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L'impact du stress précoce sur la perception hédonique des odeurs et ses bases neurales chez la souris

Anna Athanassi

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**L'impact du stress précoce
sur la perception hédonique des odeurs
et ses bases neurales chez la souris**

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-LISTE DES ABRÉVIATIONS-

- Acétylcholine (ACh)
- Aire Tegmentale Ventrale (ATV)
- Bromodésoxyuridine (BrdU)
- Bulbe Olfactif (BO)
- Cortex Orbito-Frontal (COF)
- Cortex Préfrontal Médian (CPFm)
- DARP 32 *Dopamine- and cAMP-regulated phosphoprotein 32*
- DopAmine (DA)
- *Early Life Adversity* - Adversité Précoce (ELA)
- GABA : acide γ -aminobutyrique
- *Green Fluorescent Protein* (GFP)
- Hormone Corticotrope (CRH)
- Axe Hypothalamo Hypophysaire (HPA)
- Imagerie par Résonance Magnétique Fonctionnelle (IRM(f))
- *Limited Bedding and Nesting* - Restriction des Conditions d'Hébergement (LBN)
- Locus Cœruleus (LC)
- NorAdrénaline (NA)
- *Post-natal days x* - Jours après la Naissance (Px)
- Tubercule Olfactif (TO)
- Zone Sous-Ventriculaire (ZSV)

-AVANT-PROPOS-

-Environnement, sens et cerveau-

Donner un sens aux sens. Le cerveau est un organe énigmatique dont on veut percer les mystères, reproduire la course des connexions et modéliser les schémas fonctionnels. Pour cela, on peut le disséquer afin d'étudier son anatomie, marquer et identifier ses composants, enregistrer ses signaux ou encore influencer ses états. Les questions et leurs réponses sont infinies. Dans l'étude du cerveau, l'organe le plus complexe qui soit, s'intéresser aux « simples » sens peut paraître anecdotique pour le grand public, en comparaison à la grande question de la conscience par exemple. Pourtant, le cerveau isolé, comme suspendu dans le vide, n'est tout simplement pas le cerveau. Il est au cœur d'un réseau d'interactions entre l'extérieur et l'intérieur, entre l'environnement et le reste de l'organisme. La passerelle qui relie ces deux mondes, l'une des portes d'entrée du cerveau, n'est autre que les voies sensorielles. Détection des informations extérieures à travers notre palette de sens. Transmission de ces informations de la périphérie de nos terminaisons nerveuses à la tour de contrôle que représente le cerveau. Intégration dans le flux immense de données déjà en cours de traitement. Évaluation multifactorielle de la situation. Interprétation à la lumière de l'expérience et enfin induction d'une réponse comportementale adaptée au monde qui nous entoure. Voilà l'esquisse rapide et succincte de la danse des sens que l'on nomme perception sensorielle. Chaque modalité sensorielle suit ce chemin avec des caractéristiques uniques et, parmi ces modalités, l'olfaction est le cœur battant de ce travail de thèse.

-Le sens olfactif-

La place de l'olfaction dans notre vie. L'odorat, ou l'olfaction, est le sens chimique permettant la détection et l'analyse de molécules odorantes volatiles présentes dans notre environnement, depuis leur arrivée dans notre nez, jusqu'au traitement de l'information dans notre cerveau et la production d'une réponse comportementale et/ou psychologique. Son importance dans le règne animal est capitale pour la survie, la reproduction, l'alimentation, la migration et la navigation, la prise de décision, ou encore les comportements sociaux. En réalité bien plus performant que ne le suggère la croyance collective, la perception olfactive est d'une importance capitale pour nous aussi et notamment pour notre bien-être. Dès l'Antiquité, l'olfaction avait une place centrale en médecine, en religion et en arts. Son importance dans nos vies quotidiennes a ensuite été réduite avec l'industrialisation et l'hyper-hygiénisation du XIX^e et début XX^e siècles, conduisant à la désodorisation de notre environnement. Avec le temps, la recherche a redoré le blason de l'olfaction au cours du XX^e siècle, après la découverte des récepteurs olfactifs par Buck et Axel en 1991, qui leur a valu un prix Nobel en 2004, et a permis de remettre de la lumière autour de ce sens. Enfin, très récemment, la pandémie de COVID-19 a profondément sensibilisé le monde sur l'importance de l'olfaction dans nos vies. Aujourd'hui, la valeur de nos capacités olfactives est indéniable, tout comme leurs rôles au quotidien. Ce manuscrit se propose de rappeler et d'approfondir ces arguments, en soulignant l'importance fondamentale de l'olfaction vis-à-vis de notre bien-être.

