± s.e.m. Two-way analysis of variance with pretreatment (lenti-GFP vs lenti-BDNF) and treatment (vehicle vs fluoxetine) factors was used followed by Fisher Protected Least Significance Difference *post hoc* test. In the irradiation experiments of lenti-BDNF mice, a two-way analysis of variance with irradiation (sham vs X-ray) and treatment (vehicle vs fluoxetine) factors was applied. Finally, hypothermic responses induced by 8-OH-DPAT were compared using a three-way analysis of variance with repeated measures. In specific cases, a Student's *t*-test was used to compare two groups of mice. Significant level was set at $P < 0.05$.

The detail of the statistical analysis for each figure is depicted in Supplementary Table S1.

Results

Validation of BDNF overexpression in the hippocampus of adult mice. The ability of the BDNF-engineered lentiviral vector to induce a detectable production of BDNF in the hippocampus was determined using ELISA. A significantly higher concentration of BDNF was observed in lenti-BDNF mice compared with control lenti-GFP mice treated with vehicle ($P < 0.001$; Figure 1a). The administration of fluoxetine significantly increased the concentration of BDNF in the control lenti-GFP ($P < 0.01$) but not in the lenti-BDNF mice ($P > 0.05$; Figure 1a).

We next examined whether the BDNF-engineered lentiviral vector increased the levels of activated (phosphorylated) ERK and Akt in the rat hippocampus as an indirect marker of BDNF secretion. A significant activation of both ERK and Akt was detected in lenti-BDNF mice compared with control lenti-GFP mice ($P < 0.001$ and $P < 0.001$, respectively; Figures 1b–d). When treated with fluoxetine, the activation of both kinases was significantly increased in lenti-GFP ($P < 0.01$ and $P < 0.05$, respectively) but not in lenti-BDNF mice ($P > 0.05$ and $P > 0.05$, respectively).

Validation of glial cell transduction with GFP and BDNF. The microinjection of lentiviral particles into the hippocampus resulted in diffuse GFP expression. We showed that GFP fluorescence obtained with this mock-pseudotyped lentiviral vector was localized to the subgranular layer and hilus of the DG (Figures 1e and f), while no expression was detected outside the hippocampus. Transduced proteins were selectively expressed in astrocytes as demonstrated by overlapping of GFP and GFAP immunostaining, whereas no GFP expression was detected in neurons located in the granular layer (Figure 1f; Supplementary Figure S2).

Behavioral response in lenti-BDNF adult mice administered the vehicle or fluoxetine. Given the ability of lentiviral vector to promote the production and release of BDNF from astrocytes, we therefore assessed the behavioral consequences of such manipulation in a battery of sensitive tests to the chronic administration of fluoxetine through neurogenesis-independent (EPM, TST and ST) and neurogenesis-dependent (NSF) mechanism of action.⁵

In the EPM, the time spent in the open arms was unaltered in lenti-BDNF mice compared with the control lenti-GFP mice treated with vehicle ($P > 0.05$). Nevertheless, in response to fluoxetine, a significant increase was observed in the lenti-BDNF mice ($P < 0.01$; Figure 2a) but not in lenti-GFP mice ($P > 0.05$; Figure 2a) compared with their respective control groups treated with vehicle. The total entries in the open arms (Figure 2b) did not differ between the groups.

With respect to behavioral paradigms developed to characterize antidepressant-like responses, in the TST our results showed that the duration of immobility was not altered in the control lenti-BDNF mice compared with the control

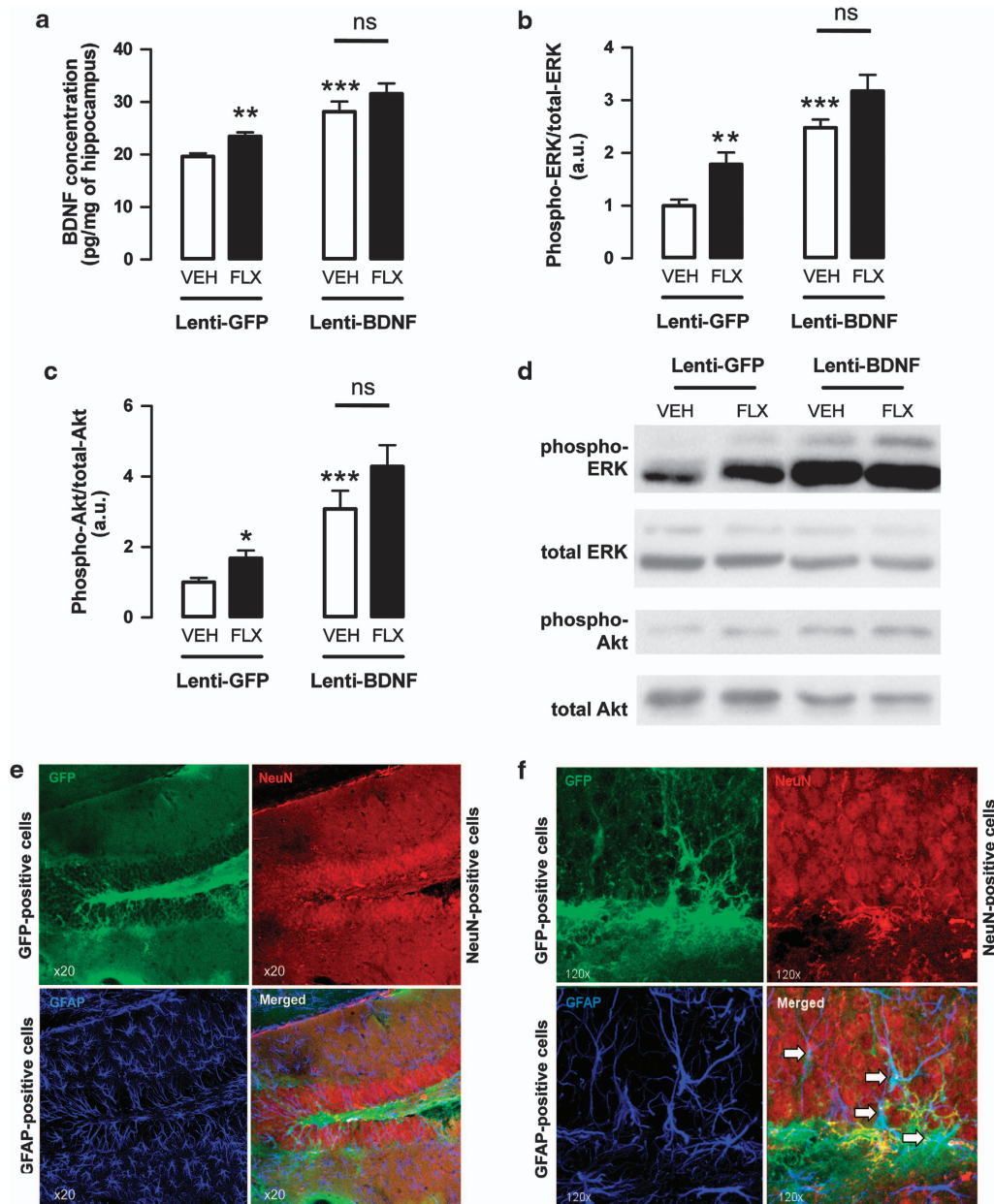


Figure 1 *In vivo* validation of lentiviral vector on brain-derived neurotrophic factor (BDNF) overexpression/signaling and cellular tropism in the adult mouse hippocampus. Mice were microinjected with either lentiviral vector-expressing green fluorescent protein (GFP) alone (Lenti-GFP) or lentiviral vector-expressing the human BDNF gene (Lenti-BDNF) within the hippocampus. (a) *In vivo* measurements of BDNF concentration in the hippocampus of adult Lenti-GFP or Lenti-BDNF mice administered either with vehicle (VEH; p.o.) or with fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks. Values are mean \pm s.e.m. of BDNF concentration (pg mg⁻¹) ($n=3-8$ per group). (b, c) Phosphorylation of extracellular signal-regulated kinase (ERK) and protein kinase B (Akt) in the hippocampus of adult mice microinjected with Lenti-GFP or Lenti-BDNF administered either with vehicle (VEH; p.o.) or with fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks. Values are mean \pm s.e.m. of phospho-ERK or phospho Akt/total ERK or total Akt (arbitrary unitary, a.u.) ($n=3-8$ per group). * $P<0.05$, ** $P<0.01$, *** $P<0.001$: significantly different from Lenti-GFP mice administered with vehicle for 4 weeks. NS, not significant. (d) Western blot showing the phosphorylation of ERK and Akt in each experimental group. (e, f) Confocal micrographs ($\times 20$ and $\times 120$) of a representative transduced dentate gyrus (DG) showing the immunostaining for GFP- (green; up left panel), GFAP- (dark blue; down left panel) and NeuN-positive cells (red, up right panel). Merged is depicted on the down right panel. The white arrows indicate transduced astrocytes.

lenti-GFP mice ($P>0.05$; Figure 2c). However, fluoxetine significantly decreased this parameter in both the lenti-GFP mice ($P<0.01$) and the lenti-BDNF mice ($P<0.001$). This antidepressant-like response to fluoxetine did not differ the lenti-GFP and the lenti-BDNF groups ($P>0.05$; Figure 2c). Similarly, in the ST fluoxetine increased grooming behaviors

in both lenti-GFP ($P<0.001$) and lenti-BDNF mice ($P<0.05$) compared with their respective control groups administered the vehicle, while no differences between the lenti-GFP and lenti-BDNF groups were detected ($P>0.05$; Figure 2d).

In a last series of experiments, in the same animals, we assessed the impact of BDNF overexpression in hippocampal

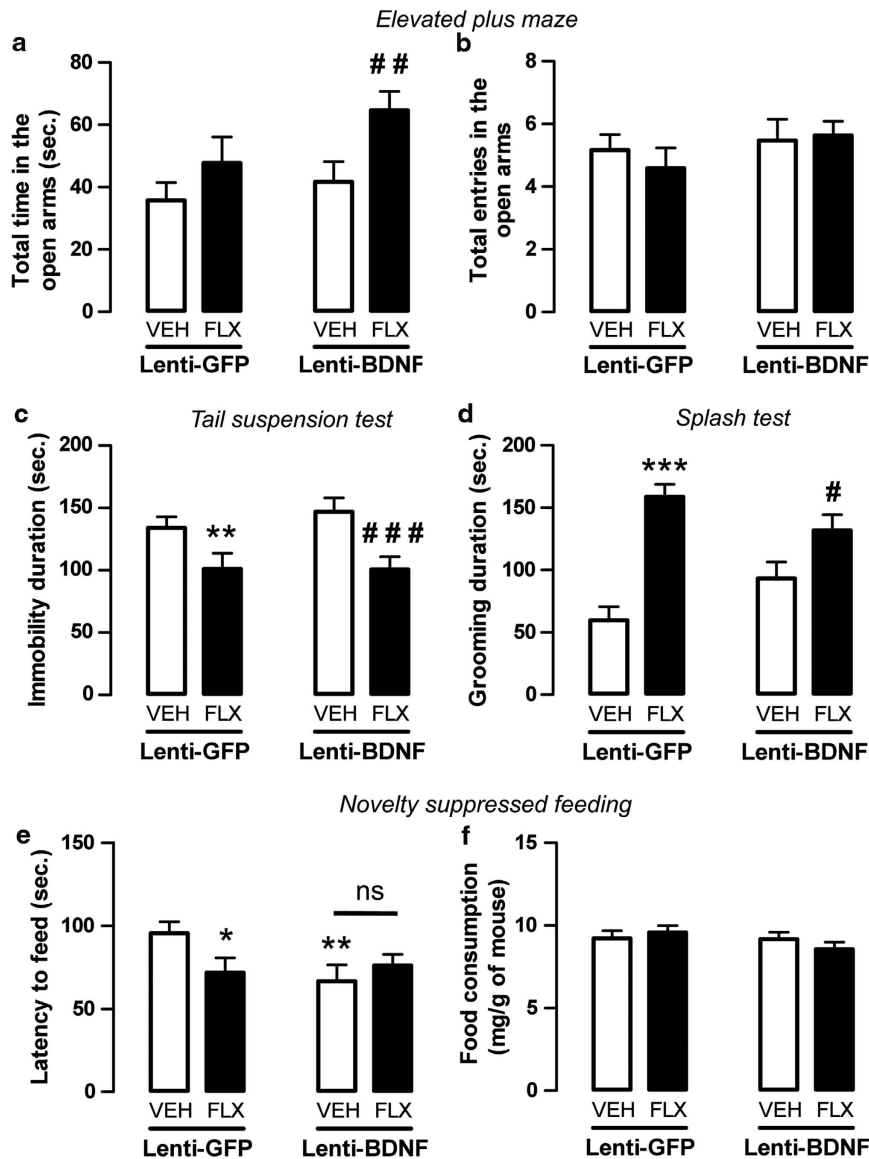


Figure 2 Anxiolytic/antidepressant-like activity of fluoxetine in adult mice displaying an overexpression of brain-derived neurotrophic factor (BDNF) in hippocampal astrocytes. (a, b) Anxiolytic-like effects of BDNF overexpression in hippocampal astrocytes of adult mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks in the elevated plus maze (EPM) test. Anxiety is measured as mean \pm s.e.m. of time spent in the open arms in seconds (a). Locomotor activity is measured as mean \pm s.e.m. of number of entries in the open arms (b). (c, d) Antidepressant-like effects of BDNF overexpression in hippocampal astrocytes of adult mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks evaluated in the tail suspension test (TST) (c) and splash test (d). Depression is measured as mean \pm s.e.m. of the immobility time or grooming duration in seconds. (e, f) Anxiolytic/antidepressant-like effects of BDNF overexpression in adult hippocampal astrocytes in mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks evaluated in the novelty suppressed feeding (NSF) test. (e) Mean \pm s.e.m. of latency to feed in seconds. (f) Mean \pm s.e.m. of food consumption (in mg g⁻¹ of mice). In all tests: $n = 10$ – 20 per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$: significantly different from lenti-GFP (green fluorescent protein) mice administered with vehicle for 4 weeks. # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$: significantly different from lenti-BDNF mice administered with vehicle. NS, not significant.

astrocytes in the NSF. We showed that the latency to feed was significantly decreased in the lenti-BDNF mice compared with the control lenti-GFP mice ($P < 0.01$; Figure 2e). When treated with fluoxetine, the latency to feed was significantly decreased in lenti-GFP ($P < 0.05$), but not in lenti-BDNF mice compared with their respective control groups ($P > 0.05$). At the end of the test, the mice were immediately transferred to their home cages, where food consumption was measured for 5 min. This parameter used as a relative control for animal hunger and

body weight did not differ between groups (Figure 2f; Supplementary Figure S3A).

Hippocampal neurogenesis in lenti-BDNF adult mice administered the vehicle or fluoxetine. To further investigate the cellular mechanisms underlying the behavioral effects of BDNF, we evaluated the impact of BDNF overexpression in hippocampal astrocytes on adult hippocampal neurogenesis, which has been proposed as relevant

for antidepressant action.^{1,5} The BDNF overexpression in hippocampal astrocytes induced a significant proliferation of progenitor cells as shown by an increase in the number of Ki67-positive cells in the lenti-BDNF mice compared with the control lenti-GFP mice ($P < 0.01$; Figure 3a). Fluoxetine also significantly increased this proliferation in lenti-GFP ($P < 0.05$), but not in lenti-BDNF mice ($P > 0.05$; Figure 3a) compared with their respective control groups treated with vehicle. A similar profile was observed for cell survival (Figure 3b) and the maturation of young neurons, particularly the number of DCX-positive cells with tertiary dendrites and the maturation index (Figures 3d–f).

We also examined the relative 'maturity' of BrdU⁺ NeuN⁺ cells according to whether they express DCX. The number of mature BrdU⁺ granule cells (BrdU⁺ NeuN⁺ DCX⁻) corresponding to young neurons increased in response to fluoxetine in the control lenti-GFP ($P < 0.05$) and lenti-BDNF mice, although the increase was not statistically significant in the latter group ($P < 0.1$). These results indicate that the chronic fluoxetine administration similarly changed the fate determination of early progenitors in lenti-GFP and lenti-BDNF mice (Figure 3g).

In an attempt to correlate the anxiolytic-/antidepressant-like effect of BDNF overexpression in hippocampal astrocytes in the NSF with neurogenesis, we showed in lenti-BDNF mice (treated with vehicle or fluoxetine) that the lower the latency to feed, the higher the number of DCX⁺ cells with tertiary dendrites. Although results did not reach statistical significance ($P < 0.1$ with an $R^2 = 0.29$; Figure 3h), they strongly suggested a neurogenesis-dependent effect of BDNF overexpression in the NSF. In marked contrast, the results obtained from the EPM and the TST tests in lenti-BDNF mice did not support this correlation ($P = 0.8$ in both tests with $R^2 = 0.01$ and $R^2 = 0.01$, respectively; Supplementary Figure S4).

Behavioral response in lenti-BDNF adult mice administered the vehicle or fluoxetine after the disruption of neurogenesis. To confirm the possibility that BDNF overexpression in hippocampal astrocytes produced anxiolytic-/antidepressant-like effects through neurogenesis-dependent and neurogenesis-independent mechanisms, we examined whether the ablation of neurogenesis through X-irradiation, which does not affect glial cells density or hippocampal morphology,^{1,26} blocked or attenuated the behavioral responses observed in lenti-BDNF mice in a new batch of animals (Figure 4; Supplementary Figure S5).

In the EPM, the application of X-irradiation mice did not alter the effect of fluoxetine relative to sham mice ($P < 0.05$). In addition, the increase in time spent in open arms induced by fluoxetine treatment was not different between sham and X-ray-treated lenti-BDNF mice ($P > 0.05$; Figure 4a). The total entries in open arms (Figure 4b) did not differ between groups.

In the TST and ST, fluoxetine produced antidepressant-like activities in both sham and X-ray-treated mice ($P < 0.01$ and $P < 0.05$, respectively, in the TST and $P < 0.05$ and $P < 0.05$, respectively, in the ST; Figures 4c and d). Taken together, these results suggest that fluoxetine has a neurogenesis-independent effect on these behavioral paradigms.

In NSF, the application of x-irradiation on lenti-BDNF mice administered the vehicle lowered the anxiolytic-/antidepressant-like response, as indicated by the tendency to increase the latency to feed compared with sham mice ($P = 0.1$). Similarly, in lenti-BDNF mice administered fluoxetine, x-ray treatment prevented the decrease in the latency to feed observed in the sham mice ($P < 0.01$), confirming that the behavioral effect of BDNF overexpression alone or in combination with fluoxetine requires neurogenesis to produce behavioral effects in this test (Figure 4e). Food consumption and body weight did not differ between the groups (Figure 4f; Supplementary Figure S3B).

Changes in presynaptic serotonergic neuronal activity in lenti-BDNF mice treated with vehicle or fluoxetine

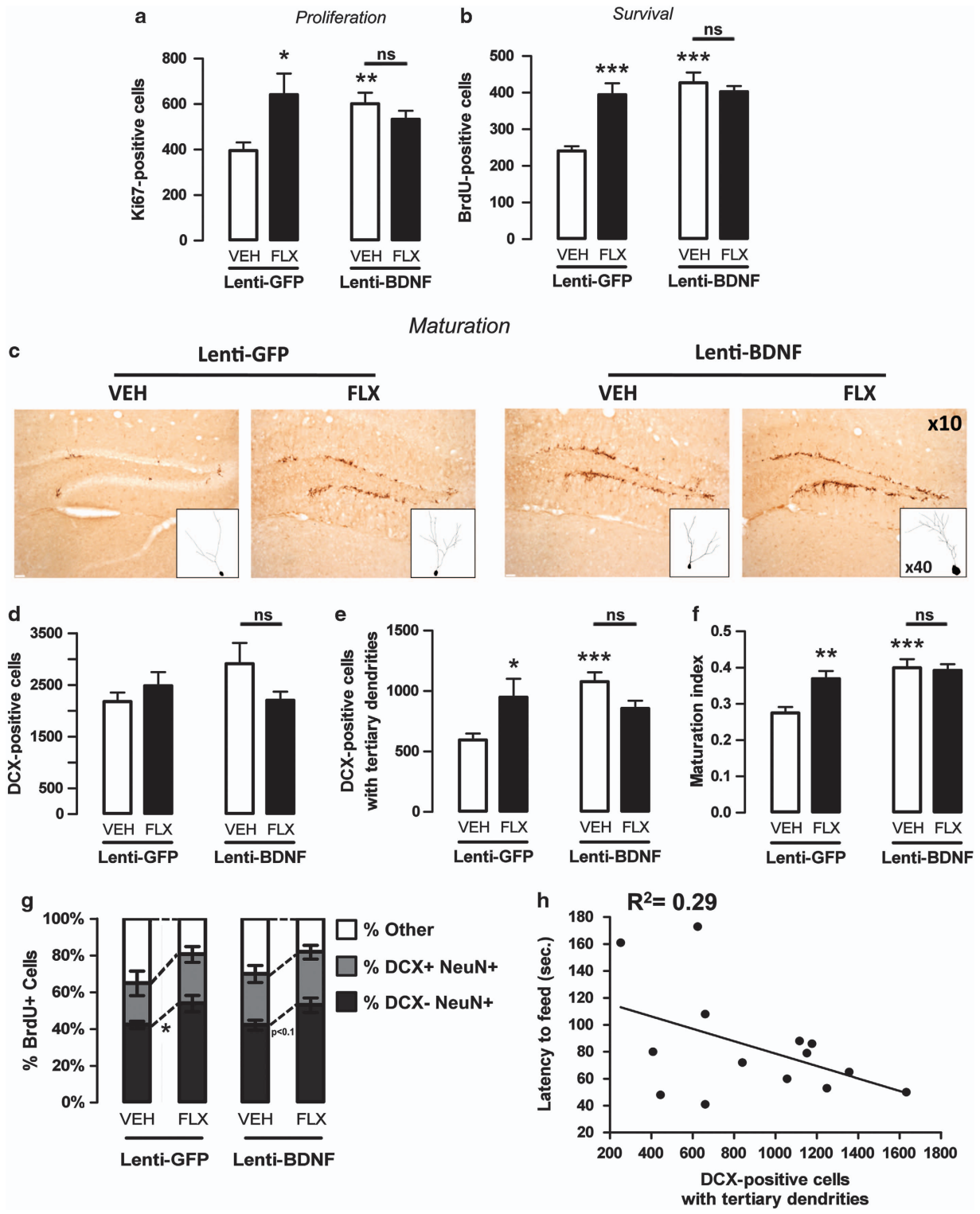
Extracellular levels of 5-HT in the hippocampus and DR. We then investigated whether the overexpression of BDNF in hippocampal astrocytes affected the local extracellular levels of 5-HT using *in vivo* intracerebral microdialysis. Figure 5a shows that the extracellular 5-HT levels in the hippocampus were not modified in the lenti-BDNF mice compared with the control lenti-GFP mice. Fluoxetine enhanced hippocampal 5-HT outflow in both lenti-GFP and lenti-BDNF mice administered fluoxetine compared with their corresponding group administered the vehicle ($P < 0.001$ and $P < 0.001$). Nevertheless, the enhancement of 5-HT outflow was significantly lower in the hippocampus of lenti-BDNF compared with lenti-GFP mice ($P < 0.05$). Similar neurochemical profile was observed at the somatodendritic level, that is, in the DR (Figure 5b).

Neuronal activity of DR 5-HT neurons. Next, we determined whether changes in the extracellular levels of 5-HT resulted from modifications of the firing rate of DR 5-HT neurons. It has been shown previously that sub-chronic administration of SSRIs decreases the DR 5-HT firing rate, and a gradual

Figure 3 Neurogenesis in response to fluoxetine in adult mice displaying an overexpression of brain-derived neurotrophic factor (BDNF) in hippocampal astrocytes. (a) Ki-67 labeling to examine the effects of BDNF overexpression in hippocampal astrocytes of adult mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks on cell proliferation. Values are mean ± s.e.m. of Ki67-positive cell counts ($n = 5–6$ per group). (b) BrdU (150 mg kg⁻¹) was given twice a day for 3 days before drug treatment to examine the effects of BDNF overexpression in hippocampal astrocytes of adult mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks on cell survival. Values are mean ± s.e.m. of BrdU-positive cell counts ($n = 5–6$ per group). (c–f) Effects of BDNF overexpression in hippocampal astrocytes of adult mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks. (c) Histological slices of the dentate gyrus showing doublecortin (DCX) immunostaining in each experimental group ($\times 10$) with 3D drawing of representative DCX-positive cells with dendritic complexity ($\times 40$). Total DCX-positive cells (d), DCX-positive cells with tertiary dendrites (e) as an index of young neurons maturation and maturation index (f) ($n = 8–10$ per group). (g) Proportion of BrdU⁺ NeuN⁺ cells that are DCX⁺ or DCX⁻ (percentage of BrdU cells) as an index of maturity. (h) Correlation between behavioral response in the NSF and the number of DCX-positive cells with tertiary dendrites. Each point represents a lenti-BDNF mouse selected on the basis of its participation of both behavioral and immunohistochemical studies. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$: significantly different from lenti-green fluorescent protein (GFP) mice administered with vehicle. NS, not significant.

recovery to baseline is observed after prolonged administration.²⁷ In the present study, the mean firing activity of DR 5-HT neurons was similar between lenti-GFP mice administered the vehicle and lenti-GFP mice administered fluoxetine (1.4 ± 0.1 vs 1.2 ± 0.1 Hz; $P > 0.05$, respectively; Figures 5c and d). In contrast, in lenti-BDNF mice

administered fluoxetine, the firing rate of DR 5-HT neurons was significantly lower than that obtained in lenti-BDNF mice administered the vehicle (0.9 ± 0.1 vs 1.5 ± 0.09 Hz; $P < 0.001$). Thus, in lenti-BDNF mice, DR 5-HT neurons did not return to baseline as rapidly as lenti-GFP mice in response to fluoxetine treatment.