Perception olfactive et hédonisme. Parmi les études replaçant l'olfaction à sa juste place dans la perception sensorielle, une capacité olfactive toute particulière, guidant les comportements humains et animaux depuis des centaines d'années, est ressortie comme majeure : la perception hédonique des odeurs. Elle est la sensation agréable, plaisante, attractive, ou à l'inverse désagréable, déplaisante, répulsive, provoquée par une source odorante. Cette composante s'impose comme essentielle -si ce n'est principale- dans la perception olfactive. Elle joue, pour l'humain comme pour le reste du monde animal, un rôle clé dans certains aspects essentiels de la vie comme le rejet d'aliments avariés, l'attachement maternel dans les premières années de vie, ou encore le choix de partenaires amoureux. Mais sa portée

est bien plus vaste car elle module également des comportements *a priori* plus superflus en termes de survie tels que l'envie de se parfumer soi-même ou son environnement, le plaisir de manger, la quête du bien-être etc. Cet aspect bien précis de la perception olfactive renforce l'existence de son lien étroit avec l'état émotionnel de l'individu. Et c'est ce lien que ce projet de thèse vise à décrire, comprendre et appréhender.

-Bien sentir pour se sentir bien-

Dans cet avant-propos, l'olfaction est abordée avant même l'évocation de la dimension pathologique, qui est au cœur également du projet. Ce choix délibéré vise à réaffirmer l'importance de l'olfaction et à rappeler qu'elle est la véritable **star** ici. Cette étude trouve en effet son origine dans l'observation d'un rapport étroit entre l'olfaction et le stress. D'un côté, les odeurs ont le pouvoir de générer et de moduler les émotions, impactant ainsi le bien-être, l'anxiété et les humeurs. D'autre part, la recherche a apporté des arguments probants quant à la dégradation de ce sens dans les troubles affectifs. Le terme « affect » a été défini par le philosophe français René Descartes (1596-1650), comme une expérience subjective d'une émotion ou d'un sentiment. Le « trouble affectif » est un terme général désignant en psychiatrie les perturbations de l'état émotionnel survenant dans diverses pathologies. Ces troubles se superposent à ceux de l'humeur, dont l'un des représentants les plus courants est le trouble dépressif majeur. Cependant, ce terme regroupe une variété de symptômes, d'états tourmentés et/ou de sentiments écorchés tels que l'anxiété généralisée, la panique, les phobies, les obsessions, et les états relatifs au stress chronique ainsi qu'aigu, traumatique et autres. C'est bien parce qu'il englobe tous ces états dysfonctionnels que ce terme apparaît dans la suite de ce manuscrit, où les manifestations émotionnelles troublées par le stress nous intéressent davantage qu'une pathologie en particulier. De nombreuses études s'efforcent donc de décrire la relation entre les troubles affectifs et le sens olfactif, pour en comprendre la nature et accéder, à terme, à des leviers d'actions s'inscrivant dans une stratégie thérapeutique. Pourtant, malgré les avancées qui ont été faites, les bases neurales régissant le lien entre la perception olfactive et le stress, en particulier précoce, sont encore à décrire, et c'est bien cela qui constitue la principale motivation de notre étude. Ainsi, la dimension psychopathologique est décrite en premier dans l'introduction de ce manuscrit, reflétant le point de départ de notre travail, même s'il apparaît en seconde position dans cet avant-propos. Ce choix, je l'espère, aura fait sens dans votre esprit.

-RÉSUMÉ-

La maltraitance lors de la petite enfance peut être à l'origine d'un état dépressif à l'âge adulte. En effet, le stress en début de vie, la négligence ou les abus, peuvent perturber le développement cérébral, affectant l'équilibre émotionnel jusqu'à l'âge adulte. Par ailleurs, la dépression est souvent associée à des perturbations de la perception des odeurs. Or les odeurs jouent un rôle primordial dans de nombreux comportements vitaux tels que l'alimentation, les interactions sociales, l'évitement des dangers. Ainsi, toute altération de la perception olfactive et plus particulièrement de celle des odeurs plaisantes est susceptible de diminuer les plaisirs quotidiens et donc d'aggraver les symptômes de la dépression. **Dans ce contexte, ma thèse vise à étudier les effets du stress précoce sur la perception des odorants plaisants à l'âge adulte et à identifier les bases neurales de ces altérations.**

Pour cela, j'ai utilisé un modèle murin de stress précoce via une restriction des conditions d'hébergement, permettant de confirmer une altération du comportement émotionnel à l'âge adulte, mais également de révéler une altération de la perception d'odorants plaisants (**ÉTUDE 1**). Nous avons ensuite exploré les mécanismes de cette perturbation olfactive en nous intéressant tout d'abord aux interneurons du bulbe olfactif, dont le rôle dans le codage de la valeur hédonique des odeurs a été préalablement démontré. Ces interneurons qui naissent en postnatal (P0-P1), sont dans leur période critique d'intégration au moment de la phase de stress précoce et donc particulièrement vulnérables. Nos observations montrent en effet que le stress précoce altère la morphologie fine des interneurons répondant spécifiquement aux odorants plaisants. Les interneurons du bulbe olfactif ont également la particularité d'être la cible d'une neurogenèse adulte. Nous avons montré que les nouveaux neurones, bien que nés bien après la période de stress, ont une survie et une plasticité modifiées. Toutes ces perturbations conduisent à une modification globale de l'activité neuronale du bulbe olfactif en réponse aux odorants plaisants, perturbant le message de sortie de cette structure. Deux autres structures cérébrales, impliquées dans le codage de la valeur hédonique des odeurs, le tubercule olfactif et l'aire tegmentale ventrale sont également touchées. Ces structures font partie du circuit de la récompense et sont particulièrement actives lors du comportement d'approche envers les odorants plaisants. Leur activité en réponse aux odorants plaisants est altérée chez les animaux ayant subi un stress précoce.

Enfin, le fait que les odorants plaisants puissent induire un comportement motivé, mobilisant le circuit de la récompense, suggère une libération de dopamine, molécule clé de ce circuit. Pour étudier directement la libération de dopamine en réponse aux odorants, j'ai mis en place au laboratoire la technique de photométrie par fibre couplée au signal vidéo (**ÉTUDE 2**). Mes premières analyses montrent une augmentation de dopamine en réponse aux odorants attractifs en comparaison aux odorants non attractifs. L'analyse des effets du stress précoce sur ce mécanisme est actuellement en cours.

En conclusion, nos résultats indiquent que le stress précoce modifie les substrats neuronaux responsables de la perception des odorants plaisants, ce qui a des implications pour la compréhension de la résilience à l'anhédonie, ou la perte d'intérêt et de motivation pour les stimuli agréables, ainsi que pour évaluer la gravité des symptômes dépressifs chez les individus avec des antécédents de stress précoce.

-ABSTRACT-

Childhood maltreatment can lead to depressive states in adulthood. Neglect, abuse, and any form of early-life stress can disrupt brain development, affecting emotional balance into adulthood. Additionally, depression is often associated with disturbances in odor perception. Odors play a crucial role in many vital behaviors, such as eating, social interactions, and avoiding danger. Therefore, any alteration in olfactory perception, particularly of pleasant odorants, may reduce daily pleasures and exacerbate depressive symptoms. **In this context, my thesis aims to investigate how early-life stress affects the perception of pleasant odorants in adulthood, and seeks to uncover the neural mechanisms underlying these alterations.**

To do so, I used a murine model of early stress induced by limited nesting and bedding, which confirmed an alteration in emotional behavior in adulthood, but also revealed a disturbance in the perception of pleasant odorants (**STUDY 1**). We then explored the mechanisms of this olfactory impairment by focusing first on the interneurons of the olfactory bulb, whose role in encoding the hedonic value of odorants has been previously demonstrated. These interneurons, generated postnatally (P0-P1), are in their critical integration period during the early stress phase and are therefore particularly vulnerable. Our observations show that early stress alters the fine morphology of interneurons specifically responsive to pleasant odorants. Olfactory bulb interneurons are also the targets of adult neurogenesis. We demonstrated that the new neurons, although born long after the stress period, have altered survival and plasticity. All these disturbances lead to an overall modification of the neural activity of the olfactory bulb in response to pleasant odorants, disrupting the output signal of this structure. Two other brain structures involved in encoding the hedonic value of odorants, the olfactory tubercle and the ventral tegmental area, are also affected. These structures are part of the reward circuit and are particularly active during approach behavior toward pleasant odorants. We revealed that their activity in response to pleasant odorants is altered in animals subjected to early stress.

Finally, the fact that pleasant odorants can induce motivated behavior, engaging the reward circuit, suggests dopamine release, a key molecule in this circuit. To directly study dopamine release in response to odors, I established the fiber photometry technique coupled with video signal analysis in the laboratory (**STUDY 2**). My initial analyses show an increase in dopamine in response to attractive odors compared to non-attractive odorants. The analysis of the effects of early stress on this mechanism is currently ongoing.

In conclusion, our results indicate that early stress modifies the neural substrates responsible for the perception of pleasant odorants, which has implications for understanding resilience to anhedonia, or the loss of interest and motivation for pleasant stimuli, and for assessing the severity of depressive symptoms in individuals with a history of early stress.