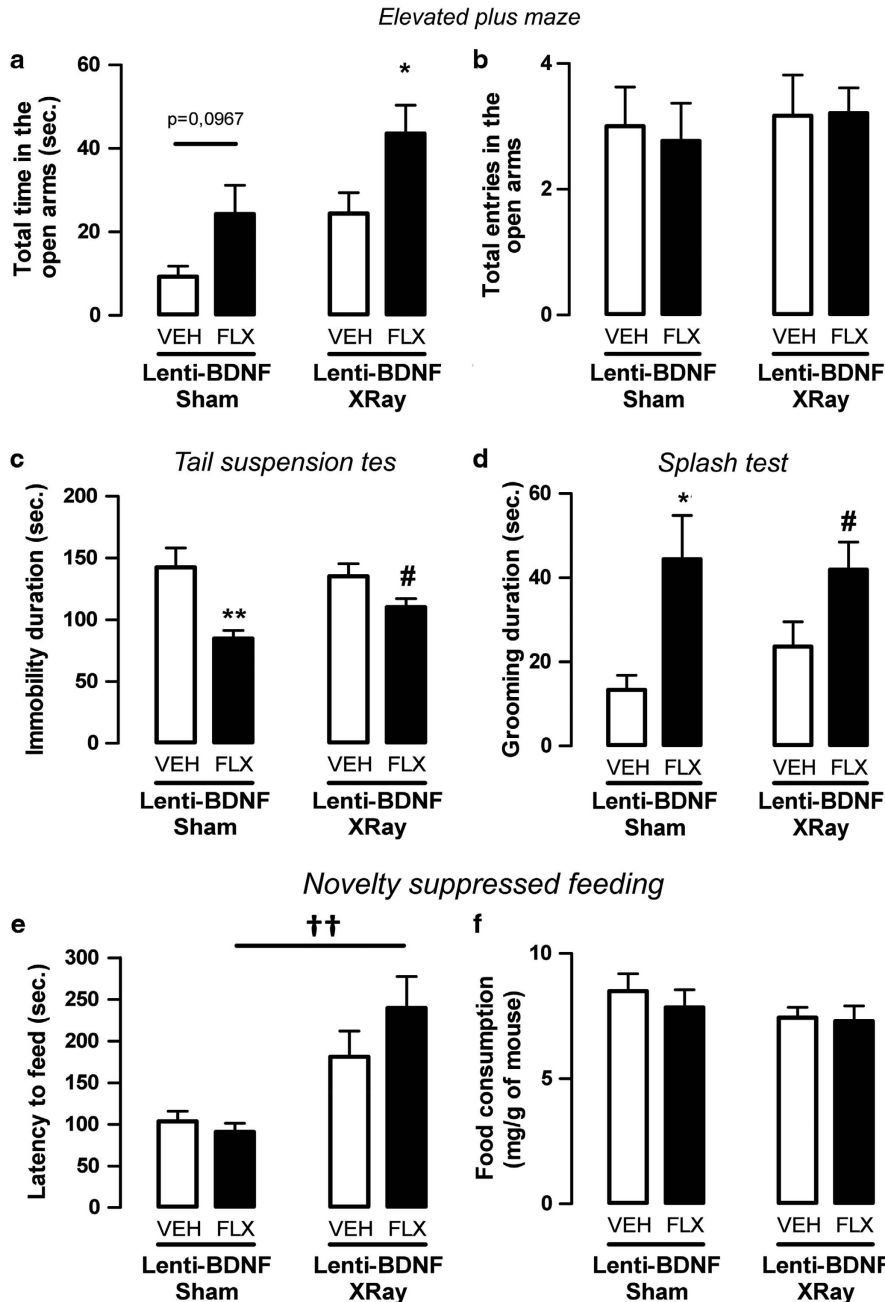


Figure 4 Effect of X-ray on fluoxetine response in adult mice with an overexpression of brain-derived neurotrophic factor (BDNF) in hippocampal astrocytes. (a, b) Anxiolytic-like effects of BDNF overexpression in hippocampal astrocytes of adult mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks in the elevated plus maze (EPM) test. Anxiety is measured as mean ± s.e.m. of time spent in the open arms in seconds (a). Locomotor activity is measured as mean ± s.e.m. of number of entries in the open arms (b). (c, d) Antidepressant-like effects of BDNF overexpression in hippocampal astrocytes of adult mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks evaluated in the tail suspension test (TST) (c) and splash test (d). Depression is measured as mean ± s.e.m. of the immobility time or grooming duration in seconds. (e, f) Anxiolytic-/antidepressant-like effects of BDNF overexpression in adult hippocampal astrocytes in mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks evaluated in the novelty suppressed feeding (NSF) test. (e) Mean ± s.e.m. of latency to feed in seconds. (f) Mean ± s.e.m. of food consumption (in mg g⁻¹ of mice). In all tests: *n* = 10–15 per group. **P* < 0.05, ***P* < 0.01: significantly different from sham lenti-BDNF mice administered with vehicle. #*P* < 0.05: significantly different from X-ray lenti-BDNF mice administered with vehicle. ††*P* < 0.01: significantly different from lenti-GFP (green fluorescent protein) mice administered with fluoxetine.

Functional status of presynaptic 5-HT_{1A} autoreceptor in the DR. To address the possibility that the decreased 5-HT neurotransmission in lenti-BDNF mice administered fluoxetine was associated with a reduced desensitization of the

inhibitory 5-HT_{1A} autoreceptor, we examined the ability of the 5-HT_{1A} receptor agonist 8-OH-*DPAT* to decrease the firing rate of DR 5-HT neurons (Figures 5f and g). A marked inhibition of DR 5-HT neuronal activity was observed in the

control lenti-GFP and lenti-BDNF mice administered the vehicle. Consistent with a functional desensitization of the 5-HT_{1A} autoreceptor, the ability of 8-OH-DPAT to suppress 5-HT neuronal firing activity was attenuated in lenti-GFP and lenti-BDNF mice administered fluoxetine compared with their respective control group ($P < 0.001$ and $P < 0.001$, respectively; Figures 5f and g). However, in fluoxetine-administered mice, the ability of 8-OH-DPAT to suppress DR 5-HT neuronal activity was lower in lenti-BDNF than in lenti-GFP mice ($P < 0.001$).

To further explore the functional status of the 5-HT_{1A} autoreceptor, we evaluated the ability of 8-OH-DPAT to decrease body temperature.²⁸ In the present study, the control lenti-GFP and lenti-BDNF mice administered the vehicle displayed a robust hypothermic response to 8-OH-DPAT. The prolonged administration of fluoxetine attenuated this response in both lenti-GFP and lenti-BDNF mice (Figure 5g). However, after fluoxetine treatment, the attenuation of 8-OH-DPAT-induced hypothermia was less pronounced in the lenti-BDNF mice than in the lenti-GFP mice (Figure 5g).

Discussion

Adaptations induced through the use of chronic antidepressants, most notably in the hippocampus, are related to the upregulation of neurotrophic factors, such as BDNF,⁷ which enhances adult neurogenesis.²⁹ This phenomenon is believed to be involved in the therapeutic activity of SSRIs.¹ As such, developing pharmacological or genetic strategies that stimulate the production and/or release of BDNF represents promising approaches for the treatment of psychiatric disorders. In physiological conditions, neurons are the primary source of BDNF in the brain whereas astrocytes have a role in the storage and secretion of this neurotrophic substance.³⁰ Growing evidence suggests however that this cell type may also synthesize BDNF at functionally relevant levels under certain conditions such as neuronal lesion¹⁷ or inflammation-like conditions.³¹ Interestingly, it was recently proposed that astrocytes synthesize BDNF *in vitro* in response to SSRIs^{20,32} and 5-HT/norepinephrine or 5-HT/norepinephrine/dopamine reuptake inhibitors.³² Such an event might have a significant role in the therapeutic activity of these classes of antidepressant drugs. The present study used a novel and efficient BDNF-gene transfer strategy to shift the tropism of lentiviral vectors toward astrocytes to determine whether these cells may be activated by SSRIs to promote the *in vivo* synthesis/release of BDNF. To our knowledge, although transgenic mice have been engineered to overexpress BDNF into astrocytes,³¹ this strategy is unique, notably due to the detargeting method using micro ribonucleic acid to eliminate residual expression of BDNF in neurons.²¹

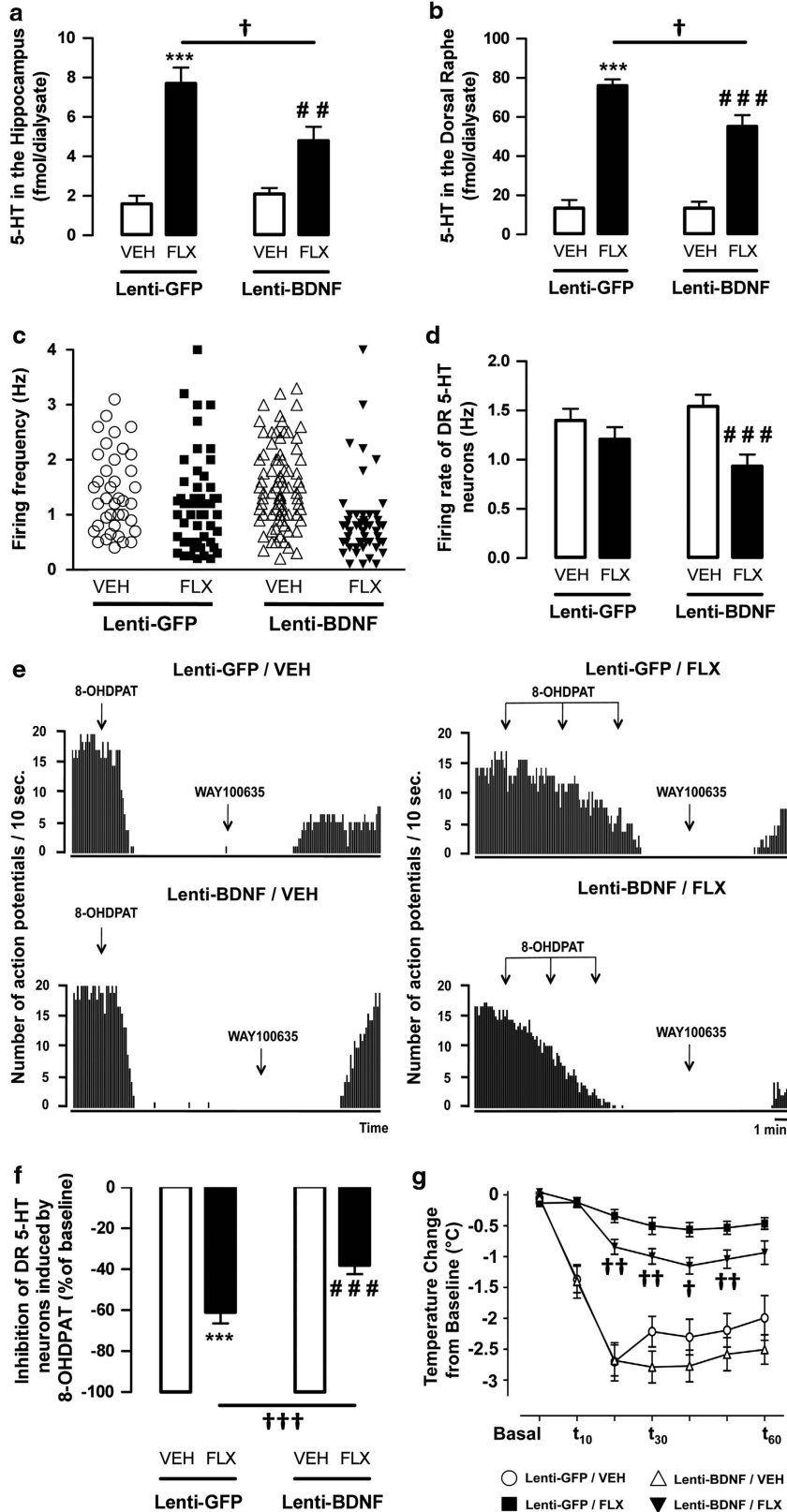
In the hippocampus of mice injected with the Mokola vector, a high density of astrocytes was transfected. As expected, the intrahippocampal injection of the lentiviral vector significantly increased local BDNF levels and related signaling as shown by the stimulation of Erk and Akt phosphorylation. These data seemingly confirm that BDNF was overexpressed and secreted, even in the absence of fluoxetine treatment.

Similarly, when fluoxetine was administered for 28 days in control lenti-GFP-transfected mice, a significant increase in BDNF levels and kinases activation was observed. These data are in agreement with the observation that the application of fluoxetine or paroxetine upregulates BDNF mRNA and protein levels in cultured cortical astrocytes or glioblastoma-astrocytoma cells.^{20,33} They also suggest that SSRIs trigger a signal stimulating astrocytes, which in turn elicits the *in vivo* upregulation of BDNF in these cells. Given that the combination of fluoxetine with lenti-BDNF did not induce more pronounced effects on BDNF levels and kinases activation than those measured with either treatment alone, it can be assumed that SSRIs and astrocytic BDNF acted through a similar downstream mechanism. Alternatively, it is possible that BDNF overexpression masked fluoxetine-induced neuronal BDNF expression. However, the strong tendency of fluoxetine to potentiate lenti-BDNF-induced increase in hippocampal BDNF levels, Erk and Akt phosphorylation is not in favor of the latter hypothesis.

Because the Mokola lenti-vector efficiently stimulated hippocampal BDNF signaling, we examined whether these molecular changes, induced through lenti-BDNF alone or in combination with fluoxetine, modified the anxiolytic-/antidepressant-like responses of mice in various paradigms. One of the most remarkable results obtained herein was the fact that the overexpression of BDNF alone in hippocampal astrocytes produced anxiolytic-/antidepressant-like activity in mice submitted to the NSF in relation to the stimulation of the adult neurogenesis in the hippocampus. These data are in agreement with previous findings showing that the fluoxetine-induced anxiolytic-/antidepressant-like activity in the NSF requires hippocampal neurogenesis.^{1,5} The good correlation between the behavioral response in this paradigm and the number of neurons with tertiary dendrites in lenti-BDNF mice along with the fact that the ablation of hippocampal neurogenesis compromised the behavioral effects of BDNF overexpression, strengthened the hypothesis that hippocampal astrocytic BDNF produces anxiolytic-/antidepressant-like effects in a neurogenesis-dependent manner. Importantly, several studies have reported a positive association between the activation of BDNF/tropomyosin-related kinase B (TrkB) signaling pathways, the upregulation of hippocampal neurogenesis and antidepressant-like activity of SSRIs^{8,34–36} but the neuronal and/or astrocytic source of this factor had yet to be determined. Because the antidepressant response might have involved an excitatory action on the density of glial cells,^{13,37} we also investigated whether BDNF overexpression in hippocampal astrocytes alone or in combination with fluoxetine modified the density of local GFAP+ cells. Our results indicate that this parameter did not differ between groups (Supplementary Figure S6), thus ruling out the possibility that the secreted BDNF acted on astrocytes themselves (autocrine mechanism) to generate newborn neurons. This is in contrast with a recent study showing that hippocampal BDNF infusion partially rescues the local decreased GFAP expression in rats submitted to chronic mild stress.³⁸ Such a discrepancy may be due to the use of non-stressed mice in the present study. Conversely, our results strongly suggest a paracrine-like mechanism where hippocampal astrocytes constitute a microenvironment stimulating

the synthesis and release of BDNF responsible for the neurogenesis and anxiolytic-/antidepressant-like effect in the NSF.

Some behavioral effects of fluoxetine do not require neurogenesis, particularly in the EPM, TST and ST.⁵ Therefore, we assessed the impact of BDNF overexpression in



hippocampal astrocytes in these paradigms. As expected, fluoxetine displayed a robust antidepressant-like activity in the TST and ST. However, BDNF overexpression within astrocytes had no effects in these paradigms. This result can be somewhat puzzling given that BDNF infusion in the hippocampus has been previously reported to improve behavioral parameters such as despair.^{39,40} Several reasons may be advanced to explain these differences such as the strain of mice tested (129sV vs Swiss) or the part of the hippocampus where BDNF or lenti-BDNF virus was injected (dorsal vs ventral). Alternatively, it is possible that chronic overexpression of BDNF produced TrkB receptor desensitization, whereas acute injection failed to do so. In the EPM, the overexpression of BDNF did not produce anxiolytic-like behavior. There is, however, accumulating evidence that hippocampal BDNF has a role in anxiety. For example, mice exhibiting decreased BDNF secretion or possessing a truncated isoform of its preferential TrkB receptor, most notably in newborn neurons,⁴¹ displayed an increased level of anxiety,^{42,43} suggesting that the facilitation of BDNF/TrkB signaling is required for anxiolysis. Although the present study did not report an anxiolytic-like effect of BDNF overexpression alone in hippocampal astrocytes, an anxiolytic-like activity of fluoxetine was unveiled specifically in the group of BDNF-transfected mice. These results are consistent with previous findings showing that SSRIs produce anxiolysis after the intrahippocampal injection of BDNF.³⁹ They are also in agreement with initial data reporting that fluoxetine was unable to reverse increased anxiety-related behaviors in BDNF^{+/-} mice.⁴² Therefore, it appears that the overexpression of BDNF in hippocampal astrocytes favored the anxiolytic-like activity of SSRI and this property did not require neurogenesis because it was insensitive to X-irradiation. It is noteworthy that the neurochemical and behavioral effects observed in the present study might have resulted from viral infection that has been shown previously to cause reactive astrocytosis and neuroinflammation.^{44,45} However, it seems unlikely that lenti-GFP alone increased BDNF signaling since the value of BDNF levels obtained in the present study was quite similar to that previously published by our group in control mice.⁴⁶ Moreover, we demonstrated that the lenti-GFP alone was devoid of behavioral activity (Supplementary Figure S7) thereby excluding the possibility of glial generation

of inflammatory molecules that would have modified behavioral parameters.

To determine a putative mechanism by which BDNF overexpression in hippocampal astrocytes regulated the anxiolytic-like activities of fluoxetine in the EPM, we directed our research on the regulation of the serotonergic system. The overexpression of BDNF in hippocampal astrocytes did not modify extracellular 5-HT concentrations in the hippocampus or DR. However, consistent with previous findings,⁴⁷ an increase in extracellular 5-HT concentrations was detected in response to the chronic administration of fluoxetine in both lenti-GFP and lenti-BDNF mice. This neurochemical effect was, however, less pronounced in the latter group of mice, suggesting that the release of BDNF from hippocampal astrocytes constituted a negative signal on 5-HT neurons. These results were confirmed from the observation that in lenti-BDNF mice, a challenge of fluoxetine significantly increased hippocampal extracellular levels of 5-HT but this neurochemical response was significantly lower than in lenti-GFP mice (Supplementary Figure S8).

Because the ability of SSRIs to enhance 5-HT release is strongly dependent on the firing rate of DR 5-HT neurons,⁴⁸ we determined how neurochemical changes were reflected at the electrophysiological level. No changes in the basal firing rate of DR 5-HT neurons were detected in lenti-BDNF mice administered the vehicle. Conversely, in response to fluoxetine treatment, the firing rate was lower in lenti-BDNF mice than in lenti-GFP mice, and this was reminiscent of the neurochemical data. The acute or sub-chronic administration of SSRIs typically reduces the neuronal activity of DR 5-HT neuronal activity in rodents;⁴⁹ therefore, the electrophysiological response reported herein after 28 days of treatment could be interpreted as a complete recovery to the basal firing rate in lenti-GFP mice. Importantly, when fluoxetine was administered in lenti-BDNF-transfected mice, this recovery of firing seemed partial and might explain the reduced increase in hippocampal and DR extracellular 5-HT levels. It is well known that SSRI-induced progressive return to basal DR 5-HT firing rate results from progressive 5-HT_{1A} autoreceptor functional desensitization.⁵⁰ Hence, we explored the functional status of this 5-HT_{1A} autoreceptor in lenti-GFP and lenti-BDNF mice administered fluoxetine. Using two distinct methods, we demonstrated that the degree of 5-HT_{1A}

Figure 5 Effects of brain-derived neurotrophic factor (BDNF) overexpression in hippocampal astrocytes of adult mice administered with vehicle or fluoxetine on serotonergic neuronal activity. **(a, b)** Extracellular levels of serotonin (5-HT) in the hippocampus and dorsal raphe (DR). Values are expressed as mean \pm s.e.m. of 5-HT levels in the hippocampus (fmol per 20 μ l) **(a)** or in the DR (fmol per 10 μ l) **(b)** of lenti-GFP (green fluorescent protein) and lenti-BDNF mice administered vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks ($n = 5-10$ mice per group). **(c, d)** Electrophysiological activity of DR 5-HT neurons. **(c)** Scattergram depicting the firing frequency of all spontaneously active 5-HT neurons encountered during systematic electrode descents through the DR of lenti-GFP and lenti-BDNF mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks ($n = 4-5$ mice per group). Lenti-GFP vehicle (39 cells recorded), Lenti-GFP fluoxetine (50 cells recorded), lenti-BDNF vehicle (81 cells recorded) and lenti-BDNF fluoxetine (52 cells recorded). **(d)** Values are mean \pm s.e.m. of firing rate of DR 5-HT neurons in lenti-GFP and lenti-BDNF mice administered with vehicle or fluoxetine. **(e-g)** Functional activity of 5-HT_{1A} autoreceptor in the DR. **(e)** Representative integrated firing rate histograms illustrating the effect of 8-OH-DPAT administrations in each group of mice. Note that at the end of each recording, the inhibitory effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT is reversed by the 5-HT_{1A} antagonist WAY100635 (300 μ g kg⁻¹; subcutaneous (s.c.)). **(f)** Values are mean \pm s.e.m. of the percentage of decrease in DR 5-HT firing rate induced by the first dose of 8-OH-DPAT (100 μ g kg⁻¹; s.c.). **(g)** Hypothermic effects of 8-OH-DPAT (100 μ g kg⁻¹; s.c.) in lenti-GFP and lenti-BDNF mice administered with vehicle (VEH; p.o.) or fluoxetine (FLX, 18 mg kg⁻¹ per day; p.o.) for 4 weeks. Values are mean \pm s.e.m. for each time point of body temperature change from baseline ($^{\circ}$ C). Baselines are calculated from the average from three temperature measures before 8-OH-DPAT administrations, each measure being performed with a 10-min interval ($n = 10-15$ per group). ** $P < 0.01$ and *** $P < 0.001$: significantly different from lenti-GFP mice administered with vehicle for 4 weeks. ** $P < 0.01$ and *** $P < 0.001$: significantly different from lenti-BDNF mice administered with vehicle. † $P < 0.05$, †† $P < 0.01$ and ††† $P < 0.001$: significantly different from lenti-GFP mice administered with fluoxetine.

autoreceptor desensitization induced through fluoxetine treatment was blunted in lenti-BDNF mice; thus, this receptor was able to maintain a significant inhibitory influence on 5-HT neurons. These findings might seem puzzling because the overexpression of BDNF was restricted to the hippocampus (Supplementary Figure S1), while the electrophysiological and hypothermic responses to 8-OH-DPAT are distally regulated by the DR.^{28,47} However, given the presence of the TrkB receptor on 5-HT neurons in rodents,^{39,51} an axonal retrograde transport of BDNF from hippocampal 5-HT terminals to their cell bodies within the DR is conceivable, as previously shown following the local infusion of BDNF in this brain region⁵² and in the rat occipital or entorhinal cortex.⁵³ In addition, because *in vitro* and *in vivo* evidence demonstrates that BDNF modulates the 5-HT system, particularly through the induction of the serotonergic phenotype,^{54,55} metabolism^{56,57} and axonal sprouting in the adult brain,^{58,59} we postulate that the overexpression of BDNF in hippocampal astrocytes might have resulted in the remodeling and growth of 5-HT dendrites in the DR, thereby increasing the density of the inhibitory 5-HT_{1A} autoreceptor in response to fluoxetine. Such a mechanism might have participate to maintain an inhibitory feedback induced on the 5-HT system leading to an attenuation of extracellular 5-HT levels, which is supposed to favor anxiolytic-like response. This hypothesis is of particular interest since a recent study has demonstrated that mice displaying a high density of 5-HT_{1A} autoreceptor display an attenuated response to stress and a lower extracellular levels of 5-HT in the hippocampus after fluoxetine treatment.²⁸ Moreover, several pieces of evidence indicate that increased 5-HT tone can increase anxiety through activation of specific post-synaptic 5-HT receptors (for example, the 5HT_{1A}, 5-HT_{2A/C} or 5-HT₃ subtypes) whereas genetic or pharmacological conditions that reduce 5-HT release would be a favorable condition for anxiolysis.⁶⁰

Conclusion

The present data highlight a role for hippocampal astrocytes in the synthesis of BDNF *in vivo*. Given that in physiological conditions astrocytes express very low levels of BDNF, our results suggest that the increase in extracellular 5-HT levels induced by SSRIs might constitute a permissive signal to stimulate such a synthesis. In addition, it clearly appears that BDNF released from astrocytes acts on post-synaptic cells in the hippocampus to stimulate neurogenesis and mediate related anxiolytic-/antidepressant-like activities. Remarkably, BDNF also influence the neuronal activity of presynaptic neurons to prevent SSRI-induced increase in 5-HT tone. This mechanism might be a prerequisite for the potentiation of the anxiolytic-like effects of fluoxetine. Taken together, our results illustrate the concept of the tripartite synapse⁶¹ in which astrocytes may contribute in concert with neurons to increase BDNF/5-HT relationship, and have an important role in both neuronal functions and antidepressant-like activity (Supplementary Figure S9). It is noteworthy that one limitation of our study relies in the fact that some of the cells transfected, albeit weak, looked like transiently amplifying progenitor cells. We cannot rule out that the *in vivo* effects described herein were not mediated, at least in part, by such a residual

overexpression of BDNF in non-glia cells. Our data require therefore further confirmation by using a complementary knockdown approach. If astrocytic BDNF effectively has a role in the antidepressant action then a decreased SSRI activity should be observed after its inactivation.

Conflict of interest

The authors declare no conflict of interest.

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CONCLUSION :

L'ensemble de ces résultats suggère qu'une partie des effets thérapeutiques des ISRS passerait par la stimulation de la synthèse et de la libération de BDNF par les astrocytes. En retour, le BDNF pourrait jouer un rôle important sur le tonus sérotoninergique nécessaire à l'obtention des effets anxiolytiques de ces antidépresseurs. En accord avec le concept de la synapse tripartite, ces données suggèrent que l'augmentation de 5-HT induite par les ISRS est favorable à l'activation des astrocytes.

Ces travaux soulèvent différentes questions et notamment celle de la pertinence physiologique de la surexpression de BDNF astrocytaire dans l'hippocampe adulte et celle de son impact dans des conditions physiopathologiques. Nous avons ainsi réalisé la même étude chez un modèle de souris CORT présentant un phénotype anxio-dépressif.

Article 2

Astrocytic BDNF and fluoxetine modulate hippocampal connexin 43 protein in a mouse model of anxiety/depression

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In preparation

RÉSUMÉ :

Nos résultats précédents suggèrent un rôle original des astrocytes hippocampiques dans la synthèse de BDNF, modulant ainsi différentes facettes de la réponse aux ISRS par des mécanismes neurogène-dépendants et -indépendants. À la lumière de ces données, nous avons appliqué la même stratégie virale que précédemment mais cette fois-ci dans un modèle de souris anxio-dépressives, le modèle CORT basé sur l'administration chronique de corticostérone, dans l'eau de boisson (David et al. 2009). Ainsi, trois semaines après l'injection du lentivirus contenant la séquence du gène codant soit pour la GFP ou le BDNF, nous avons soumis une partie des souris à la corticostérone pendant un mois. À l'issue de cette période, nous avons réalisé une première série de tests comportementaux comprenant le test du labyrinthe en croix surélevé (elevated plus maze, EPM), le test d'hypophagie induite par la nouveauté (novelty suppressed feeding, NSF), le test de suspension caudale (tail suspension test, TST) et le test d'éclaboussure (splash test, ST) dans le but d'obtenir un score comportemental global permettant d'évaluer le phénotype anxio/dépressif des souris. Ceci a pu être réalisé grâce au score "d'émotionnalité" calculé selon la méthode du Z-score (Guilloux et al. 2011). À la suite de ces test comportementaux, nous avons séparé les groupes d'animaux traités à la corticostérone en deux lots : une partie des animaux recevant alors un traitement chronique de fluoxétine (Flx : 18 mg/Kg/jour, per os) ou de véhicule (Veh), pendant un mois. À l'issue de cette période de traitement, nous avons réalisé une seconde série de tests comportementaux et évalué l'activité du réseau astrocytaire à partir de l'expression des connexines 30 et 43.