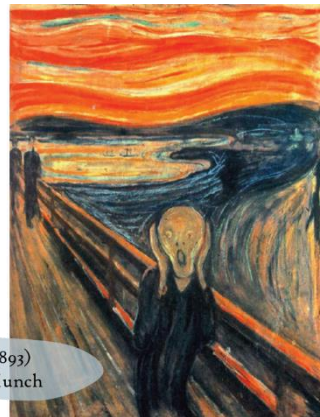
-INTRODUCTION-

-Premier Chapitre-

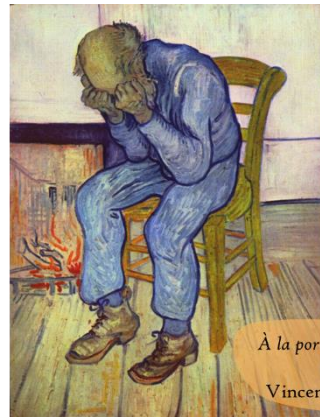
Le Stress avec un grand S

1.1) Introduction

La notion de stress n'a pas toujours eu le nom qu'on lui connaît aujourd'hui. Le mot *stress* a plusieurs origines et significations. Il vient de la langue anglaise dans laquelle « *distress* » signifie détresse, étroitesse. Ce terme vient lui-même de l'ancien français « *drestresce* » ou « *estrece/estresse* », et/ou du latin « *stringere* » qui signifie rendre raide, serrer, resserrer, étrangler. C'est ainsi que le mot stress a progressivement fait référence aux difficultés de la vie, à l'adversité et ses conséquences, qui serrent la gorge, le cœur et les tripes (*Le Figaro Santé, Centre National de Ressources Textuelles et Lexicales, Cairn.info*). Souvent associé à la vie moderne, des exercices de pensées sont pourtant retrouvés très tôt dans notre histoire sur ce que nous nommons aujourd'hui stress. Dès l'Antiquité, les philosophes et médecins évoquent les troubles émotionnels via des concepts de mélancolie, de séparation du « *psyche* », ou encore d'harmonie et de dysharmonie. La notion de stress est alors plus floue, entremêlée à celles de désespoir, de tristesse, de solitude et à d'autres troubles de l'humeur (*Haustgen, 2014*). Ce mélange de notions a longtemps été présent, notamment dans l'art, véritable reflet de l'état psychologique collectif, sa place dans la société et son évolution à travers les époques. *Le Cri* (1893) de Edvard Munch, ou *À la porte de l'éternité* (1890) de Vincent Van Gogh capturent l'angoisse d'une époque marquée par des bouleversements, la dépression, l'isolement et l'abandon complet au chagrin.



Le Cri (1893)
Edvard Munch



À la porte de l'éternité
(1890)
Vincent Van Gogh

Figure 1. Expressions artistiques du stress humain : entre angoisse et désespoir.

Au cours du XIX^e siècle, le scientifique Charles Darwin décrit le stress comme la réponse d'un organisme à des agressions extérieures (*Darwin, 1872*). Au XX^e siècle, Walter Cannon (1929), ajoute à cette première définition la notion de lutte ou fuite, et Hans Selye (1936), endocrinologue hongrois, la précise (*Pronost, 2012*). Pour Selye, les agents stressseurs produisent une série de réactions appelée « le Syndrome Général d'Adaptation ». Ce syndrome est caractérisé par trois étapes : la réaction d'alarme préparant à lutter ou à fuir, la résistance si le stressseur perdure, et enfin lorsqu'il perdure trop longtemps, le dépassement des capacités d'adaptation et une phase d'épuisement (*Selye, 1956*). Cette théorie aborde également les concepts de « eustress » (« eu » signifiant « bon »), le stress bénéfique qui motive et

favorise l'amélioration de l'individu, par opposition au « distress » (« dis » signifiant « mauvais »), le franchissement de la limite de l'individu à faire face.

Aujourd'hui, le stress est retrouvé chez l'humain dans beaucoup de circonstances : au travail, lors de la rédaction d'un manuscrit de thèse, face à la pression sociale et sociétale, en réponse à des traumatismes ou encore face à l'urgence climatique. Il correspond avant toute chose à une réaction biochimique de l'organisme, à ce qu'il considère à tort ou à raison comme un danger ou une menace. Epictète dit « Ce qui trouble les hommes, ce ne sont pas les choses, mais les jugements qu'ils portent sur les choses » (*de Nicomédie, 125apr. J.-C.*). Récemment, l'Organisation Mondiale de la Santé (OMS) décrit que « le stress apparaît chez une personne dont les ressources et les stratégies de gestion personnelle sont dépassées par les exigences qui lui sont posées », et ajoute en 2023 que « le stress est un état d'inquiétude ou de tension mentale causé par une situation difficile. Il s'agit d'une réponse humaine naturelle qui nous incite à relever les défis et à faire face aux menaces auxquelles nous sommes confrontés dans notre vie. Chacun éprouve du stress dans une certaine mesure. Toutefois la façon dont nous réagissons face au stress fait une grande différence pour notre bien-être général ». Aujourd'hui, tous ces concepts sont largement utilisés en recherche, en médecine, en psychologie et en thérapie, pour élaborer des stratégies visant à promouvoir le bien-être et la résilience. Pourtant les mécanismes du stress, « eu » ou « dis », restent obscurs. Plusieurs confusions sont d'ailleurs encore ancrées, la plus fréquente étant de confondre le stress et le stresser. Le stresser est le stimulus de différents ordres, physiques, moral, mental, émotionnel, environnemental ou social, résultant en une réponse physiologique et un état psychologique particulier, qui forment le stress. Pour des raisons de clarté, nous utiliserons le terme stress dans la suite de ce manuscrit.