Nous avons observé que la surexpression de BDNF astrocytaire a permis d'atténuer l'intensité des symptômes anxio/dépressifs induit par l'exposition chronique à la corticostérone.

En accord avec les résultats précédents obtenus chez les souris naïves, nous avons également observé que la surexpression de BDNF astrocytaire au niveau de l'hippocampe permet de révéler les effets anxiolytiques du traitement chronique à la fluoxétine. De manière intéressante, cette

potentialisation des effets de la fluoxétine par le BDNF pourrait s'expliquer par une augmentation de l'activation de la connexine 43 dans l'hippocampe. En revanche, dans ces conditions expérimentales, la surexpression de BDNF n'a pas induit d'effet de type anxiolytique/antidépresseur dans le NSF.

Une partie de ces résultats est en accord avec nos données précédentes obtenues chez les souris naïves, confirmant ainsi que la libération de BDNF astrocytaire pourrait moduler et/ou participer à la réponse aux ISRS en conditions pathologiques, notamment sur le phénotype de type anxieux des animaux.

Contribution personnelle :

Au cours de ce travail :

- j'ai réalisé les injections de lentivirus ainsi que le suivi des animaux incluant la préparation et l'administration des différents traitements pharmacologiques,
- j'ai mené l'ensemble des tests comportementaux,
- j'ai participé à la réalisation des expériences de western blot au Collège de France dans le laboratoire du Dr Giaume avec l'aide de M. Pascal Ezan pour l'étude de l'expression des connexines 30 et 43.

Figure 1

Experimental Protocol

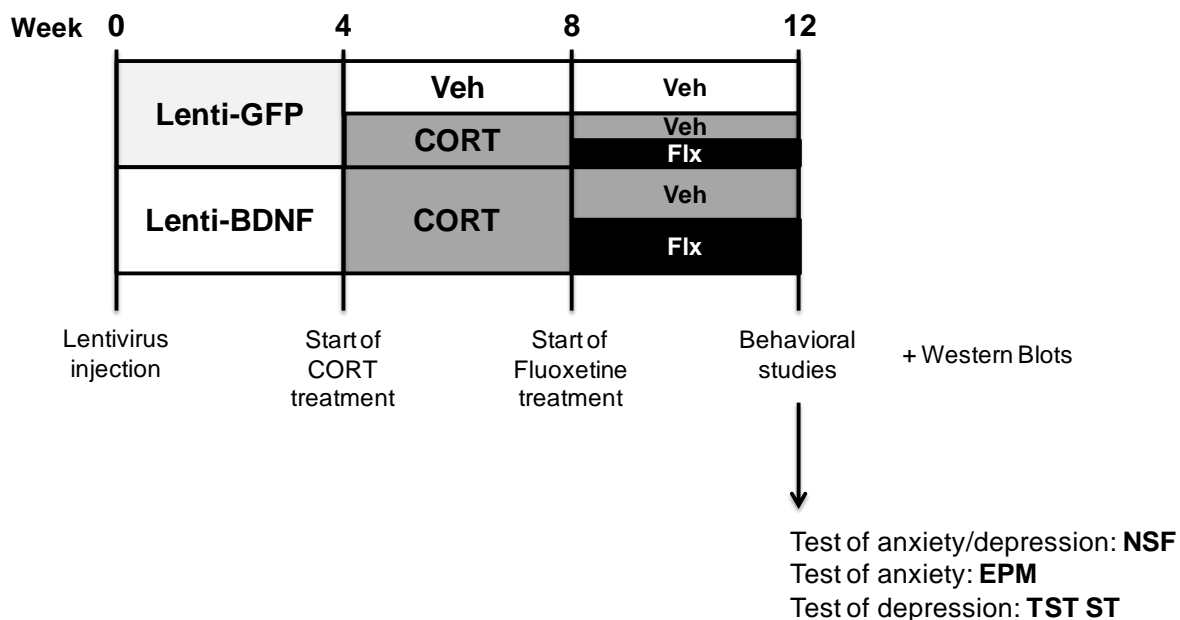


Figure 1: Experimental protocol describing the distinct groups of mice used in this study.

Experimental protocol. Mice were injected either the lentivirus containing the sequence encoding for GFP (Lenti-GFP) or BDNF (Lenti-BDNF). After 4 weeks of recovery, a part the animals was chronically treated either with corticosterone (CORT) or the vehicle (Veh) for 4 weeks. At the end of this treatment behavioral test including the elevated plus maze (EPM), the novelty suppressed feeding (NSF), the tail suspension test (TST) and the splash test (ST), were performed. Then, each groups of animal receiving CORT were separated: a part of the animals received a chronic treatment of fluoxetine (FLX) and the other group the vehicle (VEH), for 4 weeks. At the end of this period a second set of behavioral test were conducted. n= 8-10 animals per group.

Figure 2

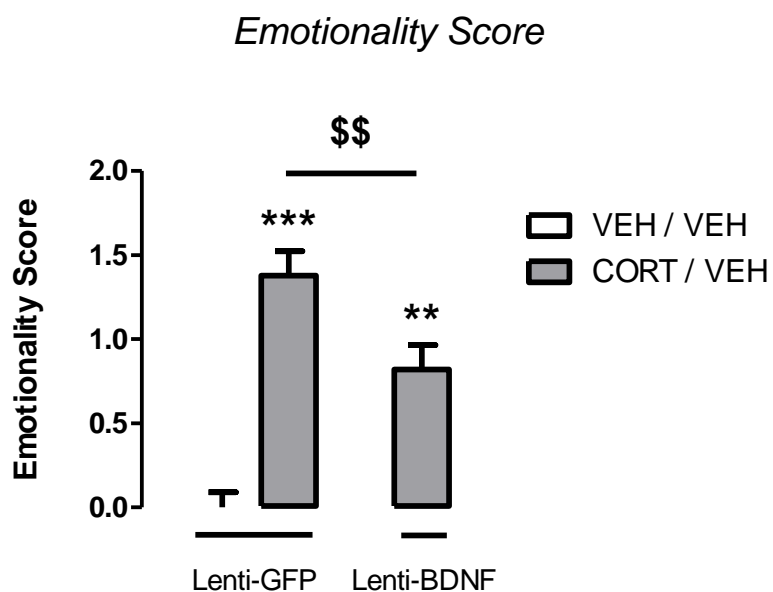


Figure 2: Impact of hippocampal astrocytic BDNF overexpression in the behavioral effects induced by chronic exposure to corticosterone.

After the first series of behavioral tests, a global emotionality score was calculated in order to evaluate the anxiogenic/depressive-like phenotype induced by chronic exposure to CORT. Data are means \pm SEM of emotionality score. In all tests: n= 8-10 animals per group. ** $p < 0,01$; *** $p < 0,001$: significantly different from the corresponding group Lenti-GFP treated with vehicle. \$\$ $p < 0,01$: significantly different from the Lenti-GFP mice administered with CORT.

Figure 3

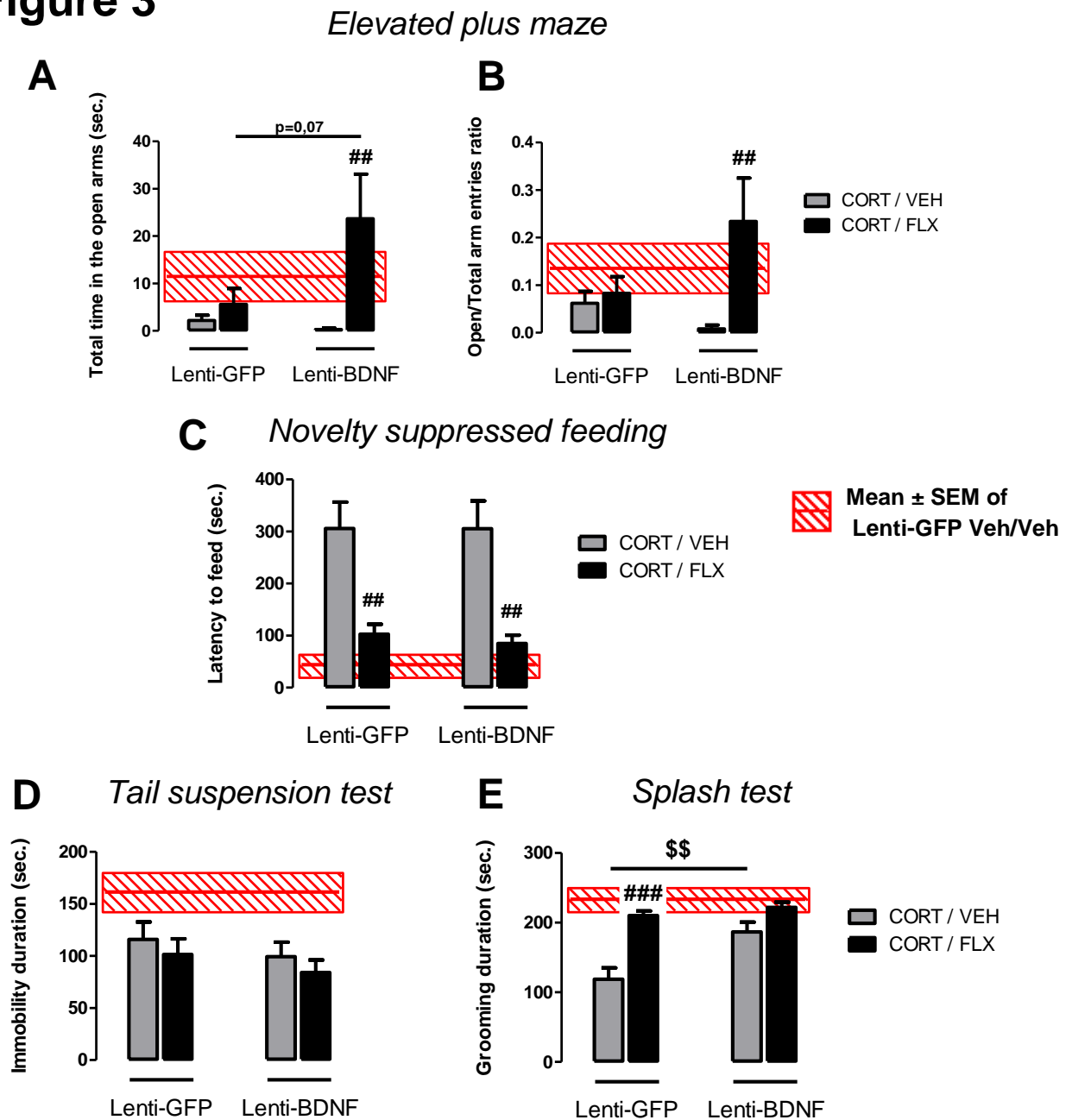


Figure 3 Impact of hippocampal astrocytic BDNF overexpression either alone or in combination with chronic fluoxetine treatment on anxio-/depressive-like phenotype induced by prolonged corticosterone administration.

Anxiolytic-/antidepressant-like effects of fluoxetine (FLX, 18 mg/kg per day; p.o) and vehicle (VEH; p.o) administered for 4 weeks in Lenti-GFP and Lenti-BDNF mice were evaluated in the elevated plus maze (EPM) (A, B), the novelty suppressed feeding (NSF) (C), the tail suspension test (TST) (D) and the splash test (ST) (E). (A, B) Data are means \pm SEM of total time in the open arms in seconds (A) or of the ratio of open/total arms entries (B). (C) Data are means \pm SEM of the latency to feed in seconds. (D) Data are means \pm SEM of the immobility duration in seconds. (E) Data are means \pm SEM of the grooming duration in seconds. In all tests: n= 8-10 animals per group.## $p < 0,01$ and ### $p < 0,001$: significantly different from the corresponding group treated with CORT/Veh. \$\$ $p < 0,01$: significantly different from the Lenti-GFP mice administered with CORT/VEH.

Figure 4

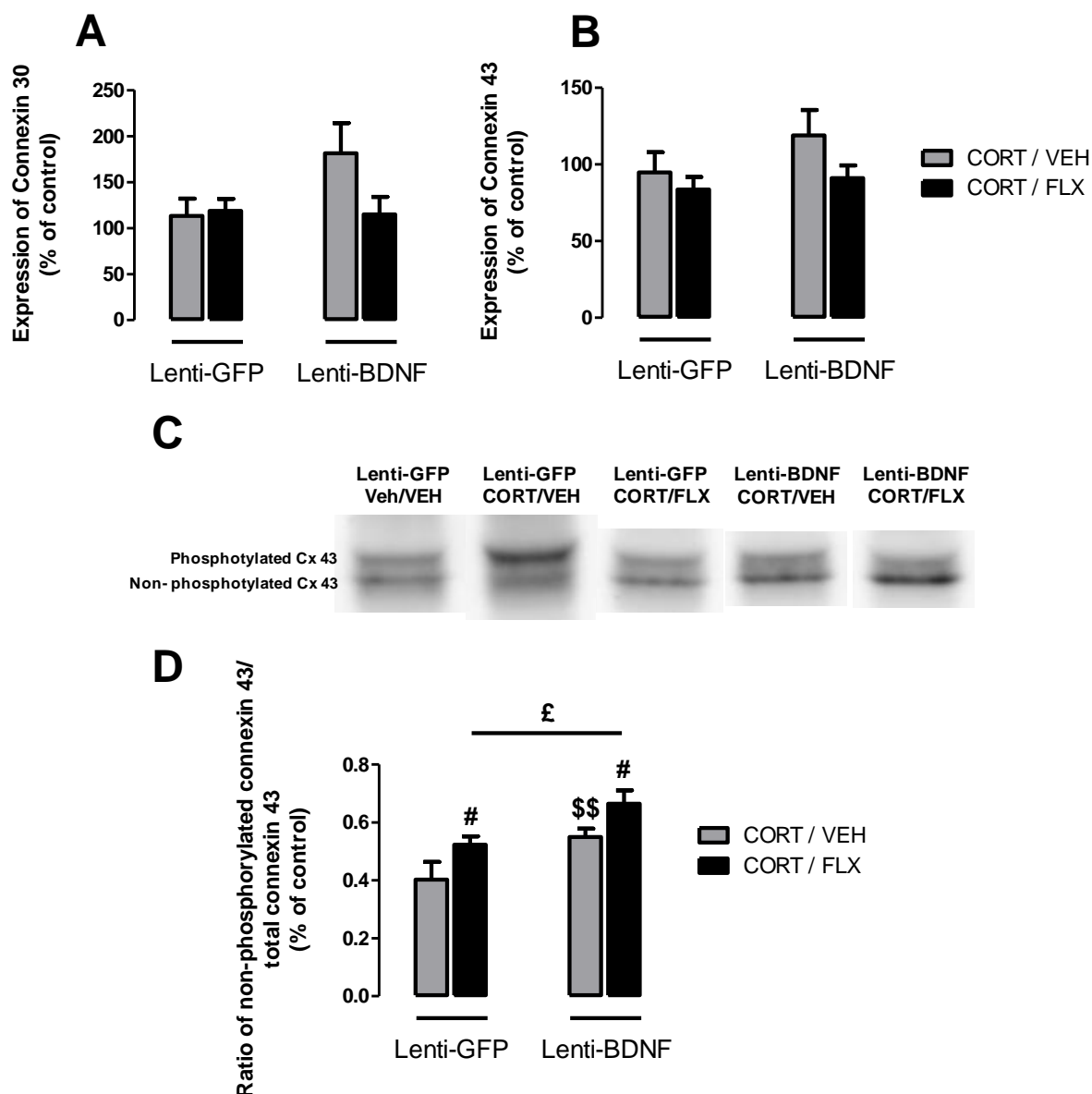


Figure 4: Impact of hippocampal astrocytic BDNF overexpression either alone or in combination with chronic fluoxetine treatment on hippocampal expression of connexin 30 and 43 in mice submitted to prolonged corticosterone administration.

Effects of fluoxetine (FLX, 18 mg/kg per day; p.o) and vehicle (VEH; p.o) administered for 4 weeks in Lenti-GFP and Lenti-BDNF mice on the expression of connexin (Cx) 30 (A), and Cx 43 isoforms (B, D) in the hippocampus. (C) Western Blot SDS-PAGE was processed on 7% Tris-acetate NuPAGE minigels and Cx43 was blotted with the Sigma antibody. Gel were analyzed using Scion Image program. (A, B et D) Expression of Cx 30 and 43 were calculated in percentage from control group (LentiGFP Veh/VEH) and normalized by expression of Tubulin. (A, B) Data are means \pm SEM of the hippocampal expression of Cx 30 (A) or Cx 43 (B). (D) Data are means \pm SEM of the ratio of non-phosphorylated Cx 43 /Total Cx 43 in the hippocampus. In all tests: n= 4-6 animals per group. # $p < 0,05$: significantly different from the corresponding group treated with CORT/Veh. \$\$ $p < 0,01$: significantly different from the Lenti-GFP mice administered with CORT/VEH. £ $p < 0,05$: significantly different from the Lenti-GFP mice

CONCLUSION :

Ces résultats suggèrent d'une part que la synthèse/libération de BDNF par les astrocytes pourrait participer à la prévention des effets délétères induit par l'exposition à un stress chronique induit par la corticostérone. Ils suggèrent également qu'une partie des effets thérapeutiques des ISRS passerait par la stimulation de la synthèse/libération de BDNF par les astrocytes dont les effets autocrines se traduirait par une activation des connexines 43. Ce point est important dans la mesure où ces effets seraient favorables au renforcement de la communication entre ces cellules gliales mais également entre astrocytes et neurones de l'hippocampe.

Les astrocytes seraient donc un des acteurs majeurs de la réponse aux ISRS. En raison de la présence de nombreux sous-types de récepteurs monoaminergiques sur ces cellules gliales, nous nous sommes ensuite intéressés à l'identification du récepteur sérotoninergique astrocytaire qui pourrait être impliqué dans la libération de BDNF. A la lumière des données de la littérature décrites notamment dans ce mémoire bibliographique, nous nous sommes focalisés sur l'implication de la famille des récepteurs 5-HT₂, et plus particulièrement sur le sous-type de récepteur 5-HT_{2A}, dans la physiopathologie de la dépression et le mécanisme d'action des ISRS.

Article 3

Converging translational evidence for the association of functional genetic variation in the serotonin 2A receptor gene with major depressive disorder

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*These authors contributed equally to this study

In preparation.

RÉSUMÉ :

Reposant sur l'hypothèse d'une diminution de la neurotransmission monoaminergique centrale comme principale cause des différentes formes de dépressions, des études génétiques ont tenté de mettre en évidence des associations entre certains polymorphismes (SNPs) des récepteurs sérotoninergiques dont le récepteur 5-HT_{2A}, et la physiopathologie des troubles dépressifs. Cette étude translationnelle visait à déterminer l'impact de l'inactivation génétique du récepteur 5-HT_{2A} sur la sévérité de la dépression 1) chez des patients dépressifs et 2) dans un modèle de souris anxio-dépressives basé sur l'exposition à la corticostérone (CORT).

Afin de comparer le phénotype anxio-dépressif des souris sauvages et mutées pour le récepteur 5-HT_{2A} (KO 5-HT_{2A}), trois tests comportementaux ont été réalisés : le labyrinthe en croix surélevé (EPM), le test de suspension caudale (TST) et le splash test (ST). Un score global "d'émotionnalité" a été déterminé chez les souris, comme le font les échelles d'Hamilton chez l'Homme. En parallèle, l'impact de deux SNPs du récepteur 5-HT_{2A} (T102C et Hys452Tyr) a été évalué sur la sévérité de la dépression à partir d'une cohorte de 485 patients dépressifs. Certains variants alléliques de ces deux SNPs sont suspectés d'entraîner une diminution de l'activité du récepteur 5-HT_{2A}.

Les souris KO 5-HT_{2A} semblent plus sensibles à l'exposition à la corticostérone, développant un phénotype anxio-dépressif plus marqué que les souris sauvages (WT). Chez les patients dépressifs, les patients porteurs de l'allèle C du polymorphisme T102C, présentent un score d'Hamilton plus élevé que les porteurs de l'allèle T. De manière remarquable, le variant TT du polymorphisme His452Tyr, a été identifié chez seulement deux patients de la cohorte, chacun présentant un score de dépression (mélancolique) très élevé.

L'ensemble de ces données suggère que la diminution de l'expression et/ou de l'activité du récepteur 5-HT_{2A} augmente sensiblement la sévérité des épisodes dépressifs majeurs.

Contribution personnelle :

Au cours de ce travail :

- j'ai réalisé le suivi des animaux incluant la préparation et l'administration des différents traitements pharmacologiques ainsi que l'ensemble des tests comportementaux et leur analyse (Z-score).

**Converging translational evidence for the association of functional genetic variation in
the serotonin 2A receptor gene with major depressive disorder**

Category : Regular research paper

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ABSTRACT

An association between serotonin 2A receptor (5-HT_{2A}R), encoded by *HTR2A* gene, and major depressive disorder (MDD) has been suggested. Here, we combined preclinical and clinical ecological approaches to explore the impact of impaired 5-HT_{2A}R-mediated transmission on MDD.

Firstly, *Htr2a* knock-out mice (*Htr2a*^{-/-}) and wild-type mice were compared for the ability of chronic corticosterone to elicit a depressive-like phenotype in three behavioral paradigms (elevated plus maze, tail suspension test and splash test). Secondly, two single nucleotide polymorphisms of the *HTR2A* gene (His452Tyr and 102C/T), which specific allelic variants may decrease 5-HT_{2A}R-mediated transmission (as in *Htr2a*^{-/-} mice), were studied in a sample of 485 Caucasian patients with MDD.

Htr2a^{-/-} mice were more prone than wild-type mice to develop a depressive-like phenotype after chronic corticosterone exposure, as shown by a significant higher “emotionality score” (p<0.01). In patients, the rare *TT* variant of His452Tyr was detected in two patients with a particularly severe melancholic major depressive episode (MDE). The *C* allele of 102C/T was more frequent in depressed patients (p=0.019) and was also associated with a more severe MDE (p=0.008).

This translational and ecological study involving constitutive *Htr2a*^{-/-} knock-out mice and related SNPs in depressed patients suggests that a lower neurotransmission at the 5-HT_{2A}R may favor the susceptibility to MDD and the severity of MDE. It also suggests that specific allelic variants of the 102C/T and His452Tyr may reduce 5-HT_{2A}R-mediated transmission.

Keywords: 5-HT_{2A} receptor, single nucleotide polymorphism, major depression, animal model, translational study

INTRODUCTION

The serotonin 2A receptor (5-HT_{2A}R) is widely expressed throughout the central nervous system, especially around serotonin (5-HT) neurons cell bodies in the raphe nuclei and near most of the serotonergic terminal areas, including the cortex, the hippocampus and the amygdala (Quesseveur et al., 2012). The role of 5-HT_{2A}R in the control of serotonergic tone has been examined and *in vivo* studies report that its acute activation attenuates the firing rate of 5-HT neurons (Wright et al., 1990; Garratt et al., 1991; Martin-Ruiz et al., 2001; Boothman et al., 2003; Bortolozzi et al., 2003; Boothman and Sharp, 2005; Quesseveur et al., 2013), reduces the extracellular 5-HT levels at nerve terminals (Martin-Ruiz et al., 2001) and produces depressive-like behaviors (Diaz and Maroteaux, 2011). In a marked contrast, pharmacological or genetic inactivation of 5-HT_{2A}R with selective antagonists or antisense oligonucleotides respectively, produces antidepressant-like activities in rodents (Sibille et al., 1997; Patel et al., 2004; Pandey et al., 2010; Zaniowska et al., 2010). Importantly, these preclinical studies were performed in naive rodents whereas data using a relevant animal model exhibiting hallmark characteristics of depression is lacking to determine the contribution of the 5-HT_{2A}R in pathological states. Animal models of depression are mainly based on stressful situations, such as unpredictable chronic mild stress (UCMS). Although UCMS has been efficiently used to assess antidepressant-like activity (Surget et al., 2009; Farley et al., 2010), it is notoriously difficult to reproduce consistently in rodents. An alternative is to supply mice with exogenous corticosterone (David et al., 2009; Gourley and Taylor, 2009), an hormone found to be elevated in several animal models of depression and in patients with major depressive disorders (MDD) (Sterner and Kalynchuk, 2010). We have recently reported, in mice administered with corticosterone (“CORT model”) some behavioral abnormalities reflecting major depression (David et al., 2009).

Given the dysfunction of the serotonergic system in major depressive episodes (MDE) of MDD (Baldwin and Rudge, 1995), genetic association studies have focused on the genetic variants at the gene encoding for the 5-HT_{2A}R, *HTR2A* (see (Anguelova et al., 2003; Serretti et al., 2007) for review). The association between MDD and three single nucleotide polymorphisms (SNPs), G-to-A substitution at nucleotide -1438 (*-1438G/A*, rs6311), C-to-T substitution at nucleotide 102 (*102C/T*, rs6313) and C-to-T substitution at nucleotide 1354 (His452Tyr, *1354C/T*, rs6314) has been investigated, showing inconsistent results for the C allele of *102C/T* (association: (Zhang et al., 1997; Du et al., 2000; Arias et al., 2001), no association: (Tsai et al., 1999; Minov et al., 2001; Zhang et al., 2008; Illi et al., 2009; Kishi et al., 2009; Wang et al., 2009)), for the A allele of the *-1438G/A* (association: (Enoch et al., 1999; Jansson et al., 2003; Lee et al., 2006; Christiansen et al., 2007; Kamata et al., 2011), opposite association: (Choi et al., 2004), no association: (Ohara et al., 1998; Illi et al., 2009; Kishi et al., 2009; Tencomnao et al., 2010)) and for His452Tyr which has been poorly studied (no association: (Minov et al., 2001)). Moreover, the functional consequences of these SNPs on 5-HT_{2A}R function and/or *HTR2A* expression remain poorly studied (Serretti et al., 2007), especially for the C allele the *102C/T* SNP, which could be submitted to methylation, a process known to prevent gene expression (Polesskaya et al., 2006) and for the 452Tyr allele of the His452Tyr SNP, which could be associated with a decreased 5-HT_{2A}R-mediated intracellular signaling (Ozaki et al., 1997). These data regarding the functional consequences of these SNPs on 5-HT_{2A}R function and/or *HTR2A* expression deserve further investigation in pre-clinical and clinical studies.