Ainsi, deux types principaux de stress ont été identifiés, caractérisés et décrits sur la base de leurs durées. **Le stress aigu** est une réponse immédiate et transitoire à une situation fortement stressante. Il peut être bénéfique et formateur. En revanche, lorsqu'il devient **chronique**, répété et/ou prolongé, il peut avoir des conséquences néfastes sur la santé mentale et physique, la cognition ou encore les comportements émotionnels et affectifs (*Ironside et al., 2018; Juster et al., 2010*). Le stress, aigu ou chronique, peut se préciser ensuite *via* des critères supplémentaires. Il est précoce lorsque le(s) stresser(s) apparaît(ssent) tôt dans la vie de l'individu, ou encore post-traumatique après un événement majeur traumatisant. Tous ces stress ont des mécanismes plus ou moins communs, peuvent être cumulés et/ou interagir (*Ironside et al., 2018; Young et al., 2019*).

Il existe de nombreux stresser et donc différents états de stress, aux propriétés et incidences variables. Cependant, tous ces états de stress ont pour point commun de résulter d'une réaction biologique, dont les mécanismes généraux connus à ce jour sont décrits dans la partie suivante.

1.2) Les mécanismes généraux du Stress

La réaction biologique au stress, ou réponse au stress, se divise en deux phases principales. La première est immédiate et survient en quelques secondes grâce à l'activation du système nerveux sympathique. Cette activation augmente les niveaux de catécholamines, qui se fixent sur des récepteurs nicotiques et muscariniques répartis dans le système nerveux central et périphérique. Ce phénomène a pour but de focaliser les ressources dans la réponse urgente au stress aigu pour produire la fameuse réponse de « lutte ou fuite » (*Masuo et al., 2021; McCormick & Mathews, 2010*). Cette réponse peut passer par une augmentation du rythme cardiaque, des phénomènes de bronchodilatation et de vasoconstriction etc. La deuxième vague, plus lente, est endocrine et implique l'Axe Hypothalamo Hypophysaire (HPA). L'axe HPA régule les mécanismes responsables de la stabilité physiologique de

l'organisme, et son activité est nécessaire au maintien de l'homéostasie. Cet axe est une composante essentielle de la réponse au stress car il est à l'origine de la sécrétion de glucocorticoïdes, principalement le cortisol chez l'humain et la corticostérone chez l'animal, qui modulent à plus long terme cette réponse (*McCormick & Mathews, 2010*). Son activité est initiée par le noyau paraventriculaire de l'hypothalamus qui sécrète la neurohormone corticotrope, marquant le début de la réponse endocrine. Cette hormone corticotrope agit ensuite sur la partie antérieure de l'hypophyse, aboutissant à la sécrétion de l'adrénocorticotrope, qui entraîne la production de glucocorticoïdes par la glande surrénale, transportés ensuite dans la circulation sanguine (*Kauffman et al., 2001 ; Nishi et al., 2020*) (Figure2).

Les glucocorticoïdes agissent sur l'hypophyse, l'hypothalamus et d'autres régions régissant la réponse au stress telles que l'hippocampe, le cortex préfrontal médian (CPFm), l'amygdale qui gère la peur et les menaces potentielles et véritables via ses projections retour sur le noyau paraventriculaire de l'hypothalamus (*Opendak et al., 2019, 2021*), et enfin le Locus Cœruleus (LC), noyau central de production de noradrénaline (NA) (*Kaufman & Charney, 2001*). En retour, certaines de ces structures comme le LC, l'hippocampe ou encore le noyau du Raphé (producteur de la sérotonine), constituent une boucle de rétrocontrôle fine du système (*Ulrich-Lai & Herman, 2009*). Les glucocorticoïdes peuvent agir par ailleurs sur d'autres structures cérébrales exprimant les récepteurs adéquats telles l'aire tegmentale ventrale (ATV) et le noyau accumbens, qui font partie du circuit de la récompense que nous détaillerons plus tard (*Marinelli & Piazza, 2002*). Ensemble, ces structures, leurs activités et leurs connexions élaborent la réponse adéquate au stress. En cas de suractivité de l'axe HPA ou de problèmes dans le fonctionnement de ce réseau, des dérégulations comportementales et des conséquences délétères sont observées dans tout l'organisme.