Thus, this translational study was aimed at assessing the functional role of 5-HT_{2A}R signaling in MDD. Indeed, in the present study, genetic evidence for a functional impact of an impairment of brain 5-HT_{2A}R-mediated transmission was studied both in mice with a constitutive genetic inactivation of the 5-HT_{2A}R exposed to corticosterone and in MDD

patients considering two *HTR2A* SNPs supposed to alter the expression of the gene or the function of 5-HT_{2A}R. One of the most remarkable results reported herein is the observation that a lower neurotransmission at the 5-HT_{2A}R is associated with the susceptibility to MDD and the severity of MDE.

METHOD

PRE-CLINICAL STUDY

Animals

Adult male 5-HT_{2A}R mutants (*Htr2a*^{-/-} mice) and wild-type mice (*Htr2a*^{+/+} mice) (Weisstaub et al., 2006) were bred on a S129/Sv genetic background at University of Paris-Sud (SCA Châtenay-Malabry, France). All *Htr2a*^{-/-} and *Htr2a*^{+/+} mice were 7–8 weeks old and weighed 23–35 g at the beginning of the experiments. They were maintained on a 12L:12D schedule, and were housed five per cage. Behavioral testing occurred during the light phase for the elevated plus maze, the tail suspension test and the splash test. All testing was conducted in compliance with the NIH laboratory animal care 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, permissions # 92-196 to Alain Gardier).

Drugs

The administration of corticosterone in the drinking water has been repeatedly used to induce a depressive-like phenotype in mice (David et al., 2009; Rainer et al., 2011). Corticosterone at the dose 70 µg/ml (Sigma-Aldrich, St.Quentin-Fallavier, France) was dissolved in hydroxypropyl-beta-cyclodextrin (β-cyclo: 0.45%, St.Quentin-Fallavier, France) and delivered for 5 weeks in opaque bottles to protect it from the light. It was available ad libitum in the drinking water (David et al., 2009).

Behavioral tests

Distinct groups of *Htr2a*^{-/-} and *Htr2a*^{+/+} mice were used for behavioral analysis in presence or not of corticosterone. All mutants and wild-type mice were submitted to the same sequence of behavioral tests.

Elevated plus maze (EPM). The EPM was performed as previously described (David et al., 2009). Animals were placed into the central area facing one closed arm and allowed to explore the maze for 5 min. Testing took place in bright dimmed light conditions (800–900 lux). Automated scoring was done using ANY-maze(tm) behavioral video-tracking software from Stoelting Co (Bioseb, Vitrolles, France). The total entries and the time spent in the open arms were measured.

Tail suspension test (TST). The TST is a mouse behavioral test useful in the screening of potential antidepressant drugs, and assessing of other manipulations that are expected to affect depression related behaviors (Steru et al., 1985). Mice are suspended by their tails with tape. In such a position animals cannot escape or hold on to nearby surfaces. During the test, typically 6 minutes in duration, the resulting escape oriented behaviors are quantified using the bioseb TST software (Bioseb, Vitrolles, France). A specific strain gauge linked to a computer quantifies properly the time spent by the animal trying to escape i.e. the time of immobility. Immobility is scored as a measure of despair (Lucki, 1997).

Splash test (ST). The ST was performed as previously described (David et al., 2009). This test consisted of squirting 200 µl of a 10 % sucrose solution on the mouse's snout. Because of its viscosity, the sucrose solution dirties the mouse fur and animals initiate grooming behavior. After applying sucrose solution, the time spent grooming was recorded for a period of 5 minutes. A decrease in grooming, which is a particular feature of mice submitted to stress such as the UCMS was used as an index of self-care and motivational behavior (Surget et al., 2008).

Emotionality z-score. Because evaluation of behavioral parameters was performed with several tests in the same animal, a z-score was calculated in *Htr2a*^{-/-} and *Htr2a*^{-/+} mice submitted to chronic corticosterone administration according to Guilloux et al. (Guilloux et al., 2011). This score evaluates consistency of behaviors across tests compared to random changes, and it provides an integrated and continuous measure of severity. Based on the depression definition (i.e. a syndrome as a collection of variable symptoms), z-score constitutes a relevant parameter to evaluate the severity of anxio-depressive-like state in mice taking into consideration different facets of the syndrom as humans scales do. Notably, it has been validated in mice submitted to UCMS and corticosterone exposure. The higher the z-score, the higher depression severity (Guilloux et al., 2011).

Statistical analysis. Statistical analyses were performed with StatView 5.0 software (SAS Institute Inc). One-way analyses of variance were applied to compare the behavioral parameters of *Htr2a*^{-/-} and *Htr2a*^{+/+} mice. When main effects were significant, genotype comparisons were analyzed using a PLSD post-hoc test.

CLINICAL STUDY

Experimental design

In a transversal multicenter study, MDD patients with a current MDE were assessed for clinical characteristics and two *HTR2A* SNPs *102C/T* and *His452Tyr* were genotyped. The study protocol was approved by the French ethics committee of Paris-Boulogne and the French national CCTIRS and CNILs.

Patients

485 Caucasian in or out-patients, aged 18-70 years, with a current MDE diagnosis in a context of MDD (Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revised (DSM-IVTR)) based on the Mini International Neuropsychiatric Interview (MINI, (Sheehan et al., 1998)), were included in 3 university psychiatry departments in Paris, France. A

minimum depression score of 18 (corresponding to 16 in the USA) on the 17-item Hamilton Depression Rating Scale (HAMD-17, (Hamilton, 1960)) was also required to ensure that patients qualify for MDE. Patients with bipolar disorders (DSM-IVTR), psychotic disorders (DSM-IVTR), current substance abuse/dependence (DSM-IVTR), organic brain syndromes or unstable medical conditions were excluded. Patients provided written informed consent for study participation and for genetic analyses.

MDD characteristics

The HAMD-17 and Clinic Global Impression Severity scale (CGI-S, (Guy, 1976)) were rated by trained clinicians to document the current MDE severity. In the whole sample, the HAMD-17 scores range from 18 to 40 and the mean score was 24.8 ± 5.0 (first quartile from 18 to 21, second quartile from 21 to 24, third quartile from 24 to 28, fourth quartile from 28 to 40). The CGI-S scores range from 0 to 7 and the mean score was 4.9 ± 0.75 . The age at onset of MDD and number of previous MDEs were also recorded.

DNA preparation and SNP genotyping

A sample of 5 mL of whole blood was collected. DNA from lymphocytes was extracted from 1 mL of blood sample using a Puregene Kit (Gentra systems, Minneapolis, USA) and cryopreserved at -20°C .

For the *HTR2A* genetic polymorphisms, *-1438G/A* (rs6311) and *102C/T* (rs6313) are known to be in complete linkage disequilibrium (Spurlock et al., 1998; Myers et al., 2007). Our analyses were then performed only on two *HTR2A* SNPs: *102C/T* and His452Tyr (rs6314). SNPs genotyping was performed by a TaqMan SNP genotyping assay (assay ID: C_3042197_1 for rs6313 and C_11696920_20 for rs6314, Applied Biosystems) according to the manufacturer's instructions. SNPs primers and probes were designed for genotyping using the Primer express software from Applied Biosystem (Forster City, CA, USA).

The G-protein beta3 subunit gene (*GNB3*), which has no link with serotonergic signaling, was also investigated (C-to-T substitution at nucleotide 825; rs5443). For *GNB3* genetic polymorphism, the forward 5'-CTGATCCCTGACCCACTTGC-3' and reverse primer 5'-CCTGCCGCTTGTTTGACCT-3' were used for PCR amplification and the "C" (5'-TCACGTCcGTGGCC-3') and "T" (5'-ATCACGTCtGTGGCCT-3') allele probes were labeled with VIC and FAM fluorescent markers respectively at their 5' extremity.

For both genes, allelic discrimination was performed with the ABI prism 7900HT Sequence Detection System (Applied biosystems, Courtaboeuf, France). Genotyping was performed blind to clinical data.

Statistical analysis

Genotype frequencies as well as deviation from the Hardy-Weinberg equilibrium were assessed using the Chi2 test. Analyses of variance (ANOVA) were used to compare quantitative variables according to genotype groups. Chi2 tests were applied to compare qualitative variables according to the genotype groups. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed with StatView 5.0 software (SAS Institute Inc).

RESULTS

PRE-CLINICAL RESULTS

The "emotionality score" was significantly higher in *Htr2a*^{+/+} mice treated with corticosterone than in *Htr2a*^{+/+} mice administered with vehicle (p<0.05; Fig. 1). Having shown the ability of corticosterone to promote depressive-like behaviors in *Htr2a*^{+/+} mice, we then compared this phenotype with that of *Htr2a*^{-/-} mice. We first examined the behavioral response of *Htr2a*^{-/-} mice in the EPM. In this test, no differences between *Htr2a*^{+/+} and *Htr2a*^{-/-} mice were detected on the time spent in the anxiogenic areas such as the open arms (p>0.05; Figure 1A), the ratio

of open/total arms entries ($p > 0.05$; Figure 1B) and the total ambulatory distance ($p > 0.05$; Figure 1C). We then explored mouse behavior in the TST. In this test, a higher immobility time was observed in *Htr2a*^{-/-} compared to *Htr2a*^{+/+} mice ($p = 0.019$; Figure 1D). Finally, in the ST, a lower time of grooming after the application of sweetened solution on the mouse coat was also detected in *Htr2a*^{-/-} compared to *Htr2a*^{+/+} mice ($p = 0.019$; Figure 1E). The determination of the z-score revealed a higher level of anxio-depressive like state in *Htr2a*^{-/-} than in *Htr2a*^{+/+} mice after chronic exposure to corticosterone ($p = 0.002$; Figure 1F).

Importantly, when tested in basal conditions i.e. without corticosterone administration, none of these parameters were significantly influenced by the genetic inactivation of the 5-HT_{2A}R (Table 1).

CLINICAL RESULTS

The socio-demographic characteristics of the sample are shown in Table 2

His452Tyr (1354C/T) HTR2A genetic polymorphism

Among our population, 407 out of 485 (84%) patients carried the *CC* (452His/His) *HTR2A* genotype, 76 (15.6%) the heterozygous *CT* (452His/Tyr) genotype and 2 (0.4%) the *TT* (452Tyr/Tyr) genotype. Allelic frequencies were 0.92 for the *HTR2A C* (452His) allele and 0.08 for the *HTR2A T* (452Tyr) allele, no significant deviation from the Hardy-Weinberg (HW) equilibrium being detected ($\chi^2 = 0.61$, $p = 0.43$). The *TT* variant (452Tyr/Tyr) was evidenced in two patients confirming that it is a rare genotype (cf. HapMap results on dbSNP, NCBI). These 2 patients were analyzed as single cases.

The first patient is a 58-year-old woman with a recurrent MDD, hospitalized for a MDE with melancholic features (DSM-IVTR). With a HAMD-17 score of 36, this patient is a member of the upper quartile of our distribution. Her CGI-S score of 7, which is the maximal score corresponding to an 'extremely ill' patient.

The second patient is a 31-year-old man hospitalized for a current isolated MDE with

melancholic features (DSM-IVTR), comprising marked depressed mood, guilt feelings, severe psychomotor retardation, marked weight loss (15 kilograms in 6 months), and recurrent suicidal ideation. His HAMD-17 score was 33, corresponding to the upper quartile of our distribution, and his CGI-S score was 4. Whereas he had no personal history of MDD, he had a significant familial history since his father, who suffered from a MDD committed suicide by drowning and voluntary self-poisoning, and one of his brothers also had a MDD. Thus, the results of these 2 patients argue for an association between the rare *TT* variant (452Tyr/Tyr) of His452Tyr *HTR2A* polymorphism and severe MDEs.

102C/T HTR2A genetic polymorphism

Among our population, 137 out of 485 (28%) patients carried the *CC HTR2A* genotype, 265 (55%) the *CT* genotype and 83 (17%) the *TT* genotype. The socio-demographic characteristics of the sample are shown in Table 2 according to this genotype. Allelic frequencies were 0.56 for the *HTR2A C* allele and 0.44 for the *T* allele. These measured genotype frequencies were significantly different from the expectations of HW equilibrium ($\chi^2=5.05$, $p=0.019$), with an over-representation of *CC* and *CT* genotypes (389 expected carriers vs 402 measured) and an under-representation of *TT* genotype (96 expected carriers vs 83 measured). Moreover, when testing another gene without direct link with the serotonergic transmission, ie the *GNB3* gene, allelic frequencies for the 825*C/T* SNP were 0.66 for *C* allele and 0.34 for *T* allele. These frequencies are in accordance with HW equilibrium ($\chi^2=0.12$, $p=0.72$). Thus, this result seems to be specific to the polymorphism studied, rather than related to the sample studied, arguing for an over-representation of the 102*C/T C* allele in patients with MDD.

Moreover, whereas the 3 groups of the 102*C/T* genotype (*CC*, *CT* and *TT*) were not significantly different regarding socio-demographic characteristics and history of MDD, HAMD-17 scores were significantly different between the 3 genotype groups (Table 2), with higher scores for *CT* and *CC* groups than for *TT* ($p=0.008$) (Table 2). These results also argue

for an association between the *C* allele of the *102C/T* polymorphism and higher MDD severity.

DISCUSSION

This study provides convergent preclinical and clinical genetic evidence for a significant association between the impairment of brain 5-HT_{2A}R-mediated transmission and both susceptibility and severity of MDE in MDD. Indeed, *Htr2a*^{-/-} mice are more prone than their wild-type littermates to develop a depressive-like phenotype in three different paradigms. And depressed patients with allelic variants suspected to decrease the expression/function of the 5-HT_{2A}R, i.e., the rare *TT* (452Tyr/Tyr) *HTR2A* genotype of His452Tyr and the *C* allele of *102C/T*, have an increased frequency and/or an increased severity of MDE.

In mice, the time of immobility in the TST and the time of grooming in the ST were higher in *Htr2a*^{-/-} than in wild-type mice. These results that can be interpreted as an exaggerated despair and decreased self-care behaviors in mutants compared to control. In the EPM, although *Htr2a*^{+/+} and *Htr2a*^{-/-} mice administered the corticosterone displayed an anxious-like behavior, no significant differences were detected between both genotypes. Importantly, locomotor activity in the EPM was not different between *Htr2a*^{-/-} and *Htr2a*^{+/+} mice thereby excluding the possibility that behavioral responses were related to a sedative effect. To the best of our knowledge, such behavioral investigations in *Htr2a*^{-/-} mice have never been performed in a mouse model of stress. In agreement with the present data (Table 1), an earlier study however did not report any basal modification of despair in *Htr2a*^{-/-} mice submitted to the forced swimming and tail suspension tests (Weisstaub et al., 2006) suggesting that corticosterone played a major role to enhance anxio-depressive-like behavior in these mutants. To better characterize the global impact of the genetic deletion of 5-HT_{2A}R on mice anxio-depressive-like phenotype, we employed the z-score method. This score, developed to integrate various

behavioral disturbances as depression scales do in human, can be used as a relevant index of depression severity. In support of this assumption, it has been shown that wild-type mice submitted to UCMS or chronic corticosterone administration treatment display a higher z-score than their respective controls (Guilloux et al., 2011). In the present study, the severity of depression in response to corticosterone exposure was significantly higher in *Htr2a*^{-/-} than in wild-type controls.

In this sample of depressed patients, 2 patients carrying the rare *TT* genotype (452Tyr/Tyr) of His452Tyr *HTR2A* SNP were hospitalized for a severe melancholic MDE, one of them occurring in a context of familial history of depression, suggesting a high genetic contribution to the development of MDD. This might be related to the fact that the 452Tyr/Tyr variant, corresponding to the *TT* genotype, has reduced ability to activate G proteins, downstream of 5-HT_{2A}R (Hazelwood and Sanders-Bush, 2004). Therefore, these 2 case reports suggest that the severity of MDD might result from a decreased expression/function of the 5-HT_{2A}R.

In this sample of depressed patients, the over-representation of *102C/T* *C* carriers suggests that this allele is associated with MDD. Indeed, this SNP fails to be in HW equilibrium. It is probably because we examined solely a population of people with MDD (Wittke-Thompson et al., 2005), the classical causes of such a disequilibrium such as the presence of non-Caucasian patients or consanguinity being ruled out in this sample. This suggests a selective pressure, which increases the *C* allele frequency in patients with MDD. Moreover, even if the clinical relevance of the difference shown might be challenged in this sample of MDE patients, a higher severity of MDE observed in *C* patients as compared to *TT* patients further supports the association of 5-HT_{2A}R and MDD. And the difference evidenced in depression scores has to be interpreted in the context of the marked difference evidenced in *Htr2a*^{-/-} mice. It might be proposed that such a robust phenotype in mice is due to the use of inbred mice living in a standardized environment whereas MDD is known to result from

complex genetic and environmental interactions (Nestler et al., 2002). Interestingly, the association of 5-HT_{2A}R and MDD has been mainly reported in severe forms of MDD such as MDE with suicidal attempts (Du et al., 2000; Giegling et al., 2006; Li et al., 2006; Saiz et al., 2008; Vaquero-Lorenzo et al., 2008) or MDD with melancholic features (Akin et al., 2004). Moreover, our results are in line with those of a postmortem brain study showing that the *102C/T C* allele (Polesskaya and Sokolov, 2002) and that His452Tyr *T* allele (Blasi et al., 2013) are associated with lower 5-HT_{2A}R mRNA and protein levels. This observation together with our preclinical data in mutant mice supports the idea that a blunted 5-HT_{2A}R-mediated neurotransmission could favor MDD, even if negative results both *in vivo* (Bray et al., 2004) and *in vitro* (Spurlock et al., 1998; Parsons et al., 2004) have been published.

Our clinical results are also in line with those showing a greater 5-HT_{2A}R binding in post-mortem brain tissue (Yates et al., 1990; Hrdina et al., 1993; Arranz et al., 1994; Pandey et al., 2002; Shelton et al., 2009) or in platelets (Hrdina et al., 1995; Sheline et al., 1995; Hrdina et al., 1997) from individuals with MDD, and those evidencing that 5-HT_{2A}R mediated phosphoinositide synthesis was reduced in fibroblasts from patients with melancholic depression compared to controls (Akin et al., 2004).

Finally, this translational study involving both constitutive *Htr2a* KO mice and related SNPs in depressed patients is particularly relevant since the impact of genetic mutation in both species is present throughout life. Moreover, the relevance of this study is due to its ecological conditions. Indeed, an ecological mouse model of depression was used, in which “depressed” animals, i.e. animals displaying features of depressive disorders, instead of normal animals, were used based on the CORT model (David et al., 2009; Sterner and Kalynchuk, 2010; Rainer et al., 2011). In depressed patients, an ecological model was also used *in vivo*, instead of using post-mortem or platelet studies.

Thus, this translational and ecological study in mice and depressed patients suggests that a lower neurotransmission at the 5-HT_{2A}R may favor the susceptibility to MDD and the severity of MDE.

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STATEMENT OF INTEREST

AC Petit, G Quesseveur, R Colle, D David, A Gardier, JP Lépine, C Verstuyft and B Guiard reported no biomedical financial interests or potential conflicts of interest. F Gressier has received consulting fees within the last 3 years from Lundbeck and Servier. F Ferreri has received consulting fees within the last 3 years from Bristol Myers Squibb, E. Lilly and Compagny, Lundbeck and Otsuka pharmaceutical. B Falissard has been consultant for E. Lilly, Sanofi, Pfizer, Roche, Lundbeck, Otsuka, GSK, Bayer, Servier, Stalergène, Baxter, BMS, Amiral, Merk, Genzyme, Shire. E Corruble has received consulting fees within the last 3 years from Lundbeck, Servier, Sanofi-Aventis, Bristol Myers Squibb, Eisai.

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Table 1. Effect of the genetic 5-HT_{2A} receptor (5-HT_{2A} R) inactivation on behavioral responses related to anxiety and depression in basal conditions (without corticosterone) in mice.

	β -cyclo <i>Htr2a</i> ^{+/+}	β -cyclo <i>Htr2a</i> ^{-/-}	P value
TST (immobility time, sec.)	169.9 ± 7.1	187.7 ± 5.5	t _{1,30} =0.07
ST (grooming time, sec.)	161.3 ± 13.1	127.4 ± 13.8	t _{1,30} =0.09
EPM (open arms time, sec.)	60.8 ± 15.8	60.8 ± 15.8	t _{1,30} =0.96

In a separate cohort of animals, *Htr2a*^{+/+} and *Htr2a*^{-/-} mice were submitted to the tail suspension test, splash test and elevated plus maze test after chronic vehicle administration in the drinking water. (A) Despair is measured as means ± SEM of the immobility time in seconds (n=10-12 mice per group). (B) Self-care is measured as means ± SEM of grooming after receiving a 10% sucrose solution of the coat. (C) Anxiety is measured as means ± SEM of time spent in the open arms.

Table 2. Socio-demographic and MDD characteristics according to 102C/T CC, CT and TT genotypes (n=485).

	Total number (n=485)	TT (n=83)	CT (n=265)	CC (n=137)	Statistical analysis	P
Socio-demographic characteristics						
Women (%)	344 (68%)	51 (61.5%)	177 (67%)	101 (74%)	Chi2=3.9	p=0.14
Age < 60 (nb (%))	414 (85%)	69 (83%)	231 (87%)	114 (83%)	Chi2=1.5	p=0.46
Marital status					Chi2=9.2	p=0.16
Single (nb (%))	147 (30.5%)	18 (22%)	87 (33%)	42 (30.5%)		
Married (nb (%))	224 (46 %)	41 (49%)	118 (44%)	65 (47.5%)		
Divorced (nb (%))	85 (17.5%)	14 (18%)	47 (18%)	24 (17.5%)		
Widowed (nb (%))	29 (6%)	10 (5%)	13 (5%)	6 (4.5%)		
Education level					Chi2=3.9	p=0.41
Low (nb (%))	46 (9.5%)	6 (7%)	31 (12%)	9 (6%)		
Medium (nb (%))	222 (46%)	42 (51%)	116 (44%)	64 (47%)		
High (nb (%))	216 (44.5%)	35 (42%)	117 (44%)	64 (47%)		
Professional status					Chi2=7.8	p=0.65
Active (nb (%))	309 (64%)	51 (61.5%)	173 (65.5%)	85 (62%)		
Pensioner (nb (%))	65 (13.5%)	14 (17%)	30 (11%)	21 (15%)		
Unfit for work (nb (%))	22 (4.5%)	7 (8.5%)	10 (4%)	5 (3.5%)		
Unemployed (nb (%))	39 (8%)	6 (7%)	21 (8%)	12 (9%)		
Student (nb (%))	27 (5.5%)	2 (2.5%)	17 (6.5%)	8 (6%)		
Other (nb (%))	22 (4.5%)	3 (3.5%)	13 (5%)	6 (4.5%)		
MDD						
Age at onset (m(sd))	35.2 (14.9)	36.1 (14.6)	35.1 (14.4)	34.7 (16.3)	F=0.22	p=0.80
Nb of previous MDEs (m(sd))	1.8 (2.0)	1.8 (2.1)	1.7 (1.9)	2.0 (2.1)	F=0.89	p=0.41
Current MDE severity						
HAMD-17 (m(sd))	24.8 (5.0)	23.8 (4.5)	25.4 (5.3)	24.3 (4.5)	F=4.8	p=0.008*
CGI (m(sd))	4.9 (0.75)	4.9 (0.7)	5.0 (0.8)	4.9 (0.7)	F=0.71	P=0.49

CC, CT and TT groups are similar according to socio-demographic and clinical history. A significant difference is shown between these 3 groups according to the 17-item Hamilton Depression Rating Scale (HAMD-17) score (* p<0.05). MDD= major depressive disorder, MDE= major depressive episode, m=mean value, sd=standard deviation.

FIGURE 1

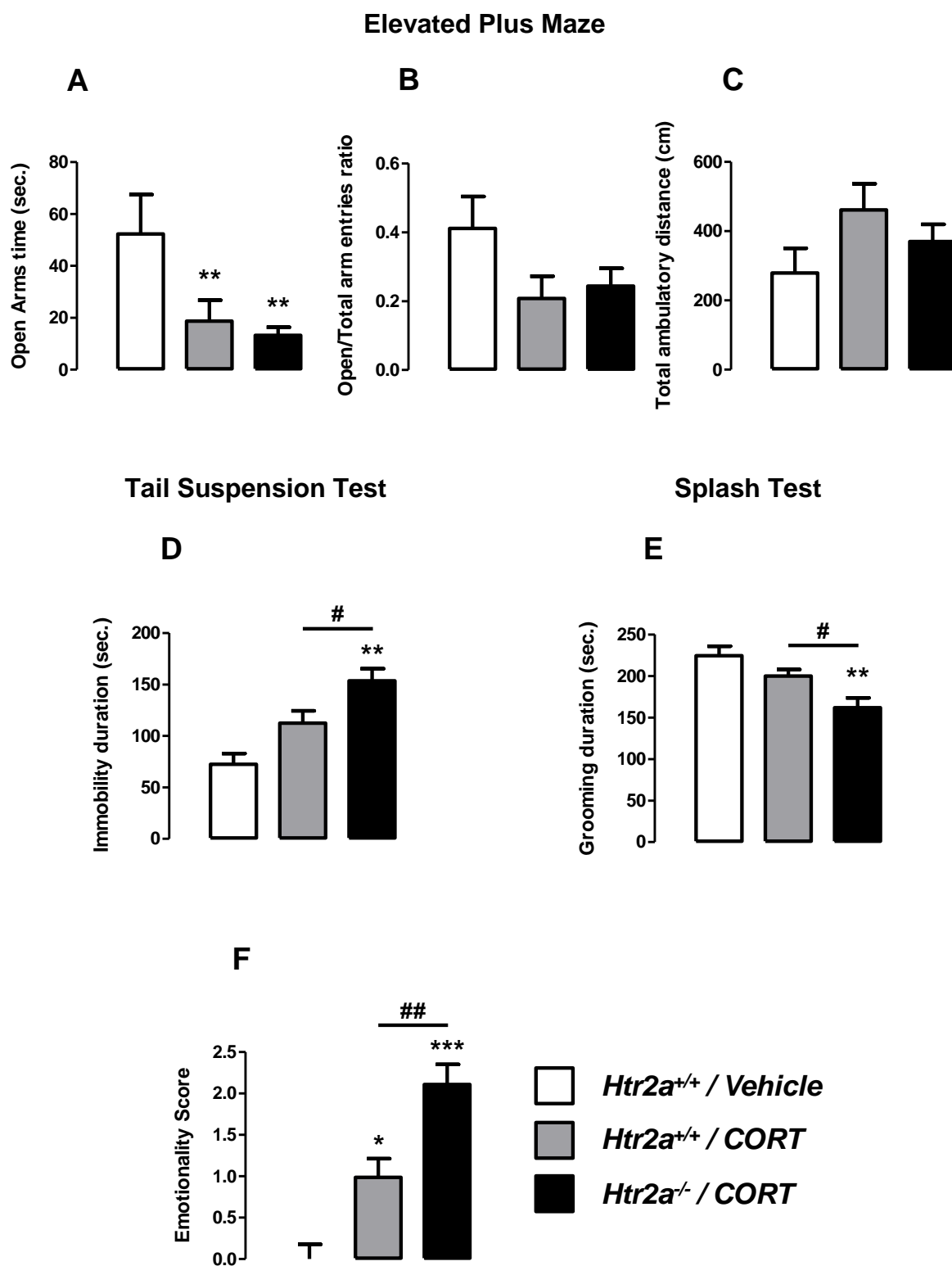


Figure 1. Effect of the genetic 5-HT_{2A} receptor (5-HT_{2A} R) inactivation on behavioral responses related to anxiety and depression after corticosterone administration in mice.