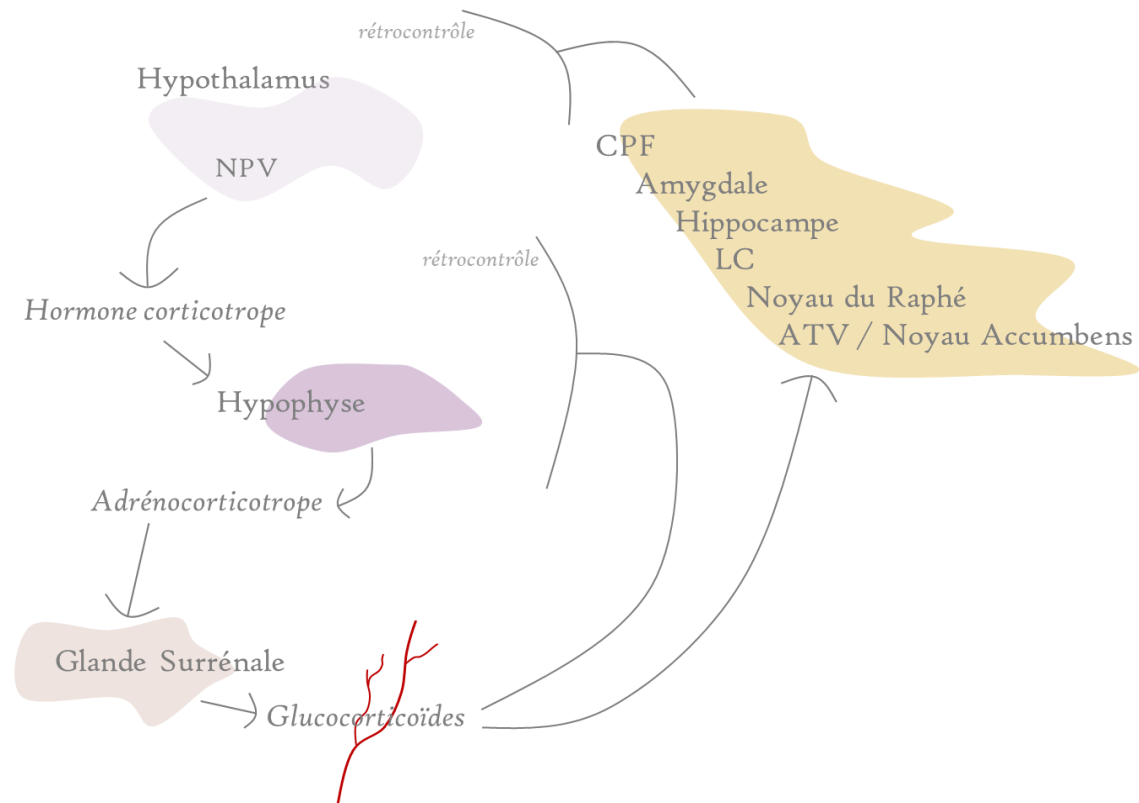


Figure 2. L'Axe HPA, initiateur et régulateur de la réponse du stress. Schéma de l'activation de l'axe du stress, aboutissant à la libération de glucocorticoïdes dans le sang qui vont se fixer à des récepteurs répartis dans le système nerveux central.

-ATV : Aire Tegmentale Ventrale ; CPF : Cortex Préfrontal ; LC : Locus Cœruleus ; NPV : Noyau Paraventriculaire-

-Inspirée de (Kaufman & Charney, 2001; McCormick & Mathews, 2010; C. E. Wood & Walker, 2015)-

De multiples situations de stress entraînent la réponse biologique que nous venons de décrire, et ce de façon plus ou moins forte. Dans la suite de ce manuscrit, nous nous focaliserons sur une forme particulière de stress : **le stress précoce**. Il répond aux mêmes définitions que celles décrites ci-dessus, mais le fait de survenir tôt dans la vie de l'individu lui confère des propriétés uniques. Il regroupe beaucoup de contextes et de situations différentes, à l'origine d'une grande variabilité d'impacts (Smith & Pollak, 2020), que nous allons aborder dans les prochaines sections.

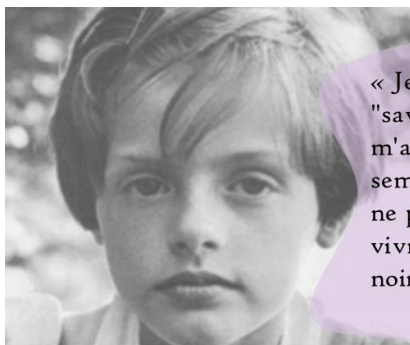
-Second Chapitre-

Le Stress Précoce

2.1) Introduction

Le traumatisme infantile dans la société. L'importance des premières années de vie a longtemps été sous-estimée. Dans les sociétés préindustrielles, les enfants étaient considérés comme des adultes en devenir, devant se rendre utiles et faire preuve de responsabilité au travail. C'est en France, au XVII^e et XVIII^e siècles, que le philosophe Jean-Jacques Rousseau a défendu l'idée selon laquelle l'enfance devait être un temps de croissance protégé, libre des contraintes de la société adulte, et que les expériences négatives précoces étaient dangereuses pour le développement. Les révolutions industrielles et sociales du XIX^e siècle ont ancré cette nouvelle perception, illustrée à travers les œuvres de Charles Dickens telles que *Oliver Twist*, qui met en lumière les effets dévastateurs d'une enfance difficile. Le XX^e siècle a ensuite vu émerger les principes de la psychanalyse avec Sigmund Freud. Bien que ses approches centrées quasi-exclusivement sur la sexualité soient légitimement critiquables, Freud fut néanmoins un des premiers à établir un lien entre les expériences et les traumatismes précoces et la santé mentale à l'âge adulte.