Htr2a^{+/+} (n=12) and *Htr2a*^{-/-} (n=18) mice were chronically exposed to corticosterone (70 µg/kg/day) in the drinking water for 5 weeks and then assessed in the elevated plus maze test (EPM), tail suspension test (TST) and splash test (ST). (A-C) Anxiety is measured in the EPM as means ± SEM of time spent in the open arms [F(1,32)=5.98; p=0.0062] and the ratio of open/total arm entries [F(1,32)=1.61; p=0.21]. (E) Locomotor activity is measured in the EPM as means ± SEM of total ambulatory distance [F(1,32)=1.28; p=0.29]. (D) Despair is measured in the TST as means ± SEM of the immobility time in seconds [F(1,32)=7.5; p=0.0021]. (E) Self-care is measured in the ST as means ± SEM of grooming after receiving a 10% sucrose solution of the coat [F(1,32)=5.87; p=0.0067]. *p<0.05, **p<0.01 and ***p<0.001: significantly different from *Htr2a*^{+/+} mice (n=5). #p<0.05 and ##p<0.01: significantly different from *Htr2a*^{+/+} mice administered the vehicle. (F) Integrated emotionality z-scores in *Htr2a*^{+/+} and *Htr2a*^{-/-} mice exposed to chronic corticosterone administration. Data represent mean S.E.M. of “emotionality score”. This score was calculated from mice behavioral performances in the TST, ST and EPM (all parameters depicted in figure 1) [F(1,32)=13.17; p=0.0001]. *p<0.05 and ***p<0.001: significantly different from *Htr2a*^{+/+} mice. ##p<0.01: significantly different from from *Htr2a*^{+/+} mice administered the vehicle.

CONCLUSION :

L'ensemble de ces résultats précliniques et cliniques suggère qu'à l'instar de l'autorécepteur 5-HT_{1A}, le récepteur 5-HT_{2A} joue également un rôle important dans la physiopathologie de la dépression. La diminution de la transmission sérotoninergique au niveau du récepteur 5-HT_{2A} serait ainsi un des éléments responsables d'une sévérité accrue aux épisodes dépressifs majeurs.

Ces travaux soulèvent différentes questions et notamment celle de l'impact de l'inactivation du récepteur 5-HT_{2A} sur l'efficacité d'un traitement chronique d'ISRS. Nous avons ainsi mené une étude chez la souris afin d'aborder ce problème, notamment dans le but de préciser le mécanisme d'action par lequel l'absence de récepteur 5-HT_{2A} chez la souris pourrait moduler l'activité thérapeutique d'un traitement chronique à la fluoxétine.

Article 4

Impact of genetic inactivation of 5-HT_{2A} receptor in the effects of chronic SSRI treatment

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*These authors contributed equally to this study

In preparation.

RÉSUMÉ :

Les inhibiteurs sélectifs de recapture de la sérotonine (ISRS) représentent la classe d'antidépresseurs la plus prescrite dans le traitement des troubles dépressifs. Cependant, 30% des patients dépressifs ne répondent pas de manière adéquate à ces thérapies. Bien que le rôle de l'autorécepteur 5-HT_{1A} dans la réponse aux ISRS soit clairement admis, des études d'associations génétiques révèlent des liens entre certains polymorphismes de gène codant pour les récepteurs 5-HT_{2A} et la réponse aux traitements antidépresseurs. Afin de préciser le rôle de ces récepteurs dans le mécanisme d'action des ISRS, nous avons comparé les effets comportementaux, électrophysiologiques et neurochimiques d'une administration chronique de fluoxétine chez des souris mutées KO 5-HT_{2A}^{-/-} et des souris sauvages 5-HT_{2A}^{+/+}.

Nos résultats montrent que l'inactivation génétique du récepteur 5-HT_{2A} atténue la capacité du traitement chronique à la fluoxétine à induire des effets de type anxiolytiques/antidépresseurs mais également à stimuler le processus de neurogenèse hippocampique adulte en agissant sur les étapes de maturation et de survie. Par ailleurs nos études électrophysiologiques et neurochimiques révèlent que la capacité du traitement chronique de fluoxétine à stimuler le tonus sérotoninergique est diminuée chez les souris 5-HT_{2A}^{-/-}, et ceci pourrait être en rapport avec une hypersensibilité de l'autorécepteur 5-HT_{1A} chez les souris mutantes.

La diminution de l'expression et/ou de l'activité de la transmission du récepteur 5-HT_{2A} semble donc jouer un rôle dans le mécanisme d'action pré- et post-synaptique des ISRS. Les liens éventuels entre ces deux évènements doivent désormais être démontrés.

Contribution personnelle :

Au cours de ce travail :

- j'ai réalisé le suivi des animaux incluant la préparation et l'administration des différents traitements pharmacologiques ainsi que l'ensemble des tests comportementaux,
- j'ai réalisé les analyses immunohistochimiques des étapes de la neurogenèse hippocampique depuis la perfusion, le prélèvement et la coupe des cerveaux jusqu'à l'incubation des lames avec les anticorps et leurs analyses (comptage),
- j'ai réalisé les études électrophysiologiques (enregistrement des neurones sérotoninergiques) et également les études de microdialyse intracérébrale (implantation des sondes et analyses chromatographiques par HPLC).

Impact of genetic inactivation of 5-HT_{2A} receptor on chronic fluoxetine treatment

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In preparation.

ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed for the treatment of major depression. However, 30% of depressive patients do not respond adequately to these medications. Although evidence incriminates the overactivation of the 5-HT_{1A} autoreceptor in this poor response, others serotonergic receptors could be recruited to modulate the therapeutic activity of SSRIs. In agreement with this hypothesis, growing arguments suggest that variants at gene encoding the 5-HT_{2A} receptor are associated with antidepressant responses but the results of pharmacogenetic studies in human are still matter of debate. The purpose of this study was to determine the effects of constitutive genetic 5-HT_{2A} receptor inactivation on the electrophysiological, neurochemical and behavioral property of SSRIs in mice.

The genetic inactivation of the 5-HT_{2A} receptor (ie. in 5HT_{2A}R^{-/-} knock-out mice) resulted in a 5-HT_{1A} autoreceptor hypersensitization since the ability of 8-OH-DPAT to dampen DR 5-HT firing rate and body temperature was greater in mutants than in wild type (5-HT_{2A}R^{+/+}). *In vivo* microdialysis data confirmed this hypothesis since an acute administration of escitalopram increased hippocampal extracellular 5-HT concentrations in both 5-HT_{2A}R^{+/+} mice and 5HT_{2A}R^{-/-} mice whereas this effect was potentiated by the co-administration of the 5-HT_{1A} receptor antagonist WAY100635 in 5HT_{2A}R^{-/-} mice only. Moreover, the fluoxetine-induced a progressive recovery of dorsal raphe (DR) 5-HT neuronal activity over the time in 5-HT_{2A}R^{+/+} mice, was significantly attenuated in 5HT_{2A}R^{-/-} mice. At the immunohistochemical and behavioral levels this functional adaptation has a dramatic impact on SSRI response since the enhancement of adult hippocampal neurogenesis and the decrease in the latency to feed in the novelty suppressed feeding induced by prolonged administration of fluoxetine was blunted in 5HT_{2A}R^{-/-} mice compared to their wild type littermates.

Altogether, these data indicate that the genetic inactivation of the 5-HT_{2A} receptor gene is associated with a poor fluoxetine response resulting, at least in part, from a decreased serotonergic tone and an impaired adult hippocampal neurogenesis. Although this study has to be confirmed by clinical studies, these results could be of particular importance to predict antidepressant response according to the patients' genotype.

INTRODUCTION

Major depression disorders (MDD) are one of the most widespread psychiatric disorders affecting about 7% of the world's population (Murray et al. 1996; Kessler et al. 2003). While many classes of antidepressant drugs such as selective serotonin reuptake inhibitors (SSRI) have been developed and approved (Wong et al. 2010), approximately one third of patients do not respond adequately to these medications (Trivedi et al. 2006). SSRIs produce a rapid blockade of the serotonin (5-HT) transporter (SERT) thereby inducing an increase in extracellular levels of 5-HT in brain regions such as the frontal cortex or the hippocampus (Gardier et al. 1996). This results in the activation of multiple 5-HT pre- and post-synaptic receptors. One of the major limitations of these drugs is their long delay of action. Electrophysiological and neurochemical studies suggest that such a delayed action depends on the time necessary for 5-HT_{1A} autoreceptors to desensitize (Gardier et al. 1996; Blier et al. 2003; Blier and Ward, 2003). The causal relationship between antidepressant activity of SSRIs and 5-HT_{1A} autoreceptor has also been confirmed by original approaches such as conditional mutant mice (Richardson-Jones et al. 2010; Richardson-Jones et al. 2011) or siRNA injection in the dorsal raphe (Bortolozzi et al. 2012; Ferres-Coy et al. 2013). In accordance with the neurogenic hypothesis of MDD, other evidence has also linked the onset of action of SSRIs with the stimulation of adult hippocampal neurogenesis (Samuels et al. 2011). Indeed, it has been postulated that the increase in extracellular 5-HT levels in the hippocampus following chronic administration of SSRIs, stimulates the expression and release of neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF) which in turn favors cell proliferation and neuronal maturation in rats (Duman et al. 2006). Clinical evidences also demonstrated the impact of BDNF in the adult hippocampal neurogenesis (Boldrini et al. 2009; Boldrini et al. 2012). This latter mechanism would be required for some beneficial effects of these medications (Malberg et al. 2000; Santarelli et al. 2003; David et al. 2009).

Among the long-term targets of sustained administration of SSRIs, the 5-HT_{2A} receptor has drawn a particular attention given its positive coupling to excitatory Gq protein (Raymond et al. 2001; Masson et al. 2012) and its expression in the hippocampus (Pompeiano et al. 1994; Tanaka et al. 2012). Accordingly, numerous studies suggest a role of 5-HT_{2A} receptor in the regulation of adult hippocampal neurogenesis in rodents (Klempin et al. 2010). For example, acute administration of the non-selective 5-HT₂ receptor agonist alpha-methyl-5-HT, decreases cell proliferation in the dentate gyrus (Klempin et al. 2010), whereas that of the selective 5-HT_{2A} receptor agonist, DOI, leads to a significant decrease in BDNF mRNA in the dentate gyrus of the rat hippocampus (Vaidya et al. 1997; Cavus and Duman, 2003). Conversely, the sustained administration of ketanserin, a non-selective 5-HT₂ receptor antagonist, induces a robust increase in progenitor cells proliferation in rats (Banasr et al. 2004) without affecting other hippocampal neurogenesis steps such as maturation of new neurons (Banasr et al. 2004; Jha et al. 2008).

Despite these data, it is difficult to anticipate the role of the 5-HT_{2A} receptor on the therapeutic effects of SSRIs particularly because chronic treatment studies have yet to be conducted. For this purpose, the present study investigated the effect of global genetic 5-HT_{2A} receptor inactivation (i.e. in the 5HT_{2A}R^{-/-} mice) on the antidepressant-like effects of sustained fluoxetine treatment through combined behavioral, electrophysiological and neurochemical approaches.

MATERIALS AND METHODS

Animals

Male 5-HT_{2A} receptor deficient mice (5HT_{2A}R^{-/-}) and wild-type littermates (5-HT_{2A}R^{+/+}), 8-10 weeks old, weighing 30 g, were used in this study. Experimental animals were housed in our animal care facility in groups of 3–6 and kept under standard conditions (room temperature of 22–23°C, 12:12 light–dark cycle, free access to food and water). Mice were tested between 9.00 a.m. and 5.00 p.m. during the light phase. Procedures were conducted in conformity with the institutional guidelines in compliance with national policy (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, permissions #92.196 to A.M. Gardier).

Drugs and administration

Fluoxetine (18mg/kg per day) was purchased from ANAWA (Wangen, Switzerland) and administered in the drinking water in opaque bottles for 4 weeks. Escitalopram oxalate (H. Lundbeck A/S, Denmark) dissolved in DMSO 5 % was administered at a dose of 4 mg/ kg by the subcutaneous (s.c.) route. For hypothermia, electrophysiological and microdialysis studies, 8-OH-DPAT (8-Hydroxy-N,N-dipropyl-2-aminotetralin) (50–250 mg/kg; s.c.) and was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). The choice of doses indicated herein were based on previous data showing the ability of fluoxetine to produce antidepressant-like activity (David et al. 2009), of 8-OH-DPAT to stimulate inhibitory 5-HT_{1A} autoreceptor (Rainer et al. 2012) and escitalopram to increase extracellular 5-HT without interfering with another monoaminergic system (Nguyen et al. 2013).

Behavioral testing

Behavioral procedure starts with the less stressful paradigm and finishes with the most stressful one. So each animal, was successively tested in the novelty suppressed feeding (NSF) and the tail suspension test (TST) paradigm. A 2-day period between each test was respected.

NSF

The novelty suppressed feeding paradigm is a conflict test that elicits competing motivations: the drive to eat and the fear of venturing into the center of brightly lit arena. Latency to begin eating is used as an index of anxiety-/antidepressant-like behavior, because classical anxiolytic drugs or sustained administration of SSRIs 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. An 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. Each mouse was weighed before food deprivation and before testing to assess the percentage of body weight loss.

TST

The tail-suspension test is a mouse behavioral test useful in the screening of potential antidepressant drugs, and assessing of other manipulations that are expected to affect depression related behaviors. Mice are suspended by their tails with tape, in such a position that it cannot escape or hold on to nearby surfaces. During this test, typically 6 minutes in duration, the resulting escape oriented behaviors are quantified using the bioseb tail suspension test (Bisoeb, Vitrolles, France). A specific strain gauge linked to a computer quantifies properly the time spent by the animal trying to escape.

Immunohistochemistry

BrdU labeling for survival study. Mice were administered with BrdU (150mg/kg , i.p. dissolved in saline), twice a day during 3 days before the start of the treatment. After anesthesia with ketamine and xylazine (75mg/kg ketamine; 10mg/kg xylazine; i.p.), mice were perfused transcardially (cold saline, followed by 4% cold paraformaldehyde at 4°C). The identification of survival cells was performed as previously described (David et al. 2009). Briefly, brains were removed and cryoprotected in 30% sucrose PBS with 0.1% NaN₃ and stored at 4°C. Serial sections (35 µM) were cut through the entire hippocampus (plate 41-61; Franklin and Paxinos, 1997) on a cryostat and stored in PBS with 0.1% NaN₃. For DAB staining, 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 min. Brain sections were incubated for 30 min 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 1 hr with secondary antibody (1:200 biotinylated goat anti-mouse) followed by amplification with an avidin-biotin complex. The staining was visualized with DAB. For the quantification of BrdU labeling, a stereological procedure was used as previously described using an Olympus BX51 microscope (Germany).

Doublecortin labeling for maturation studies. The identification was performed by doublecortin (DCX) as previously described (David et al. 2009). Briefly, sections were rinsed in PBS, treated with 1% H₂O₂ in 1:1 PBS and methanol for 15 min to quench endogenous peroxidase activity (and to enhance dendritic staining), incubated in 10% normal donkey serum and 0.3% Triton X-100 for 30 min, and then incubated overnight at 4°C in primary antibody for doublecortin (goat; v1:500; Santa Cruz Biotechnology, Santa Cruz, CA). The secondary antibody was biotinylated donkey anti-goat (v1:500) (Jackson ImmunoResearch, West Grove, PA) in PBS for 2 hr at room temperature. Sections were developed using avidin-biotin complex (Vector, USA) and DAB kit. Bright-field images were taken with an Olympus BX51 microscope. Stereological procedure was used to quantify labeled cells. Once performed, DCX⁺ cells were subcategorized according to their dendritic morphology: DCX⁺ cells with no tertiary dendritic processes and DCX⁺ cells with complex, tertiary dendrites. The maturation index was defined as the ratio of DCX⁺ cells possessing tertiary dendrites over the total DCX⁺ cells.

8-OH-DPAT-induced hypothermia

Body temperature was assessed intrarectally, using a lubricated probe (BIO-BRET-3) inserted approximately 2 cm and monitored (BIO-TK9882, BIOSEB, Vitrolles, France). Mice were singly housed in clean cages for 10 min, and three baseline body temperature measurements were taken to control that stress induces hyperthermia according to Van der Heyden et al., (1997). Ten minutes after the third baseline measurement, animals received 8-OH-DPAT (100 µg/kg; s.c.) and body temperature was monitored every 10 min for a total of 60 min. Decreases in body temperatures were represented as a change from the mean baseline measurement.

In vivo electrophysiological recordings

Mice were anaesthetized with chloral hydrate (400 mg/kg; i.p) and placed in a stereotaxic frame (using the David Kopf mouse adaptor) with the skull positioned horizontally. Additional anesthesia (50–100 mg/kg; i.p.) was given as necessary to maintain a full anesthetic state, characterized by the absence of response to a nociceptive tail pinch. Body temperature was maintained at 37 °C throughout the experiments using a thermistor-controlled heating pad (Astro-Med, Elancourt, France). The extracellular recordings of 5-HT neurons in the dorsal raphe (DR) were performed using single glass micropipettes (R&D Scientific Glass, USA) preloaded with fibre glass strands to promote capillary filling with a 2 M NaCl solution. Single glass micropipettes pulled on a pipette puller (Narishige, Japan) with impedances ranging from 2.5 to 5 MΩ, were positioned 0.2–0.5 mm posterior to the

interaural line on the midline and lowered into the DR, usually attained at a depth of 2.5–3.5 mm from the brain surface (Hof et al. 2000). The DR 5-HT neurons were then identified according to the following criteria : a slow (0.5–2.5 Hz) and regular firing rate and a long duration, positive action potential (Aghajanian and Vandermaelen, 1982). All neuronal activity was recorded in real time using Spike2 software (Cambridge Electronic Design, Cambridge, UK), which was also used to analyze neurons offline. For all dose–response curves, only one neuron was recorded and tested from each animal.

In vivo intracerebral microdialysis

Mice anaesthetized with chloral hydrate (400 mg.kg⁻¹ i.p.), were implanted with probes (CMA7 model, Carnegie Medicin, Stockholm, Sweden) located in the ventral hippocampus (stereotaxic coordinates in mm from bregma (Hof et al., 2000): A= +2.8, L= +3.0, V= -4.0; A, anterior; L, lateral; and V, ventral) (Guiard et al. 2008; Richardson-jones et al., 2010). Animals were allowed to recover from the surgery overnight. The next day, ≈ 20h after surgery, the probes were continuously perfused with aCSF (composition in mmol/L: NaCl 147, KCl 3.5, CaCl₂ 1.26, MgCl₂ 1.2, NaH₂PO₄ 1.0, pH 7.4 ± 0.2) at a flow rate of 1.5 µl/min in ventral hippocampus using CMA/100 pump (Carnegie Medicin, Stockholm, Sweden), while animals were awake and freely moving in their cage. One hour after the start of aCSF perfusion stabilization period, four fractions were collected (one every 15 min) to measure the basal values (mean ± S.E.M. corresponding to B₀ at t₀) calculated for each mouse before systemic administration of vehicle or escitalopram. Subsequent dialysate samples were then collected for a 0- 120 min post-treatment period and analyzed for 5-HT by a high-performance liquid chromatography (HPLC) system (XL-ODS, 4.6 x 75 mm, particle size 3 µm; Beckman) coupled to an amperometric detector (1049 A, Hewlett- Packard, Les Ulis, France). The mobile phase for 5-HT contained 107 or 100 mM NaH₂PO₄, 140 or 151 µM disodium EDTA; 0.77 or 3 mM I-octanesulphonic acid; respectively, and 20% (v/v) methanol (pH adjusted between 4.1 and 4.3 with phosphoric acid). Its flow rate through the HPLC column was set at 0.7 mL/min using a 118 pump (Beckman). The limit of sensitivity for 5-HT was ≈ 0.5 fmol/sample (signal-to-noise ratio=2). At the end of the experiments, localization of microdialysis probes was verified histologically (Bert et al., 2004; Guilloux et al., 2006).

Data statistical analysis

Statistical analyses were performed using the computer software StatView 5.0. (Abacus Concepts, Inc., Berkley, CA, USA). For behavioral and neurogenesis studies a two-way analysis of variance (ANOVA) with treatment (vehicle vs fluoxetine) and genotype (5-HT_{2A}R^{+/+} vs. 5-HT_{2A}R^{-/-}) factors was used. For chronic electrophysiological studies, a two-way ANOVA with duration of fluoxetine treatment (0, 2, 14, 28) and genotype (5-HT_{2A}R^{+/+} vs. 5-HT_{2A}R^{-/-}) factors was applied to compare the experimental groups. For acute electrophysiological and hypothermia studies, area under the curve

(AUC) values in 5-HT_{2A}R^{+/+} and 5-HT_{2A}R^{-/-} mice were compared using a student-t-test. Finally for acute microdialysis studies a two-way ANOVA with pre-treatment (vehicle/vehicle, vehicle/escitalopram and WAY100635/escitalopram) and genotype (5-HT_{2A}R^{+/+} vs. 5-HT_{2A}R^{-/-}) factors was performed to compare the experimental groups. When ANOVA were statistically significant, pair wise comparisons were performed using Fisher Protected Least Significance Difference *post hoc* test. The level of statistical significance was set at $p < 0.05$.

RESULTS

Functional status of 5-HT_{1A} autoreceptor in 5-HT_{2A}R^{-/-} mice in basal conditions.

8-OH-DPAT-induced inhibition of DR 5-HT neuronal activity

To address the possibility that the genetic inactivation of 5-HT_{2A} receptor affected the functional sensitivity of 5-HT_{1A} autoreceptors, we examined the ability of the 5-HT_{1A} receptor agonist 8-OH-DPAT to decrease the firing rate of the DR 5-HT neurons (Figure 1A, B). 8-OH-DPAT (50-250 µg/kg; s.c.) significantly and dose-dependently decreased this firing activity in both 5-HT_{2A}R^{+/+} and 5HT_{2A}R^{-/-} KO mice (Figure 1A). A student-t-test on the area under the curve (AUC) reflecting the percent of decrease in DR 5-HT firing rate, revealed that this inhibitory was higher in the 5HT_{2A}R^{-/-} KO mice than in 5-HT_{2A}R^{+/+} mice (-90.9±1.9, $n = 4$, -65.9±7.0, $n = 4$ $p < 0.05$; Figure 1B)

8-OH-DPAT induced hypothermia

To further explore the functional status of the 5-HT_{1A} autoreceptor, we evaluated the ability of 8-OH-DPAT to decrease body temperature (Richardson-Jones et al. 2010). 8-OH-DPAT (100 µg/kg; s.c.) significantly decreased the body temperature in both 5-HT_{2A}^{+/+} and 5HT_{2A}R^{-/-} mice (Figure 1C). A student-t-test on the AUC of the decrease in body temperature revealed that the hypothermic effect of 8-OH-DPAT was also higher in the 5HT_{2A}R^{-/-} KO than in 5-HT_{2A}^{+/+} (-0.56±0.19 °C, $n = 6$, -1.247±0.14 °C, respectively; $p < 0.05$; Figure 1D).

Potentiation of SSRI-induced increased in extracellular levels of 5-HT by WAY100635 in 5HT_{2A}R^{-/-} mice

Finally, we used *in vivo* intracerebral microdialysis to investigate the effects of acute administration of escitalopram alone or in combination with the 5-HT_{1A} receptor antagonist WAY100635 on 5-HT extracellular levels in the ventral hippocampus. A two-way ANOVA on AUC values of hippocampal extracellular 5-HT concentrations indicated an overall significant effect of treatment [$F(2,30) = 25.66$; $p < 0.001$] and genotype [$F(1,30) = 4.13$; $p = 0.05$]. A significant interaction between these factors was also detected [$F(2,30) = 4.21$; $p < 0.05$]. Escitalopram significantly increased 5-HT extracellular concentrations in the hippocampus of both 5-HT_{2A}^{+/+} (98.36±3.19 vs 319.77±5.6 $p < 0.001$) and 5HT_{2A}R^{-/-} KO mice (108.55±8.9 vs 305.56±56.9; $p < 0.05$) compared to their respective control groups administered with vehicle (Figure 1G). Co-administration of WAY100635 potentiated the neurochemical effects of escitalopram in 5HT_{2A}R^{-/-} KO mice compared to escitalopram alone treated-mice (305.56±56.9 vs 501.60±95.07; $p < 0.001$), but not in 5-HT_{2A}^{+/+} (319.77±5.6 vs 268.7±35.49; $p > 0.05$; Figure 1G).

Effect of genetic inactivation of 5-HT_{2A} receptor on dorsal raphe serotonergic firing rate in after chronic fluoxetine.

At the electrophysiological level, SSRIs have the peculiarity to decrease dorsal raphe (DR) 5-HT firing rate in response to their acute administration due to 5-HT_{1A} autoreceptor overactivation. This firing rate of DR 5-HT neurons progressively returns to baseline when the treatment is prolonged (Guiard et al. 2011). In this context, 5-HT_{2A}R^{+/+} and 5-HT_{2A}R^{-/-} mice were treated for 0, 2, 14 and 28 days with fluoxetine (18mg/kg per day) and the firing rate of 5-HT neurons was recorded (basal firing rate of DR 5-HT neurons of 5-HT_{2A}R^{+/+} and 5-HT_{2A}R^{-/-} mice was 1.35±0.07 Hz and 1.84±0.15, respectively). A two-way ANOVA for firing rate of DR 5-HT neurons indicated an overall significant effect of treatment duration [F(3,310) = 30.71; *p*<0.0001] but not of genotype [F(1,310) = 0.032; *p* = 0.86]. A significant interaction between these factors was also detected [F(3,310) = 8.91; *p*<0.0001]. Two days of treatment with fluoxetine resulted in a 73% and 70% decrease in DR 5-HT neuronal firing rate in 5-HT_{2A}R^{+/+} (0.36±0.06 Hz, *n* = 20; *p*<0,001) and 5-HT_{2A}R^{-/-} (0.6±0.13 Hz, *n* = 26; *p*<0,001) mice, respectively (Figure 2A) while no significant differences were noticed between both genotypes (*p*>0,05). In contrast, although sustained treatment with fluoxetine (14 and 28 days) produced a gradual recovery of DR 5-HT neuronal firing rate in 5-HT_{2A}R^{+/+} (0.88±0.09 Hz, *n* = 32, 1.47±0.24 Hz, after 14 and 28 days respectively, *n* = 19), it failed to do so in 5-HT_{2A}R^{-/-} (0.76±0.09 Hz, *n* = 35, 0.78±0.10 Hz, respectively, *n* = 33). Interestingly, after 28 days regimen of fluoxetine, the firing rate of DR 5-HT neurons in 5-HT_{2A}R^{-/-} was half of that in 5-HT_{2A}R^{+/+} mice (0.78±0.10 vs 1.47±0.24, respectively; *p*<0,001).

Effect of genetic inactivation of 5-HT_{2A} receptor in the behavioral activity of chronic fluoxetine.

The TST is a behavioral paradigm developed to screen drugs with antidepressant-like activities (Steru et al. 1985). A two-way ANOVA revealed a significant effect of genotype [F(1,42) = 17.21; *p* = 0.0002] but not of treatment [F(1,42) = 0.19; *p* = 0.89] whereas the interaction between both factors was statistically significant [F(1,42) = 6.20; *p* = 0.017]. In 5-HT_{2A}R^{+/+} mice, fluoxetine induced an antidepressant-like effect characterized by a decrease in the immobility duration compared to the corresponding group of mice administered with the vehicle (154.01±9.68 vs 113.75±10.5 respectively; *p*<0,05; Figure 3A). In 5HT_{2A}R^{-/-} mice, fluoxetine failed to decrease immobility duration compared to the corresponding group of mice administered with the vehicle (179.41±13.756 vs 215.41±17.858 respectively; *p*>0,05). As expected from the above-mentioned results, fluoxetine induced a more pronounced antidepressant-like effect in 5-HT_{2A}R^{+/+} mice than in 5HT_{2A}R^{-/-} mice (113.75±10.5 vs 215.41±17.858 respectively; *p*<0,001).

In the NSF, a two-way ANOVA revealed a tendency toward a significant effect of genotype [$F(1,42) = 3.58$; $p = 0.059$] but not of treatment [$F(1,42) = 0.057$; $p = 0.81$] factor. No interaction between these variables was detected [$F(1,42) = 1.293$; $p = 0.26$]. In 5-HT_{2A}R^{+/+} mice, fluoxetine induced a tendency to decrease the latency to feed compared to the corresponding group of mice administered with the vehicle (118.5 ± 17.03 vs 84.87 ± 12.83 respectively; $p = 0,059$; Figure 3B). However, this trend was not observed in 5HT_{2A}R^{-/-} mice compared to the corresponding group of mice administered with the vehicle (138.5 ± 15.36 vs 160.0 ± 32.66 respectively; $p > 0,05$). Interestingly, fluoxetine induced a more pronounced anxiolytic-/antidepressant-like effect in 5-HT_{2A}R^{+/+} mice compared to 5HT_{2A}R^{-/-} mice (84.87 ± 12.83 vs 160.0 ± 32.66 respectively; $p < 0,05$). At the end of the test, the mice were immediately transferred to their home cages, where food consumption was measured for 5 min. This parameter used as a relative control for animal hunger and body weight. Two-way ANOVA revealed a no significant effect of genotype [$F(1,42) = 0.32$; $p = 0.57$] treatment [$F(1,42) = 3.17$; $p = 0.08$] and interaction between both factors [$F(1,42) = 0.01$; $p = 0.90$; Figure 3C].

Effect of genetic inactivation of 5-HT_{2A} receptor in the neurogenic activity of chronic fluoxetine.

Due to the implication of hippocampal neurogenesis in the therapeutic effects of SSRIs particularly in the NSF (David et al. 2009), we then investigated this cellular mechanism after chronic fluoxetine treatment in 5-HT_{2A}R^{+/+} WT and 5HT_{2A}R^{-/-} KO mice. For the survival step, a two-way ANOVA revealed a significant effect of genotype [$F(1,16) = 13.16$; $p = 0.002$] and treatment [$F(1,16) = 4.674$; $p = 0.04$] factors without any interaction between both factors [$F(1,16) = 3.48$; $p = 0.08$; Figure 4A]. In 5-HT_{2A}R^{+/+} mice, fluoxetine induced a tendency to increase the number of BrdU-positive cells compared to the corresponding group of mice administered with the vehicle (1501.20 ± 255.48 vs 2348.40 ± 296.04 respectively; $p = 0,06$). In 5HT_{2A}R^{-/-} mice, fluoxetine failed to increase the number of BrdU-positive cells compared to the corresponding group of mice administered with the vehicle (1130.4 ± 96.93 vs 1192.8 ± 121.24 respectively; $p > 0,05$). Interestingly, fluoxetine-induced increase in cell survival was higher in 5-HT_{2A}R^{+/+} than in 5HT_{2A}R^{-/-} mice (2348.40 ± 296.04 vs 1192.8 ± 121.24 respectively; $p < 0,01$).

For the maturation step, two-way ANOVA similarly revealed a significant effect of genotype [$F(1,14) = 8.40$; $p = 0.012$] and treatment [$F(1,14) = 22.07$; $p = 0.0003$] factors without any interaction between both factors [$F(1,14) = 3.79$; $p = 0.07$; Figure 4B]. In 5-HT_{2A}R^{+/+} mice, fluoxetine induced an increase in the number of DCX-positive cells compared to the corresponding group of mice administered with the vehicle (3480 ± 484.97 vs 6501.6 ± 550.89 respectively; $p < 0,01$). In 5HT_{2A}R^{-/-} mice, fluoxetine also increased the number of DCX-positive cells compared to the corresponding group of mice administered with the vehicle (3048 ± 400.86 vs 4298 ± 292.23 respectively; $p < 0,05$), however this

effect was less pronounced in 5HT_{2A}R^{-/-} mice than in 5HT_{2A}R^{+/+} mice after fluoxetine treatment (6501.6±550.89 vs 4298±292.23 respectively; $p < 0,01$).

A similar profile was observed for the number of DCX-positive cells with tertiary dendrites (Figure 4C). Two-way ANOVA revealed a significant effect of genotype [$F(1,14) = 5.61$; $p = 0.03$], treatment [$F(1,14) = 18.63$; $p = 0.0007$] factors but no interaction between both factors [$F(1,14) = 2.45$; $p = 0.14$]. Fluoxetine induced an increase of DCX-positive cells with tertiary dendrites count in both 5-HT_{2A}R^{+/+} mice (1124±205.56 vs 2498.4±347.82 respectively; $p < 0,01$) and 5-HT_{2A}R^{-/-} mice (936±98.88 vs 1579±142.82 respectively; $p < 0,05$). However, this effect was attenuated in 5-HT_{2A}R^{-/-} mice compared to the corresponding group of 5HT_{2A}R^{+/+} mice administered with fluoxetine (2498.4±347.82 vs 1579±142.82 respectively; $p < 0,05$).

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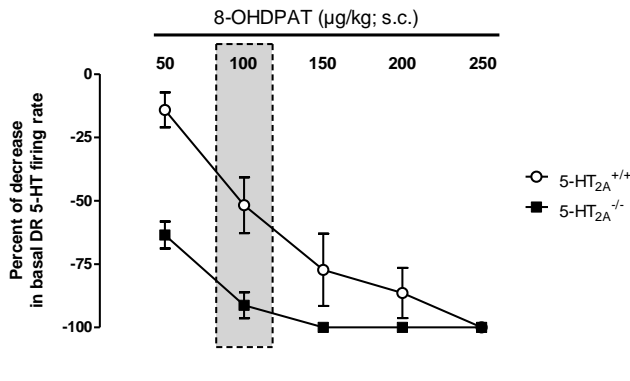
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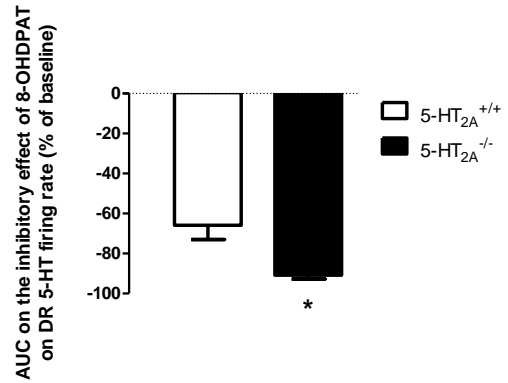
Figure 1

Electrophysiological

A

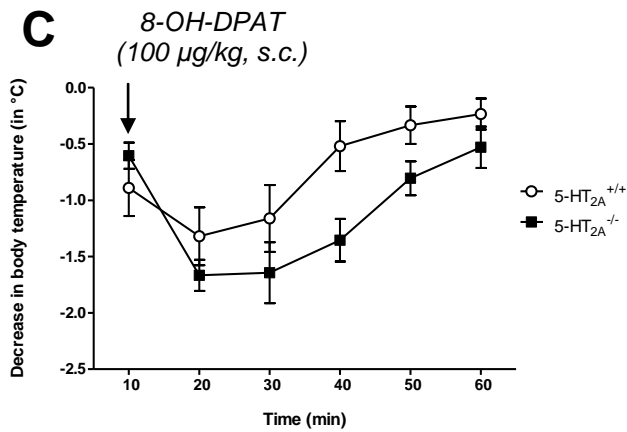


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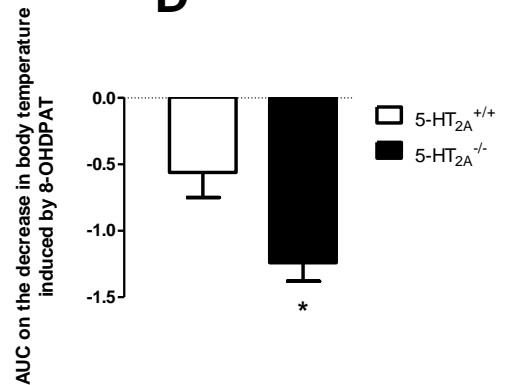


Hypothermia

C

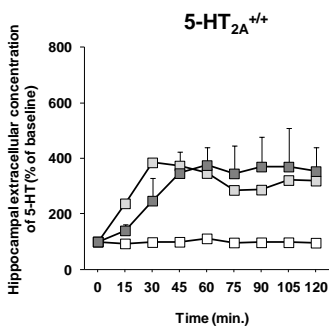


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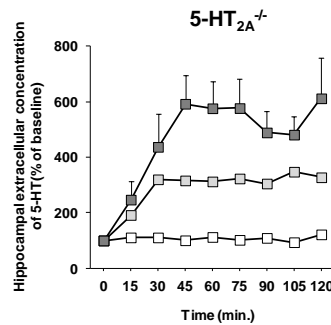


Intracerebral microdialysis

E



F



G

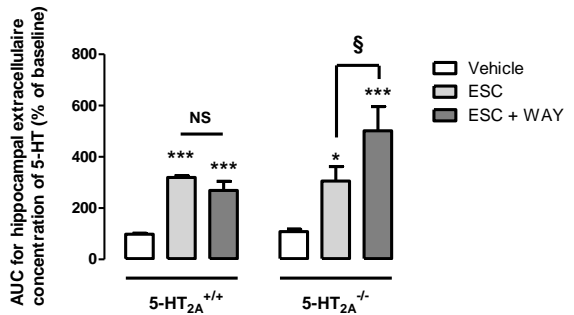


Figure 1: Functional status of 5-HT_{1A} autoreceptor in 5-HT_{2A}R^{-/-} mice in basal conditions.

(A, B) Effect of 8-OH-DPAT administration in 5-HT_{2A}^{+/+} and 5-HT_{2A}^{-/-} mice, on DR 5-HT firing rate (n= 6 per group). (A) Percent of decrease in DR 5-HT firing rate induced by cumulative dose of 8-OH-DPAT (50-250 µg/Kg; s.c.). (B) Data are means ± SEM of AUC for percent of decrease in DR 5-HT firing. (C, D) Effect of 8-OH-DPAT (100µg/Kg; s.c.), on body temperature (n= 4 per group). (C) Decrease of body. (D) Data are means ± SEM of AUC for decrease body temperature induced by 8-OH-DPAT. (E, F, G) Effects of acute administration of escitalopram alone or in combination with the 5-HT_{1A} receptor antagonist WAY100635 on 5-HT extracellular levels in the hippocampus (G) Data are means ± SEM of AUC for hippocampal 5-HT extracellular concentrations expressed as percentage of baseline in 5-HT_{2A}^{+/+} and 5-HT_{2A}^{-/-} mice, administered with vehicle/vehicle, escitalopram (ESC)/vehicle or escitalopram/WAY100635 (n= 4-10 per group). * p<0,05 and *** p<0,001: significantly different from the corresponding group treated with vehicle/vehicle. § p<0,05: significantly different from the corresponding group treated with escitalopram/vehicle. NS: no significant.

Figure 2

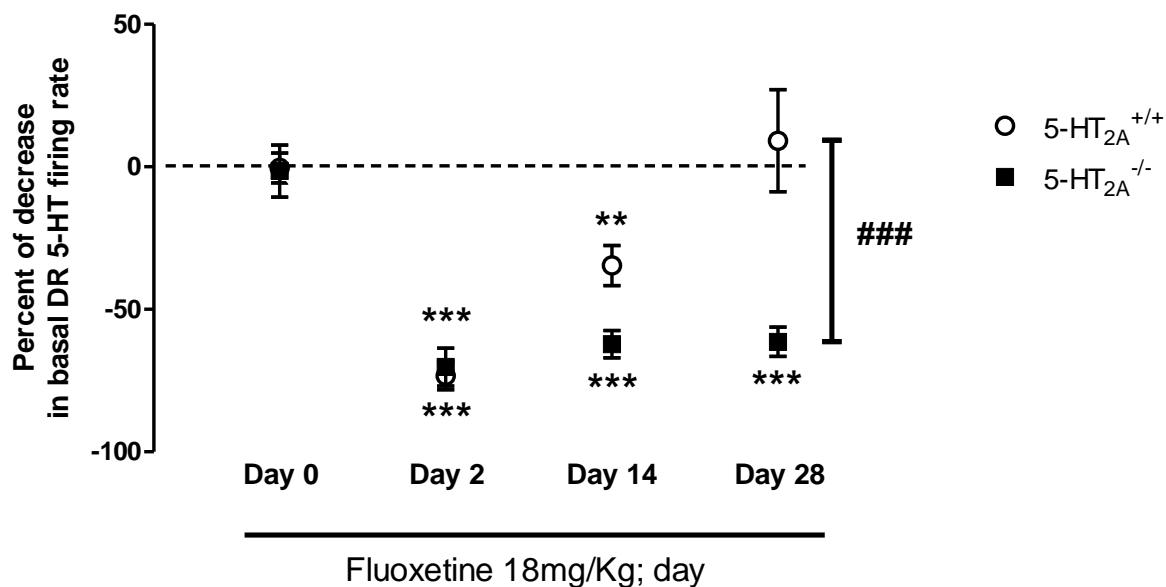


Figure 2: Effect of genetic inactivation of 5-HT_{2A} receptor on dorsal raphe serotonergic firing rate after chronic fluoxetine.

(A) Percent of decrease in basal dorsal raphe (DR) 5-HT firing rate in 5-HT_{2A}^{+/+} and 5-HT_{2A}^{-/-} mice administered with fluoxetine (FLX, 18 mg/kg per day; p.o) after 0, 2, 14 and 28 days of treatment. (n= 20-40 neurons per group). ** p<0,01 and *** p<0,001: significantly different from the corresponding group treated with vehicle. ### p<0,001: significantly different from 5-HT_{2A}^{+/+} mice administered with fluoxetine for 28 days.

Figure 3

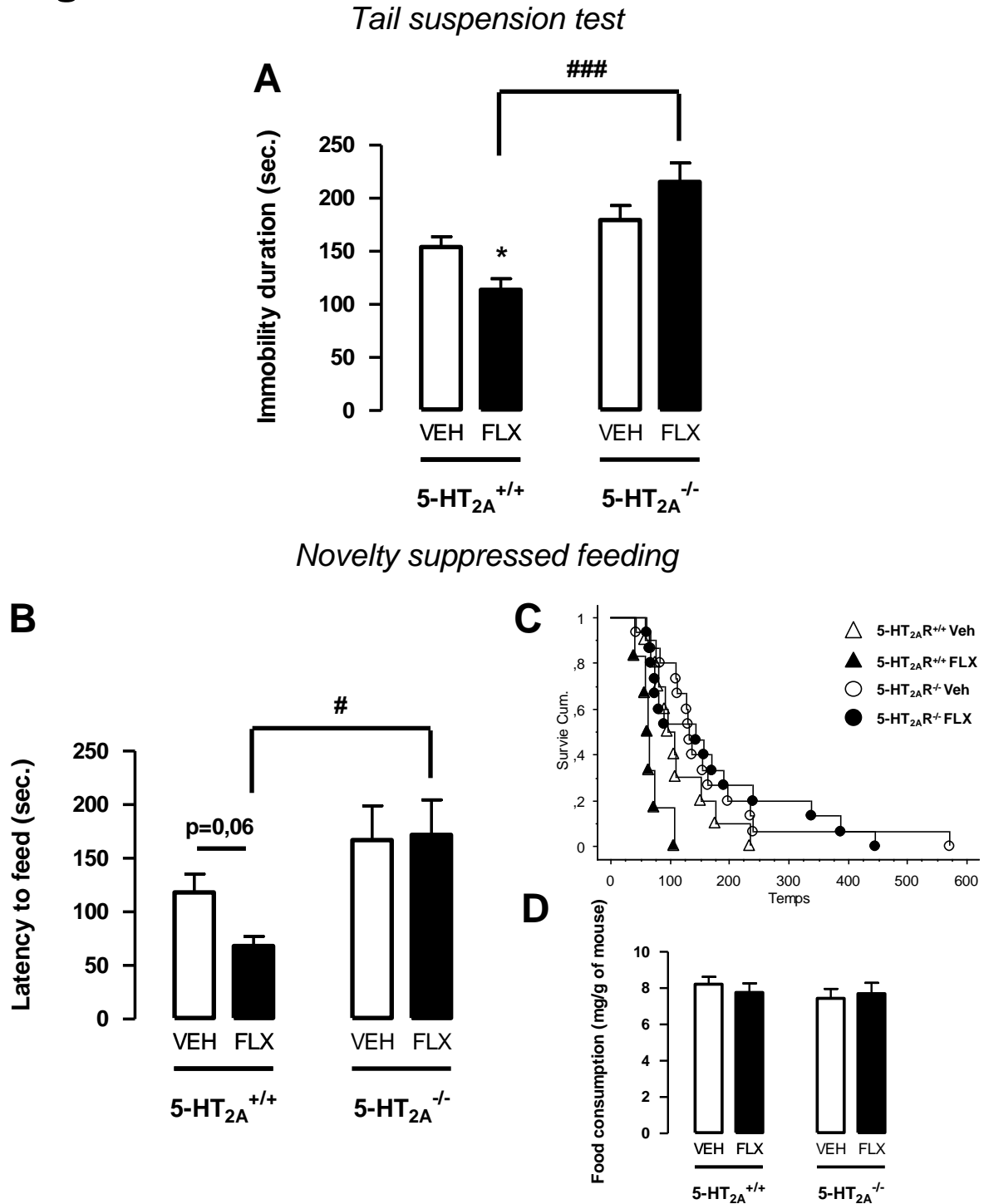


Figure 3: Effect of genetic inactivation of 5-HT_{2A} receptor in the behavioral activity of chronic fluoxetine.

Anxiolytic-/Antidepressant-like effect of fluoxetine (FLX, 18 mg/kg per day; p.o) and vehicle (VEH; p.o) administered for 4 weeks in 5-HT_{2A}^{+/+} and 5-HT_{2A}^{-/-} mice evaluated in the tail suspension test (TST) (A) and the novelty suppressed feeding (NSF) (B-D). (A) Data are means ± SEM of the immobility duration in seconds. (B, C) Data are means ± SEM of the latency to feed in seconds (B) or cumulative survival of animals that have eaten over 10 min (C). (D) Data are means ± SEM of the food consumption (in mg/g of mice). In all tests: n= 8-14 per group. * p<0,05: significantly different from the corresponding group administered with vehicle. # p<0,05 and ## p<0,01: significantly different from 5-HT_{2A}^{+/+} mice administered with fluoxetine.

Figure 4

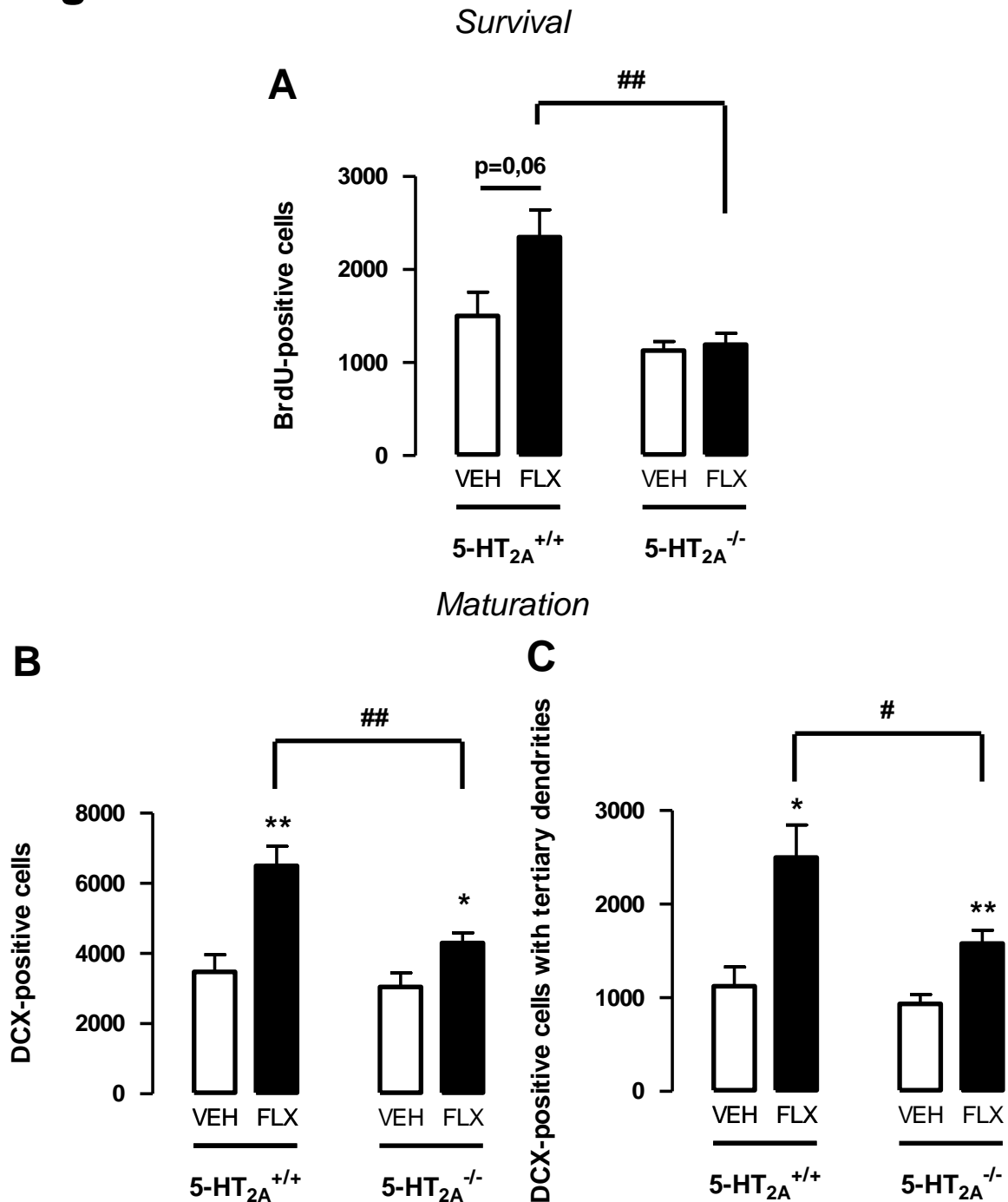


Figure 4: Effect of genetic inactivation of 5-HT_{2A} receptor in the neurogenic activity of chronic fluoxetine.

(A) Survival steps. BrdU (150 mg/kg) was given twice a day for 3 days before fluoxetine (FLX, 18 mg/kg per day; p.o) and vehicle (VEH; p.o) administered for 4 weeks in 5-HT_{2A}^{+/+} and 5-HT_{2A}^{-/-}. Data are means ± SEM of BrdU-positive counts (n= 5 per group). (B,C) Maturation step. Effect of fluoxetine (FLX, 18 mg/kg per day; p.o) and vehicle (VEH; p.o) administered for 4 weeks in 5-HT_{2A}^{+/+} and 5-HT_{2A}^{-/-} mice, on doublecortin (DCX)-positive cells. (B) Data are means ± SEM of DCX-positive cell counts (n= 5 per group). (C) Data are means ± SEM of DCX-positive cell with tertiary dendrite counts (n= 5 per group). * p<0,05 and ** p<0,01: significantly different from the corresponding group treated with vehicle. # p<0,05 and ## p<0,01: significantly different from 5-HT_{2A}^{+/+} mice administered with fluoxetine.

CONCLUSION :

L'ensemble de ces résultats suggère que le récepteur 5-HT_{2A} joue également un rôle important dans les effets thérapeutiques d'un traitement chronique d'ISRS. En effet, dans cette étude nous avons démontré qu'une perte de l'expression du récepteur 5-HT_{2A} atténue les effets thérapeutiques de la fluoxétine suggérant au contraire que son activation serait bénéfiques. Ceci contraste avec les nombreux travaux montrant l'intérêt de bloquer le récepteurs 5-HT_{2A} pour stimuler la neurotransmission sérotoninergique et noradrénergique. Des études complémentaires sont donc nécessaires pour préciser le lien entre les récepteurs 5-HT_{2A} et la réponse aux ISRS, notamment dans un modèle murin de dépression, tel que le modèle CORT. Ces études devraient également permettre d'identifier la nature des cellules post-synaptiques qui pourraient exprimer ce récepteur 5-HT_{2A}.

Discussion

Générale

III. DISCUSSION GÉNÉRALE :

Le but de ce travail était d'identifier les mécanismes post-synaptiques sous-tendant l'effet des antidépresseurs appartenant à la classe des ISRS, et plus particulièrement ceux de la fluoxétine, puisque leur action pré-synaptique a été largement documentée chez le rongeur. En effet, à l'aide entre autres de la technique de microdialyse intracérébrale *in vivo*, on sait désormais que l'administration aiguë d'ISRS entraîne une augmentation des concentrations extracellulaires de 5-HT ($[5-HT]_{ext}$) dans les terminaisons neuronales sérotoninergiques suite au blocage du transporteur de la 5-HT, le SERT. La forte densité de ce transporteur dans la région des noyaux du raphé (NR), riche en corps cellulaires sérotoninergiques, provoque également une augmentation locale de $[5-HT]_{ext}$. Ceci a pour conséquence de provoquer une forte stimulation des autorécepteurs somatodendritiques $5-HT_{1A}$ par la 5-HT endogène à l'origine d'une diminution de l'activité électrique des neurones sérotoninergiques des NR, et donc d'une augmentation modérée de $[5-HT]_{ext}$ dans les régions terminales. Lorsque le traitement se prolonge, on observe alors une désensibilisation des autorécepteurs $5-HT_{1A}$ favorisant un retour à une activité électrique normale (Blier et al. 2003) qui s'accompagne d'une élévation significative des $[5-HT]_{ext}$ dans les aires de projections (Gardier et al. 1996). Il est intéressant de noter que le délai nécessaire à cette adaptation fonctionnelle coïncide avec l'apparition des premiers signes cliniques bénéfiques des ISRS chez l'Homme, mettant ainsi en évidence un lien entre l'activité thérapeutique des antidépresseurs et l'augmentation du tonus sérotoninergique.