L'exploration artistique des traumatismes infantiles s'est alors parallèlement intensifiée au XX^e et XXI^e siècles. Le film *Le labyrinthe de Pan* (2006) de Guillermo del Toro, mélangeant réalité et fantastique, illustre comment une jeune fille trouve refuge dans son imagination pour échapper aux horreurs de la guerre et aux abus de son beau-père. D'autres œuvres continuent d'explorer les répercussions profondes des traumatismes infantiles comme *Le Voile noir* écrit par Anny Duperey en 2014, un récit autobiographique poignant illustré de photographies en noir et blanc, qui aborde la douleur du deuil lors de l'enfance et explique comment la vie de l'adulte en est imprégnée. La reconnaissance et la représentation des traumatismes infantiles évoluent dans l'histoire, la philosophie et l'art, témoignant d'une meilleure compréhension de leurs impacts profonds et durables.



« Je crois que je n'ai jamais voulu vraiment "savoir". L'atrocité de l'événement a dû m'atteindre si profondément qu'il m'a semblé préférable d'étouffer ce souvenir, de ne pas fouiller dans ce désastre. À force de vivre avec, on finit par s'habituer à ce voile noir, à cette amnésie partielle qui protège. »

-Le Voile Noir (2014) Anny Duperey-

Figure 3. Illustration littéraire des effets dévastateurs des traumatismes infantiles.

Qu'est-ce que le stress précoce ? Plus factuellement, le stress précoce, le traumatisme infantile, ou encore l'adversité en début de vie (*Early Life Adversity* - ELA) est une problématique sanitaire majeure. Selon l'OMS en 2022, près de trois enfants sur quatre - soit 300 millions d'enfants dans le monde - âgés de 2 à 4 ans subissent des châtements physiques et/ou des violences psychologiques de leurs parents et/ou tuteurs. Une femme sur cinq et un homme sur treize déclarent avoir été abusés sexuellement entre 0 et 17 ans. Aux États-Unis, 62 % des adultes recensent au moins un événement traumatique dans la petite enfance (*Merrick, 2019*). De manière très large, le stress précoce se réfère à

des expériences traumatisantes vécues in utéro, à la petite enfance, et jusqu'à l'adolescence. Chez l'humain, les formes les plus sévères sont les soins fragmentés (séparations plus ou moins longues, négligence), les privations (attachement limité ou inexistant, abandon) ou la maltraitance (Nishi, 2020). Le stress précoce peut aussi découler de la pauvreté, de la maladie, ou encore de comportements à risque des figures parentales tels que la consommation de drogues et/ou d'alcool. Cependant, les adversités en début de vie sont généralement classées en deux grandes catégories : menace ou manque (Smith & Pollak, 2020). Expérimentalement et cliniquement, des questionnaires standardisés existent pour évaluer la nature du stress précoce tels que *The Adverse Childhood Experience Scale* ou *The Childhood Trauma Questionnaire*. La qualité des soins à l'enfance, en tant que critère majeur, est souvent évaluée. Pourtant, la prédictibilité ou non du comportement parental semble également très importante. En effet, lorsque les soins sont imprédictibles, la notion de maltraitance est discutable tandis que l'impact sur le développement est indéniable. Les patterns de signaux parentaux semblent ainsi influencer la maturation des réseaux cérébraux, favorisant le développement d'un phénotype résilient ou vulnérable à de futurs troubles psychiatriques (Glynn et al., 2019). Enfin, la complexité et la gravité des effets du stress précoce repose sur le fait que les adversités vécues sont souvent multiples et cumulables.

Dans l'évaluation de la qualité de la vie précoce, beaucoup de travaux se regroupent autour de **la théorie de l'attachement**, qui met en exergue l'importance des interactions entre les petits et leurs figures parentales. Cette théorie souligne que le cerveau de l'enfant n'est pas simplement une version miniature et immature du cerveau adulte, mais plutôt une structure distincte et complexe possédant des caractéristiques uniques adaptées aux exigences et aux demandes de cette période de vie. Alors incapable de se protéger des prédateurs, de réguler sa température ou de s'alimenter, la survie de l'individu dépend de ses figures parentales, qui deviennent centrales, prioritaires (Opendak et al., 2021; Opendak & Sullivan, 2019; Sullivan & Opendak, 2021). Le sens olfactif joue à ce moment-là un rôle majeur. À la naissance, et même *in utero*, les odeurs sont primordiales pour les bébés, afin de reconnaître l'odeur de la mère et plus tard, d'autres membres de la famille. L'information olfactive joue chez l'humain et le mammifère un rôle essentiel dans les processus d'attachement (Cox et al., 2024). Chez le rongeur, en plus de subvenir aux premiers besoins essentiels, la mère transmet les éléments sensoriels extérieurs que le petit ne perçoit pas seul, étant dépourvu à la naissance de la vue et de l'ouïe (Bolton et al., 2017). Les odeurs sont donc cruciales pendant la période critique, elles régulent les émotions du petit, sous-tendent des processus et des apprentissages non olfactifs comme celui de peur et façonnent le futur sens olfactif (Schaal et al., 2020). En termes de structures cérébrales, **le circuit de l'attachement** implique le LC, l'amygdale et le système olfactif (Czarnabay et al., 2019; Moriceau et al., 2009, 2010; Moriceau & Sullivan, 2006; Roth & Sullivan, 2005; Shionoya et al., 2006). Toute altération de ce circuit due à un stress précoce peut donc à long terme avoir des conséquences néfastes sur les fonctions émotionnelles et cognitives essentielles à la vie de l'individu devenu adulte.