Malgré ces connaissances, récemment confortées par des études génétiques (Richardson-Jones et al. 2010 ; Feres-Coy et al. 2013), le mode d'action post-synaptique des ISRS doit être mieux précisé. Les travaux de Ron Duman de l'Université de Yale, ont permis de proposer dès 1995 que le mécanisme d'action post-synaptique des ISRS reposait, du moins en partie, sur la synthèse d'un facteur neurotrophique, le brain-derived neurotrophic factor (BDNF). Ce facteur est capable de promouvoir la croissance, le remodelage, la prolifération, la survie et la maturation des neurones de l'hippocampe, c'est-à-dire le processus de neurogenèse chez l'adulte (Samuels et al. 2011). L'observation récente que l'ablation pharmacologique, physique ou génétique de ce processus de neurogenèse, bloque certaines propriétés comportementales des ISRS chez le rongeur, laisse entrevoir son rôle majeur dans certaines réponses aux antidépresseurs (Santarelli et al. 2003). Bien qu'une pathologie psychiatrique aussi complexe, que sont les troubles dépressifs majeurs, ne puisse être simplement expliquée par l'altération de la neurogenèse, cette caractéristique a toutefois été rapportée chez le primate et chez l'Homme (Boldrini et al. 2009 ; Knoth et al. 2010 ; Perera et al. 2011; Boldrini et al. 2012).

Dans ce travail de thèse, nous nous sommes focalisés sur l'importance de ce processus de neurogenèse dans la réponse aux ISRS en essayant de répondre à plusieurs questions encore non élucidées. Nous avons tenté, en particulier, de déterminer le type cellulaire non-neuronal et le

récepteur sérotoninergique post-synaptique qui pourraient participer à la synthèse de BDNF, et donc indirectement aux effets comportementaux des antidépresseurs dépendants de la neurogenèse.

Nous apportons ici de nouvelles données concernant le mécanisme d'action des ISRS et le rôle fondamental non seulement des astrocytes, mais aussi du récepteur 5-HT_{2A} dans la réponse comportementale et neurochimique de cette classe d'antidépresseurs.

Considérations méthodologiques

Depuis quelques années, nous travaillons au laboratoire sur les liens entre la réponse aux antidépresseurs et la stimulation de la neurogenèse hippocampique chez l'adulte. Pour ce faire, nous avons besoin de tests comportementaux adéquats dont certaines réponses aux traitements sont corrélées à une ou plusieurs étapes de la neurogenèse. Plusieurs études comportementales ont mis en évidence que le test d'hypophagie induite par la nouveauté (novelty suppressed feeding : NSF), était pertinent dans la mesure où l'activité de type anxiolytique/antidépresseur des ISRS dépendait de ce processus (Santarelli et al. 2003; Surget et al. 2008 ; David et al. 2009). En effet, ces études démontrent que l'irradiation aux rayons X du gyrus dentelé (DG) de l'hippocampe bloque les effets neurogéniques et comportementaux, notamment ceux obtenus dans le test d'hypophagie induite par la nouveauté, provoqué par un traitement chronique d'ISRS (David et al. 2009). Pour mieux comprendre le mécanisme d'action des antidépresseurs, il est également nécessaire d'avoir recours à des modèles animaux pertinents. Il existe des modèles génétiques des pathologies dépressives révélant des différences héréditaires de sensibilité et/ou de susceptibilité à des facteurs stressants (Paré et al. 1993; Lahmame et al. 1997; El yacoubi et al. 2003). D'autres modèles ont ainsi été développés à partir de l'observation que la dépression résultait, chez certains sujets, d'un stress chronique lié à des facteurs exogènes. C'est ainsi que le modèle de stress chronique imprévisible modéré a été établi (unpredictable chronic mild stress : UCMS) (Papp and Moryl, 1994; Mineur et al. 2003; Mineur et al. 2006). Cependant, certains symptômes typiquement observés chez l'Homme comme la faible estime de-soi ou les pensées suicidaires sont impossibles à modéliser chez l'animal. La validité phénoménologique de ce modèle peut donc être questionnée et constitue une des limites de l'approche préclinique chez les rongeurs. En revanche, ce modèle présente une validité prédictive (réponse aux traitements chronique d'antidépresseurs) intéressante. Au cours de ce travail de thèse et pour des raisons de reproductibilité chez la souris, nous n'avons pas utilisé ce modèle d'UCMS mais un modèle basé sur l'administration chronique de glucocorticoïdes. Ce modèle, utilisant l'administration chronique de corticostérone dans l'eau de boisson, permet de reproduire les effets neuroendocriniens d'un stress chronique conduisant à un état de type anxio-dépressif chez la souris (David et al. 2009; Bacq et al. 2012). A l'instar du modèle UCMS, les altérations comportementales sont donc corrigées

par un traitement chronique, et non aigu, de différentes classes d'antidépresseurs tels que les tricycliques (Gourley et al. 2008), les ISRS (David et al. 2009), et plus récemment l'agomélatine (Rainer et al. 2012) ; ce modèle présente aussi l'avantage d'une mise en œuvre simple. Enfin, il permet de pouvoir s'affranchir des rythmes circadiens de sécrétion de cette hormone (pic en fin de journée) ainsi que de la réponse au stress aigu, qui peuvent varier d'un individu à l'autre (Droste et al. 2008; Sarabdjitsingh et al. 2010).

Dans le but de répondre à la première question posée sur la nature des cellules non-neuronales susceptibles de synthétiser et/ou de libérer le BDNF dans l'hippocampe, nous avons utilisé une stratégie de transfection virale originale consistant à injecter un lentivirus ayant un fort tropisme astrocytaire et contenant le gène codant pour le BDNF (Colin et al. 2009).

Enfin, la détermination de la nature du récepteur sérotoninergique susceptible d'être impliqué dans la synthèse et/ou la libération de BDNF par les astrocytes hippocampiques, a été abordée à l'aide d'une lignée de souris disponible au laboratoire : les souris mutées privées de manière constitutive du récepteur 5-HT_{2A} (KO 5-HT_{2A}^{-/-}) (Weisstaub et al. 2006). Au regard de cette problématique, il aurait probablement été plus intéressant de travailler chez des souris privées spécifiquement du récepteur 5-HT_{2A} dans l'hippocampe mais, à notre connaissance, cette lignée de souris n'a pas encore été caractérisée. Ceci ne nous permet pas non plus d'envisager à l'heure actuelle une stratégie génétique d'inactivation du récepteur 5-HT_{2A} spécifiquement sur la membrane des astrocytes de l'hippocampe. En revanche, sous l'impulsion du Dr Nicole Déglon (Université de Lausanne), des outils de biologie moléculaire (lentivirus contenant des siARN pour le BDNF ou le récepteur 5-HT_{2A} par exemple) et des approches lentivirales sont désormais envisageables (Delzor et al. 2013).

BDNF astrocytaire et réponse de type antidépresseur

L'idée princeps de l'étude du rôle du BDNF astrocytaire dans la réponse aux ISRS provient de différentes données indiquant que diverses classes d'antidépresseurs stimulent la synthèse de ce facteur neurotrophique dans des cultures primaires d'astrocytes de rat (Allaman et al. 2011; Kittel-schneider et al. 2012; Takano et al. 2012). À partir de ces données *in vitro*, nous avons émis l'hypothèse que les astrocytes pourraient constituer une source de BDNF dans l'hippocampe pour stimuler le processus de neurogenèse, et ainsi participer aux effets thérapeutiques des ISRS. Cette hypothèse est loin d'être validée *in vivo* et se heurte à de nombreuses études selon lesquelles la principale source de BDNF dans le système nerveux central (SNC) serait neuronale. Pourtant quelques données montrent que dans certaines conditions notamment pathologiques, telle que l'induction d'une mort neuronale par excitotoxicité (acide quinoléique), les astrocytes pourraient sécréter du BDNF (Giralt et al. 2010).

Dans ce travail de thèse, nous avons mis en évidence que la surexpression de BDNF dans les astrocytes de l'hippocampe provoque un effet de type anxiolytique/antidépresseur dans le test d'hypphagie induite par la nouveauté, mimant ainsi celui d'un traitement chronique de 4 semaines à la

fluoxétine. Il est intéressant de noter que ces données comportementales vont dans le même sens que celles observées sur les différentes étapes de la neurogenèse, puisque la surexpression de BDNF astrocytaire, ainsi qu'un traitement à la fluoxétine, stimulent la prolifération, la maturation et la survie neuronale. L'hypothèse d'un effet dépendant de la neurogenèse a été confirmée à la faveur d'une collaboration avec l'équipe du Pr. R. Hen (Université de Columbia à New-York), puisque nous avons montré que l'ablation de la neurogenèse hippocampique (à l'aide de rayons-X) bloque les effets comportementaux du lentivirus, mais aussi ceux de la fluoxétine dans le test d'hypophagie induite par la nouveauté (**ARTICLE 1**). L'observation que la combinaison du lentivirus avec un traitement prolongé à la fluoxétine, ne produit pas d'effets additifs ou synergiques, suggère l'existence d'une voie commune entre le BDNF astrocytaire et la fluoxétine menant aux mêmes effets comportementaux. En particulier, on peut se demander si l'augmentation des $[5\text{-HT}]_{\text{ext}}$, en réponse à la fluoxétine, ne participe pas à la stimulation d'un récepteur sérotoninergique situé sur les astrocytes responsables de la synthèse et/ou de la libération du BDNF par ces cellules gliales. En outre, l'augmentation de synthèse de BDNF, décrite dans la littérature, en réponse à un traitement chronique d'ISRS (Nibuya et al. 1995; Conti et al. 2002; Duman et al. 2006), pourrait résulter d'une action neuronale et astrocytaire faisant de ce dernier type cellulaire un partenaire potentiel dans la réponse anxiolytique/antidépresseur de la fluoxétine. Pourtant différentes évidences (indirectes) laissent entrevoir un rôle majeur des neurones dans cette réponse. En effet, dans les études du Pr Duman (Nibuya et al. 1995; Nibuya et al. 1996), l'analyse *in situ* de la quantité de BDNF ou de la protéine CREB a été réalisée dans la couche de cellule granulaire du DG de l'hippocampe, constituée essentiellement de neurones. Concernant l'absence d'effets additifs ou synergiques, il est également possible qu'on ait atteint un « effet plateau » illustrant ainsi les limites des systèmes biologiques qui ne permettraient pas, selon le principe d'homéostasie, d'induire des effets plus marqués que ceux observés dans notre étude. Une autre explication de cette absence de potentialisation pourrait venir du fait que durant notre étude nous avons utilisé une séquence chimérique du BDNF (preproNGF-BDNF). En effet, de nombreuses données de la littérature révèlent que les antidépresseurs sont capables de moduler l'expression du gène codant pour le BDNF via l'activation de certains ARNm issus de l'épissage alternatif impliquant différents exons (Dias et al. 2003; De Foubert et al. 2004; Khundakar and Zetterstrom, 2006). Ainsi, il est tout à fait envisageable que les effets que nous avons obtenu auraient pu être majorés si nous avions utilisé dans la construction du transgène, une forme du gène codant pour le BDNF régulé positivement par les antidépresseurs (exons V/VI). Quoi qu'il en soit, ces travaux mettent ainsi en lumière la participation des astrocytes dans la régulation de l'humeur notamment via leur production du BDNF. L'idée d'un rôle des astrocytes dans les troubles de l'humeur et le mécanisme d'action des antidépresseurs reste très peu connue. Toutefois, il existe quelques évidences *in vivo* qui sont en faveur de cette nouvelle hypothèse. Par exemple, une étude récente montre que le blocage de la gliotransmission, et notamment la diminution de la libération d'ATP par les astrocytes, induit des

comportements de types dépressifs (Cao et al. 2013a) qui seraient en rapport avec une baisse de la neurogenèse hippocampique (Cao et al. 2013b). D'autres études montrent que les astrocytes participent à la régulation de certaines fonctions qui sont altérées lors de troubles de l'humeur tels que l'apprentissage ou certaines formes de mémoires (Suzuki et al. 2011; Han et al. 2013), le sommeil (Florian et al. 2011; Haydon et al 2006; Hines et al. 2013; Nadjar et al. 2013), ou encore la sensibilité douloureuse (Gosselin et al. 2010). Enfin, lors de cette étude nous nous sommes focalisés sur l'implication des astrocytes dans la dépression et l'activité des antidépresseurs mais à la lumière de nos résultats et des données bibliographiques, nous ne pouvons pas exclure la participation d'autres cellules gliales dans les effets observés (Rajkowska and Michel-Hidalgo, 2007). De manière intéressante, de nombreux travaux montrent que la microglie régule le processus de neurogenèse hippocampique adulte (Kohman et al. 2013) et que les propriétés neuroprotectrices des antidépresseurs (ISRS et ISRN) pourraient passer par la régulation de l'état d'activation de ce type cellulaire (Lee et al. 2011; Tynan et al. 2012). Au regard de données récentes montrant un rôle du BDNF dans les interactions neurones/microglie dans la moelle épinière (Trang et al. 2011), il serait intéressant d'étudier dans quelle mesure la libération de BDNF neuronal/astrocytaire pourrait participer à la modulation de l'activité de la microglie et aux effets thérapeutiques des antidépresseurs. De la même manière, il serait intéressant d'analyser les effets des antidépresseurs sur la capacité des cellules microgliales à libérer le BDNF.

Nous nous sommes ensuite questionnés sur les effets de type anxiolytiques/antidépresseurs de la surexpression de BDNF dans les astrocytes de l'hippocampe dans notre modèle de souris déprimées exposées à la corticostérone. Comme précédemment décrit, nous avons montré dans ce travail de thèse que la corticostérone provoque des anomalies comportementales pouvant être corrigées par la fluoxétine (David et al. 2009 ; Bacq et al. 2012). Toutefois, dans ces conditions de stress, nous n'avons pas mis en évidence d'effets bénéfiques de la surexpression de BDNF dans le test d'hypophagie induite par la nouveauté (**ARTICLE 2**) comme nous l'avions observé chez des souris naïves, non déprimées. Plusieurs hypothèses peuvent être avancées pour tenter d'expliquer ce résultat. Puisque des études montrent que le stress diminue la synthèse de BDNF dans l'hippocampe (Vaidya et al. 1999 ; Suri et Vaidya, 2013), il est possible que la surexpression de BDNF, spécifiquement dans les astrocytes, n'ait pas été suffisante pour corriger les altérations comportementales observées en réponse à la corticostérone. Au contraire, les effets bénéfiques de la fluoxétine pourraient s'expliquer par la capacité de cet ISRS à augmenter suffisamment les taux de BDNF par une action simultanée sur les neurones et les astrocytes. Différentes régions du SNC sont impliquées dans l'activité de type antidépresseur, tel que le cortex préfrontal ou encore l'amygdale (Résus et al. 2012). Il est donc également possible que la surexpression de BDNF, spécifiquement dans l'hippocampe, n'ait pas été suffisante pour corriger les effets comportementaux induits par l'administration de corticostérone. Une

autre explication serait inhérente au protocole expérimental utilisé. En effet, dans notre étude, la surexpression de BDNF a été réalisée avant le traitement à la corticostérone. Il est possible que les effets bénéfiques de la surexpression de BDNF dans les astrocytes de l'hippocampe auraient pu être mis en évidence si l'injection du lentivirus avait été réalisée au même moment que l'administration de fluoxétine, c'est-à-dire 4 semaines avant les tests comportementaux, au lieu de 8 semaines dans nos conditions. Enfin, comme l'indique la figure ci-dessous, nous avons observé qu'une exposition chronique à la corticostérone réduit la densité de cellules GFAP-positives chez la souris (Figure 18). Bien que ces résultats préliminaires doivent être confirmés, ils sont en accord avec des données de la littérature démontrant une réduction la densité de cellules GFAP-positives dans l'hippocampe (Czeh et al. 2006), le cortex frontal (Banasr et al. 2008) et l'amygdale (Gosselin et al. 2009) dans différentes situations de stress chronique chez l'animal. Si la densité d'astrocytes synthétisant le BDNF décroît en réponse à la corticostérone, il est envisageable que les taux de ce facteur neurotrophique n'aient été atteints pour contrecarrer les effets délétères de l'exposition à la corticostérone. Par conséquent, il aurait été intéressant de vérifier si l'absence d'effets comportementaux de la surexpression de BDNF astrocytaire était corrélée à une absence d'effets pro-neurogéniques dans le DG de l'hippocampe.

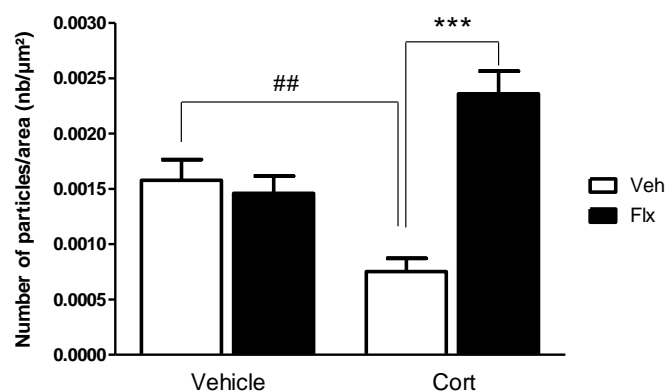


Figure 18 : Impact d'un stress chronique sur le nombre de cellules GFAP-positives dans l'hippocampe corrigé par l'administration chronique de fluoxétine.

*Effet d'un stress chronique et de l'administration chronique de fluoxétine sur le nombre de cellules GFAP-positives dans l'hippocampe. Une ANOVA à deux facteurs révèle un effet significatif du traitement (Veh vs Flx) [$F(1,10)=19,13$; $p=0,0014$], mais pas du pré-traitement (Veh vs Cort) [$F(1,10)=0,05$; $p=0,828$]. L'interaction entre ces deux facteurs est statistiquement significative [$F(1,10)=25,66$; $p=0,0005$]. Ainsi, l'administration chronique de corticostérone semble induire une diminution significative du nombre de cellules GFAP-positives ($p<0,01$). Cet effet délétère est corrigé par un traitement chronique à la fluoxétine ($p<0,001$). ## : $p<0,01$ statistiquement significatif par rapport au groupe Veh/Veh ; *** : $p<0,001$ statistiquement significatif par rapport au groupe Cort/Veh ($n=4$ animaux par groupe).*

L'une des propriétés des astrocytes repose sur leur organisation en réseau qui assure une communication intercellulaire à l'origine d'échanges de petites molécules de signalisation (IP3), d'ATP ou encore de glucose. La base de ce réseau est constituée de protéines appelées connexines (30 et 43) et il a récemment été proposé qu'un dysfonctionnement de ce réseau pourrait être à l'origine de la dépression et/ou d'une altération de la réponse aux antidépresseurs (Mitterauer, 2010). En accord avec

cette hypothèse, il a été observé chez des rats soumis à un stress chronique imprévisible modéré (UCMS), une diminution des connexines astrocytaires (Sun et al. 2012). Grâce à l'aide du Dr Giaume et de P. Ezan, nous avons cherché à étudier ces connexines chez nos souris exposées à la corticostérone. Ainsi, l'administration prolongée de corticostérone tend à diminuer le ratio « connexine 43 non-phosphorylée/connexine 43 total » (**ARTICLE 2**) suggérant qu'une altération de la communication entre astrocytes pourrait être un des éléments moléculaires/cellulaires à l'origine des troubles anxio-dépressifs. Au contraire, nous avons pu mettre en évidence que la surexpression de BDNF dans les astrocytes ré-augmente l'expression de cette connexine 43 non phosphorylée ainsi que le ratio « connexine 43 non-phosphorylée/connexine 43 total ». A l'heure actuelle, il n'existe pas de relations directes entre le degré de phosphorylation des connexines 43 et leur fonction dans les astrocytes mais une étude récente montre que cette forme non-phosphorylée est favorable à une meilleure association avec d'autres protéines impliquées dans la structure et fonction des jonctions communicantes : les occludines (Tencé et al. 2012). Des études complémentaires sont donc nécessaires pour déterminer l'impact que pourrait avoir cette forme non-phosphorylée de la connexine 43 dans nos conditions expérimentales sur la fonction des jonctions communicantes et/ou des hémicanaux. De même, le mécanisme par lequel la fluoxétine modifie l'état de phosphorylation de la connexine 43 reste inconnue. La sérotonine pourrait directement moduler l'expression/fonction des jonctions communicantes dans les astrocytes de l'hippocampe, comme cela a été rapporté au niveau des myocytes cardiaque, dans lequel l'activation des récepteurs 5-HT_{2A}/5-HT_{2B} stimule la formation de ces jonctions (Derangeon et al. 2010). L'étude du rôle des récepteur 5-HT_{2A}/5-HT_{2B} dans la régulation du réseaux astrocytaire induit par un traitement à la fluoxétine constitue ainsi une piste pour de futures investigations. Nos résultats suggèrent également que le BDNF pourrait avoir un rôle autocrine en agissant sur les récepteurs TrkB astrocytaires pour moduler le réseau nécessaire astrocytaire au sein de la synapse tripartite. Cette hypothèse reste à être vérifiée dans la mesure où les astrocytes expriment à leur surface la forme tronquée du récepteur TrkB (Rudge et al. 1994), une forme qui serait inactive. Or, une étude plus récente parue dans Nature met clairement en évidence la capacité de ce récepteur tronquée, présents sur les cellules gliales, à stimuler des voies de signalisations intracellulaires et notamment calciques (Rose et al. 2003). A l'heure actuelle, les études portant sur le rôle du BDNF dans le mécanisme d'action des antidépresseurs se focalisent sur son impact sur l'étape de maturation de la neurogenèse hippocampique adulte, en mettant de côté la possibilité d'un effet bénéfique de la gliogenèse. On sait que lors de l'étape de différenciation, 75% des cellules progénitrices du gyrus dentelé sont destinées à devenir des neurones mais les 25% de futures cellules astrocytaires restantes pourraient exercer un rôle important dans la réponse aux ISRS, notamment grâce à leur rôle de soutien aux cellules avoisinantes. Les mécanismes à l'origine de la formation des astrocytes et de leur activation restent donc un axe pertinent à explorer.

Les substrats anatomiques de la dépression étant multiples, nous nous sommes ensuite intéressés à l'impact de la surexpression de BDNF sur des symptômes de la pathologie ne mobilisant pas le processus de neurogenèse hippocampique. Chez des souris naïves ou traitées à la corticostérone, la surexpression de BDNF n'a pas eu d'effet de type antidépresseur dans le test de suspension caudale (tail suspension test, TST), alors qu'un traitement chronique à la fluoxétine s'est avéré efficace comme précédemment rapporté dans la revue de la littérature (David et al. 2009). En revanche, l'un des résultats les plus intéressants obtenu dans ce travail de thèse, concerne les effets de la surexpression de BDNF dans les astrocytes de l'hippocampe dans le test du labyrinthe en croix surélevé (elevated plus maze, EPM). Dans ce test, nous avons montré chez les souris naïves ou traitées à la corticostérone, qu'en dépit d'une absence d'effets propres, la surexpression de BDNF astrocytaire a permis de révéler un effet anxiolytique de la fluoxétine chronique (**ARTICLE 2**). Ce résultat rappelle des données préliminaires du laboratoire montrant que l'injection intra-hippocampique de BDNF potentialise les effets anxiolytiques d'un autre ISRS, la paroxétine, dans le même test (Deltheil et al. 2009). Pourtant, les mécanismes sous-tendant cette potentialisation restent mal connus, c'est pourquoi nous avons cherché à comprendre comment le BDNF pouvait interférer avec cette propriété d'un traitement chronique à la fluoxétine. Il a été proposé que les ISRS pouvaient entraîner des effets anxiogènes lors des premiers jours de traitement, notamment suite à l'activation de certains récepteurs sérotoninergiques post-synaptiques tel que le récepteur 5-HT_{2C} (Hamon, 1994). Ainsi, toute augmentation de [5-HT]_{ext} au niveau des terminaisons neuronales pourrait s'avérer, du moins dans les premiers jours de traitement chronique aux ISRS, favorable à un comportement de type anxieux, tandis qu'au contraire, une atténuation de la neurotransmission sérotoninergique au niveau du récepteur 5-HT_{2C} par exemple, pourrait avoir des effets de types anxiolytiques. Les effets de la surexpression de BDNF sur le tonus sérotoninergique ont donc été étudiés à l'aide de la technique de microdialyse intracérébrale dans l'hippocampe. Nos résultats montrent que la capacité d'un traitement chronique à la fluoxétine à augmenter les [5-HT]_{ext} hippocampique est atténuée de manière significative par la surexpression de BDNF astrocytaire. Ces résultats mettent en évidence un rôle inhibiteur du BDNF sur le système sérotoninergique central probablement pour limiter l'augmentation intra-synaptique de ce facteur en réponse à un traitement chronique à la fluoxétine. Ces analyses sont également en accord avec des données précédentes du laboratoire montrant, au contraire, que la diminution partielle des concentrations cérébrales de BDNF chez les souris hétérozygotes BDNF^{+/-} entraîne un phénotype hyper-sérotoninergique (Guiard et al. 2008). L'effet inhibiteur du BDNF sur les [5-HT]_{ext} pourrait résulter de différents mécanismes tels qu'une augmentation de l'expression ou de la fonction des transporteurs de haute ou faible affinité de la 5-HT (SERT et OCT2) identifiés dans l'hippocampe, des enzymes du catabolisme de la 5-HT (MAO et COMT) ou encore de l'autorécepteur terminal 5-HT_{1B}. Toutes ces hypothèses seraient intéressantes à tester mais nous nous sommes focalisés sur un acteur majeur de la régulation du système sérotoninergique : l'autorécepteur

somatodendritique 5-HT_{1A} situé dans la région des noyaux du raphé. Nos analyses électrophysiologiques *in vivo* chez la souris anesthésiée révèlent que la capacité du traitement chronique à la fluoxétine à désensibiliser cet autorécepteur est diminuée suite à la surexpression de BDNF astrocytaire. Il est donc envisageable qu'un « pool » d'autorécepteurs inhibiteurs 5-HT_{1A} soit resté fonctionnel en réponse à la surexpression de BDNF astrocytaire, maintenant ainsi un rétrocontrôle inhibiteur sur le système sérotoninergique (Figure 19). Il est difficile de réconcilier ce résultat avec le fait que nous avons injecté le lentivirus dans l'hippocampe. Cependant, on sait que ce facteur neurotrophique peut être transporté de manière rétrograde des terminaisons neuronales (site de l'injection) vers les corps cellulaires en se fixant sur son récepteur TrKB, dont la présence a été identifiée sur les neurones sérotoninergiques (Delteil et al. 2009). Pour des raisons techniques, et notamment de détection immunohistochimique du BDNF, nous n'avons malheureusement pas pu vérifier cette hypothèse au cours de ce travail de thèse. On pourrait imaginer toutefois que la libération de BDNF dans les NR, suite à son transport rétrograde de l'hippocampe vers les corps cellulaires sérotoninergiques, favorise la croissance des dendrites des neurones 5-HT comme cela a déjà été démontré dans d'autres régions cérébrales (Mamounas et al. 1995), augmentant ainsi la densité des autorécepteurs 5-HT_{1A}. Cette hypothèse pourrait être évaluée par des études immunohistochimiques à l'aide d'un anticorps dirigé contre le récepteur 5-HT_{1A}, et d'un marqueur spécifique dendritique (MAP-2). De même, le couplage de cet autorécepteur à sa protéine Gi pourrait être étudié à l'aide de la technique du GTPγS35 (Froger et al. 2001). Notons que ces études, concernant les effets potentiels du BDNF astrocytaire sur l'activité des neurones sérotoninergiques pré-synaptiques centraux, n'ont été évalués que chez des souris naïves, et que les effets auraient pu être complètement différents chez des souris traitées à la corticostérone. On sait, en effet, que les glucocorticoïdes administrés de façon chronique peuvent interférer directement sur la sensibilité de l'autorécepteur 5-HT_{1A} chez la souris (Froger et al. 2004; Rainer et al. 2012).

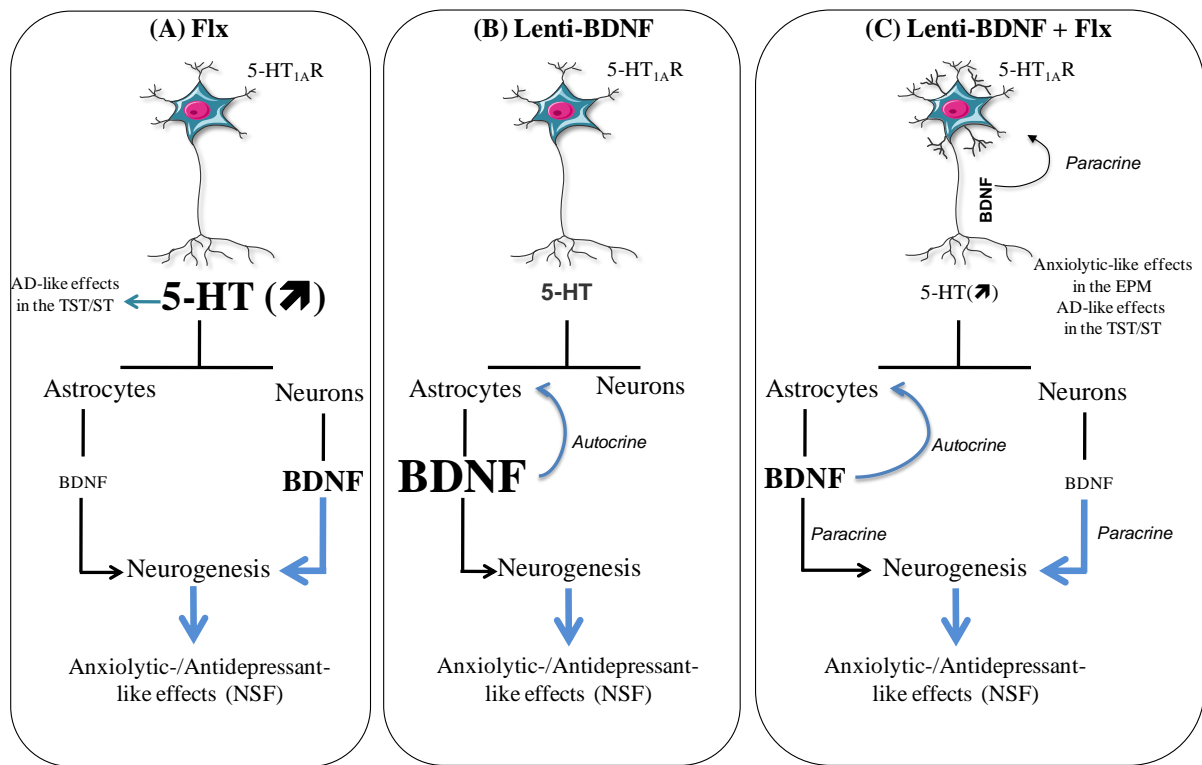


Figure 19 : Mécanisme d'action potentiel de la fluoxétine, seul et/ou associée à la surexpression de BDNF dans les astrocytes de l'hippocampe chez la souris adulte.

Après s'être questionnés sur le rôle du BDNF astrocytaire dans la régulation du comportement anxio-dépressif et la réponse à la fluoxétine chez des souris naïves ou exposées à la corticostérone, nous nous sommes intéressés à l'identification d'un sous-type de récepteur sérotoninergique éventuellement impliqué dans la synthèse et/ou la libération de ce facteur neurotrophique. Nous avons identifié le sous-type de récepteur 5-HT_{2A} comme un candidat potentiel dans ce mécanisme. En effet, certains travaux *in vitro* (chez le rat) soulignent la présence de ce sous-type de récepteur sur les astrocytes (Hansson et al. 1987; Hirst et al. 1997). Ces données ont par la suite été confirmées *ex vivo* sur du tissu cérébral post-mortem chez l'Homme (Wu et al. 1999). De plus, son activation sur les cellules gliales serait à l'origine de modifications intracellulaires menant à la synthèse de métabolites énergétiques et de substances neurotrophiques nécessaires à une activité neuronale normale (**REVUE 1**). Dans ce contexte, si on considère plausible l'hypothèse selon laquelle l'activation du récepteur 5-HT_{2A} astrocytaire participe au métabolisme du BDNF, alors au contraire, son inactivation pourrait avoir des effets délétères sur la vulnérabilité au stress et/ou la réponse aux ISRS. Il est important de noter que cette hypothèse peut paraître contradictoire avec les nombreuses données cliniques et précliniques montrant que les antagonistes des récepteurs 5-HT_{2A}, dont les antipsychotiques sont utilisés, seuls ou associés aux ISRS, dans le traitement des épisodes dépressifs majeurs (Hamon et Blier, 2013). Cependant, ces propriétés ont été attribuées à la capacité de ces agents pharmacologiques à stimuler la neurotransmission monoaminergique (sérotoninergique et noradrénergique) pré-synaptique sans que

leur propriété post-synaptique dans l'hippocampe ne soit clairement caractérisée. Nous avons donc choisi d'étudier le génotype de souris sauvages et mutées, privées de manière constitutive du récepteur 5-HT_{2A} (souris KO 5-HT_{2A}^{-/-}) suite à une exposition chronique à la corticostérone. Cette approche nous a paru pertinente dans la mesure où certains polymorphismes de ce récepteur ont été identifiés chez l'Homme, et que des études génétiques cliniques suggèrent l'existence d'associations entre certains polymorphismes du récepteur 5-HT_{2A} (responsables de modifications fonctionnelles globales depuis la naissance des individus) et l'anxiété, la dépression ou la réponse aux ISRS. En accord avec ces études, nos résultats montrent que les souris 5-HT_{2A}^{-/-} sont plus aptes à développer un comportement anxio-dépressif, évalué dans le test du labyrinthe en croix surélevé, le test de suspension caudale et le test d'éclaboussures, en réponse à la corticostérone que les souris sauvages 5-HT_{2A}^{+/+} (**ARTICLE 3**). Le dosage des concentrations de BDNF dans l'hippocampe des souris 5-HT_{2A}^{-/-} exposées à la corticostérone est en cours afin de corrélérer les altérations comportementales, et notamment celles liées à l'anxiété (fortement perturbée chez nos souris mutantes) avec une éventuelle baisse des taux de ce facteur neurotrophique dans des sous régions de l'hippocampe. En effet, différents travaux ont mis en évidence un lien entre certains polymorphismes du BDNF et un phénotype anxieux. Par exemple, en examinant les profils comportementaux de six lignées de souris, il a été observé que les souches portant l'allèle "Met" du SNP "Leu32Met" présentaient une réactivité émotionnelle et des comportements anxieux plus marqués que les souris portant l'allèle "Leu" (Ibarguen-Vargas et al. 2008). D'autres études portant sur le SNP "Val66Met" du BDNF rapportent une anxiété exacerbée chez les souris knock-in porteuses de l'allèle "Met" (Chen et al. 2006). Cet allèle "Met" est lui-même associé à une diminution de la sécrétion de BDNF et du volume de l'hippocampe chez l'Homme (Peszawas et al. 2004; Szeszko et al. 2005). Il existerait donc bien un lien entre le BDNF et l'anxiété, mais il n'y a pas de démonstration directe d'une participation éventuelle du récepteur 5-HT_{2A} dans cette interaction. De manière intéressante, il a récemment été montré qu'un polymorphisme du récepteur 5-HT_{2A}, le variant allélique "TT" du rs6314, responsable d'une diminution de l'expression de ce récepteur, est associé à une diminution du volume hippocampique et des performances cognitives (Blasi et al. 2013) ; deux aspects qui pourraient mettre en jeu une diminution du BDNF. De notre côté, et à la faveur d'une collaboration avec une équipe de psychiatres de l'Université Paris-Sud, dont le Pr. E. Corruble, nous avons montré que les patients porteur du variant "TT" de ce polymorphisme souffrent de dépression mélancolique dont la sévérité est supérieure à celle observée chez les patients porteurs de l'allèle "C" (**ARTICLE 3**). De même, nous avons observé dans cette étude translationnelle, qu'un autre SNP, le rs6313, était associé à une plus forte sévérité de la dépression notamment chez les patients porteurs de l'allèle, responsable d'une diminution de la fonction du récepteur 5-HT_{2A} comme nous l'avons observé chez les souris mutantes 5-HT_{2A}^{-/-}. Différentes données cliniques et précliniques soulignent que la surexpression de l'autorécepteur 5-HT_{1A} constitue un facteur de risques de vulnérabilité au stress et à certaines maladies psychiatriques,

telle que la dépression (Le François et al. 2008; Richardson-Jones et al. 2009; Czesak et al. 2012). Nous avons donc comparé la sensibilité de cet élément entre les souris $5\text{-HT}_{2A}^{+/+}$ et $5\text{-HT}_{2A}^{-/-}$ (**ARTICLE 4**). Différentes approches nous ont permis d'identifier que cet autorécepteur était hypersensibilisé chez les souris $5\text{-HT}_{2A}^{-/-}$. En effet, une potentialisation de la capacité d'un agoniste sélectif, le 8-OHDPAT, à diminuer l'activité électrique des neurones 5-HT, a été observée chez ces souris mutantes, tout comme la capacité d'un antagoniste, le WAY100635 à augmenter les $[5\text{-HT}]_{\text{ext}}$ dans l'hippocampe, induite lors d'un challenge d'ISRS. Ces données pourraient donc être une explication au fait que la sévérité du score "d'émotionnalité", reflétant un phénotype anxio-dépressif, soit plus forte chez des souris ou des patients présentant un déficit fonctionnel du récepteur 5-HT_{2A} . Il est intéressant de noter que la perte du récepteur 5-HT_{2A} s'accompagne d'une augmentation de la fonction du récepteur 5-HT_{1A} dans la mesure où ces deux récepteurs exercent un contrôle négatif sur le tonus sérotoninergique par des mécanismes distincts. Le récepteur 5-HT_{1A} agit en tant qu'autorécepteur inhibiteur (Gardier et al. 1996; Blier et al. 2003; Artigas et al. 2006; Hamon et Blier, 2013), tandis que le récepteur 5-HT_{2A} est un hétérorécepteur dont l'effet inhibiteur sur le système sérotoninergique a été attribué à une diminution de l'influence excitatrice des neurones noradrénergiques provenant du locus coeruleus et se projetant dans le raphé (Szabo et al. 2001; Dremencov et al. 2007; Quesseveur et al. 2013) ou à l'excitation d'interneurones GABAergiques localement dans le noyau du raphé dorsal (Boothman et al. 2003; Boothman and Sharp, 2005) (**REVUE 2**). Ainsi, cette hypersensibilité de l'autorécepteur 5-HT_{1A} chez les souris $5\text{-HT}_{2A}^{-/-}$ pourrait être analysée comme un mécanisme compensatoire visant à maintenir un frein biologique sur le système sérotoninergique, pouvant ainsi conduire à une exacerbation des troubles de l'humeur. En raison de l'affinité de la fluoxétine vis à vis du récepteur 5-HT_{2A} (Ni and Miledi, 1997), nous ne pouvons pas écarter la possibilité que la perte d'effet de cet ISRS chez les souris $5\text{-HT}_{2A}^{-/-}$ soit liée à l'absence de ce récepteur chez les souris mutées.

Dans la dernière partie de cette thèse, nous avons cherché à déterminer l'impact de la mutation du récepteur 5-HT_{2A} sur la réponse à un traitement chronique à la fluoxétine. Nous avons réalisé cette étude uniquement chez des souris $5\text{-HT}_{2A}^{+/+}$ et $5\text{-HT}_{2A}^{-/-}$ naïves, non stressées, mais les résultats sont encourageants. Ils montrent que la perte du récepteur 5-HT_{2A} conduit à une diminution significative des capacités de la fluoxétine à provoquer des effets de types anxiolytiques/antidépresseurs, notamment dans le test d'hypophagie induite par la nouveauté, et ceci en rapport avec une atténuation de la neurogenèse hippocampique (**ARTICLE 4**). Bien que ces résultats méritent d'être confirmés, ils sont opposés aux effets observés lors de la surexpression du BDNF dans les astrocytes de l'hippocampe (Tableau 2 et Tableau 3). L'évidence reste indirecte mais le lien entre l'activation du récepteur 5-HT_{2A} et la synthèse de BDNF par les astrocytes de l'hippocampe semble possible à la lumière de nos résultats expérimentaux. Afin d'apporter des éléments supplémentaires, une étude en collaboration avec les Drs Luc Maroteaux et Anne Roumier afin de vérifier si la capacité de la

fluoxétine à stimuler la synthèse de BDNF dans des cultures primaires d'astrocytes (Allaman et al., 2012) est perdue dans des cultures issues de souris 5-HT_{2A}^{-/-}. Ces études, combinées à nos observations *in vivo* pourraient souligner l'importance que représente la stimulation du sous-type de récepteur 5-HT_{2A} astrocytaire pour favoriser la synthèse et/ou la libération de BDNF par ces cellules gliales. Pourtant, des études indiquent que l'administration unique d'un agoniste des récepteurs 5-HT_{2A} chez le rat diminue l'expression du BDNF dans l'hippocampe (Vaidya et al. 1997; Cavus and Duman, 2003), tandis qu'un antagoniste prévient l'effet délétère du stress sur les taux d'ARNm codant pour le BDNF dans l'hippocampe chez le rat (Vaidya et al. 1999). Ces effets sont inattendus au regard de notre hypothèse mais peuvent s'expliquer par le fait que l'administration d'une dose unique d'agoniste du récepteur 5-HT_{2A} exerce un effet inhibiteur sur les neurones sérotoninergiques pré-synaptiques, induisant une diminution des [5-HT]_{ext} dans l'hippocampe de manière à limiter l'activation des récepteurs 5-HT_{2A} post-synaptiques nécessaires à la synthèse locale de BDNF. D'ailleurs, un travail réalisé sur des lignées de cellules C6 glioma montre que l'augmentation de la synthèse de BDNF induite par l'application du 5-HT peut être prévenue par le MDL100907, un antagoniste sélectif des récepteurs 5-HT_{2A} (Meller et al. 2002). Le lien entre ces deux acteurs de la réponse de type antidépresseur a également fait l'objet d'investigations centrées sur l'impact réciproque du BDNF sur le récepteur 5-HT_{2A}. Ainsi des études *in vivo* chez la souris montrent que la délétion de BDNF à l'âge adulte diminue l'expression du récepteur 5-HT_{2A} dans le cortex frontal (Rios et al. 2006; Klein et al. 2010a). En adéquation avec ces résultats, d'autres études *in vivo* rapportent qu'à l'inverse, l'injection de BDNF stimule l'expression de ce récepteur dans l'hippocampe (Naumenko et al. 2012). Cependant, ces données restent controversées. En effet, des études *in vitro* utilisant des cultures hippocampiques de rats rapportent un effet inhibiteur de l'administration de BDNF sur l'expression du récepteur 5-HT_{2A} (Trajkovska et al. 2009). En accord avec ces observations, des études d'imagerie chez l'Homme n'ont révélé aucune association entre le polymorphisme Val66Met du BDNF et le récepteur 5-HT_{2A} (Klein et al. 2010b). Ceci laisse entrevoir la possibilité d'un rôle dépendant de la région cérébrale étudiée dans la nature de ces interactions entre le BDNF et le récepteur 5-HT_{2A} (Klein et al. 2010a).

Durant notre étude nous avons envisagé l'impact du récepteur 5-HT_{2A} astrocytaire dans les interactions neurones/astrocytes. Ceci représente une autre limite de ce travail. Nous sommes conscients que d'autres récepteurs sérotoninergiques astrocytaires, et notamment les récepteurs 5-HT_{2B}, pourraient également être impliqués dans ces interactions. En effet, des études *in vitro* récentes ont démontré que la sérotonine mais également certains classes d'antidépresseurs sont capables de stimuler les propriétés métaboliques et neurotrophiques astrocytaire via l'activation du récepteur 5-HT_{2B} (Li et al. 2008; Li et al. 2009; Zhang et al. 2010) qui lui même est favorable à une activité de type antidépresseur (Diaz et al. 2012). Ainsi, une des perspectives de notre étude pourrait être d'étudier l'impact de l'activation ou inactivation des récepteurs 5-HT_{2B} dans le contexte de notre travail.

Test		Manip Lenti-BDNF							
		Veh				CORT			
		Lenti-GFP	Lenti-GFP/ FLX	Lenti-BDNF	Lenti-BDNF /FLX	Lenti-GFP	Lenti-GFP/ FLX	Lenti-BDNF	Lenti-BDNF /FLX
EPM		∅	∅	∅	+	∅	∅	∅	+
TST		∅	+	∅	+	∅	∅	∅	∅
ST		∅	+	∅	+	-	+	+	+
NSF		∅	+	+	+	-	+	∅	+
Neurogenèse hippocampique	Prolifération	∅	+	+	+				
	Survie	∅	+	+	+				
	Maturation	∅	+	+	+				

Tableau 2: Bilan des effets comportementaux et neurogéniques de la surexpression de BDNF astrocytaire seul ou associé à un traitement chronique de fluoxétine chez des souris naïves ou traité à la corticostérone.

∅ : absence d'effets significatif ; + : effets de type anxiolytique/antidépresseur ; - : effets de type anxiogénique/dépressiogènes.

Test		Manip 5-HT _{2A}					
		Veh				CORT	
		5-HT _{2A} ^{+/+}	5-HT _{2A} ^{+/+} / FLX	5-HT _{2A} ^{-/-}	5-HT _{2A} ^{-/-} / FLX	5-HT _{2A} ^{+/+}	5-HT _{2A} ^{-/-}
EPM						-	-
TST		∅	+	∅	∅	∅	-
ST						∅	-
NSF		∅	p=0,06	∅	∅		
Neurogenèse hippocampique	Survie	∅	p=0,06	∅	∅		
	Maturation	∅	+	∅	+		

Tableau 3: Tableau bilan des effets comportementaux et neurogéniques de l'inactivation génétique du récepteur 5-HT_{2A} seul ou associé à un traitement chronique de fluoxétine chez des souris naïves ou traité à la corticostérone.

∅ : absence d'effets significatif ; + : effets de type anxiolytique/antidépresseur ; - : effets de type anxiogénique/dépressiogènes.

CONCLUSIONS ET PERSPECTIVES :

En conditions normales (physiologiques), le système sérotoninergique, via la libération de 5-HT dans l'hippocampe, stimulerait la synthèse et la libération de BDNF par les neurones et les astrocytes. Ce mécanisme serait nécessaire à un comportement non-pathologique (Figure 20).

En conditions pathologiques, et notamment lors d'un stress chronique induit par l'administration prolongée de corticostérone, on observe une diminution du nombre de neurones et d'astrocytes associée à des changements morphologiques participant à la diminution du volume de l'hippocampe. Toutefois, cet aspect n'a pas encore été démontré par des études d'imagerie chez le petit animal. Par contre, il nous semble possible que la corticostérone provoque une diminution de synthèse et de libération de BDNF, et par conséquent, une diminution du processus de neurogenèse hippocampique induisant l'apparition d'un comportement de type anxieux/dépressif (Figure 20).

L'administration prolongée d'ISRS induit une désensibilisation progressive des autorécepteurs 5-HT_{1A} somatodendritiques provoquant la levée du rétrocontrôle inhibiteur exercée par cet autorécepteur. D'ailleurs, ce processus de désensibilisation serait potentialisé en conditions pathologiques sous corticostérone (Rainer et al. 2012). La désensibilisation des autorécepteurs 5-HT_{1A} se traduit au niveau des aires de projections sérotoninergiques par une forte augmentation des concentrations synaptiques de 5-HT renforçant la neurotransmission sur les récepteurs post-synaptiques neuronaux et/ou astrocytaires (5-HT_{2A}). Ainsi, la synthèse et la libération de BDNF par ces cellules seraient restaurées. Ce facteur neurotrophique va à son tour activer le processus de neurogenèse hippocampique nécessaire à l'obtention d'une partie des effets comportementaux des ISRS. Le BDNF pourrait aussi réguler le système sérotoninergique via la modulation de l'activité et/ou de l'expression des récepteurs 5-HT_{1A} et 5-HT_{2A}. En plus de ces effets paracrines, le BDNF astrocytaire pourrait enfin induire des effets autocrines en activant les récepteurs TrkB-T1 sur les cellules gliales, stimulant ainsi leur activité et notamment leurs propriétés de support métabolique et neurotrophique nécessaires au bon fonctionnement des neurones (Figure 20).

Nous sommes conscients que la surexpression de BDNF ne constitue pas une approche suffisante pour conclure définitivement sur l'implication des astrocytes dans la synthèse de ce facteur neurotrophique, et qu'une stratégie d'inactivation ciblée aurait été plus convaincante. Ceci est désormais possible grâce au lentivirus infectant spécifiquement les astrocytes et contenant l'ARNsi, bloquant l'expression du BDNF. De plus, il est important de noter que les effets du BDNF (neuronale et/ou astrocytaire), s'ils devaient être confirmés, ne constituent pas la seule explication pouvant rendre compte de l'activité thérapeutique des ISRS. De nombreuses régions, impliquées dans le contrôle de l'humeur, sont également la cible de ces antidépresseurs, et ceci en accord avec nos résultats montrant des effets neurogenèse-indépendants de la fluoxétine. Ces aspects doivent également être explorés.

Discussion

Des considérations techniques (anticorps anti-BDNF) font qu'une des limites de cette étude a été de pas avoir tenté de déterminer la proportion de proBDNF et de BDNF mature surexprimé par les astrocytes chez les souris ayant reçu l'injection de lentivirus. Or, Comme nous l'avons présenté en introduction le proBDNF et le BDNF mature ne se lient pas aux mêmes récepteurs et induisent des effets différents (Barker et al. 2009; Fenner et al. 2012). Ainsi, dans le but d'approfondir les mécanismes cellulaires induit par la surexpression de BDNF astrocytaire, il aurait été judicieux de distinguer la part de proBDNF et BDNF mature libérée par les astrocytes.

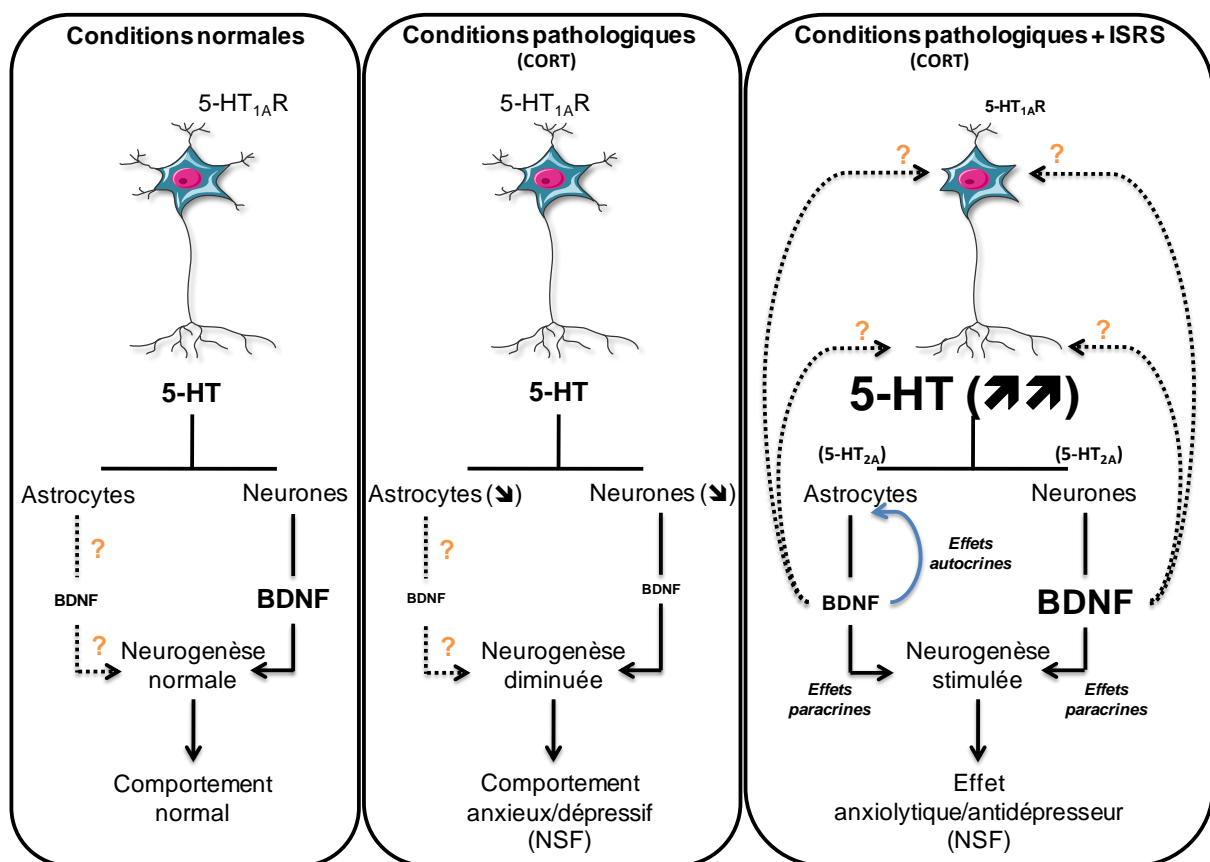


Figure 20 : Bilan du rôle du BDNF astrocytaire dans la physiopathologie de la dépression et le mécanisme d'action des ISRS proposé sur la base de nos résultats.

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