Le stress précoce regroupe tous les événements et phénomènes néfastes survenant dans les premiers moments de vie, souvent dépendants de l'environnement et de la qualité des interactions avec les figures parentales, et influençant le développement de l'individu (Perry et al., 2017). Pour être plus précis, on parle de stress précoce lorsqu'une perturbation survient pendant la période critique du développement cérébral, décrite dans la partie suivante.

2.2) La période critique de neurodéveloppement

La particularité du stress précoce repose sur le moment où il apparaît, **la période dite critique**, car particulièrement sensible à l'environnement et cruciale pour le développement physiologique, physique et cérébral. Le cerveau des espèces dites altriciales, où les jeunes vertébrés sont incapables de

subvenir seuls à leurs besoins, continue de se développer après la naissance (*Opendak et al., 2019*). Cette période critique coïncide avec des processus clés de développement et de maturation cérébraux dépendant de l'expérience et des indices environnementaux (*Kane & Ismail, 2017*). Cette période critique s'étend plus précisément sur les cinq premières années de vie chez l'humain et les deux premières semaines chez la souris. Néanmoins, dans les périodes qui suivent, certaines régions restent sensibles aux perturbations environnementales. Même s'il est difficile de transposer le développement du cerveau humain à celui de l'animal, des études documentent aujourd'hui très bien l'existence de similitudes inter-espèces (*Bolton et al., 2017*) (*Figure 4*).

La période critique est associée à **des évènements de plasticité intense**, conservés entre les espèces tels que : l'établissement et l'élimination de synapses et/ou de matière grise, l'expansion des fibres de projection et l'extension dendritique, ou encore le développement morphologique des cellules (*Chakraborty et al., 2021; Teicher et al., 2006; Teicher & Samson, 2016*). La synaptogenèse est un mécanisme favorisant l'équilibre entre les informations excitatrices (principalement le glutamate) et les informations inhibitrices (principalement l'acide γ -aminobutyrique ou GABA). Au cours de la période critique, cet équilibre fragile façonne de façon spécifique le développement des régions cérébrales (*Danese & McEwen, 2012; Danese & van Harmelen, 2017; Sorg et al., 2016; Takesian & Hensch, 2013*). Chaque région suit en effet sa propre trajectoire développementale, correspondant à des fenêtres de sensibilité environnementale qui leurs sont propres (*Andersen, 2022*). Il est ainsi difficile de définir à quel âge le cerveau dans son ensemble -ou même une région entière- est réellement fonctionnel (*Opendak et al., 2019*). Si on se penche sur les structures citées plus haut pour leurs implications dans la boucle de rétrocontrôle de l'axe HPA, des études chez l'humain montrent que la connectivité entre l'hippocampe et l'amygdale apparaît assez tard chez l'enfant par rapport à d'autres régions (*Gee, 2016; Gee et al., 2013; Gee & Casey, 2015*) et que les deux structures ont des chronologies développementales différentes. Le cortex frontal mature également plus lentement, chez l'humain comme chez le rongeur, le rendant particulièrement sensible à l'environnement et ses perturbations jusqu'à l'adolescence (*Perry et al., 2021*).

Dans le contexte de notre étude, **le système olfactif en développement** des mammifères subit lui aussi de profonds processus de maturation morphologique, physiologique et fonctionnelle lors de la période critique. Chez le rongeur, ces remaniements sont nombreux et intenses les dix premiers jours de vie, notamment dans le Bulbe Olfactif (BO) : dans son innervation par les systèmes neuromodulateurs noradrénergique, cholinergique et sérotoninergique, dans l'organisation interne de ses couches et la morphologie de ces cellules, et enfin dans ses projections visant les structures olfactives secondaires (*Tufo et al., 2022*). Dès la naissance, le BO est capable grâce à ses connexions et sa synchronisation avec le cortex piriforme, de former des représentations d'odeurs et de guider le comportement des nouveau-nés. Malgré une structure encore immature, ces circuits sont fonctionnels pendant la période néonatale et favorisent l'apprentissage des odeurs, notamment celles associées aux soins maternels. Le cortex piriforme subit lui aussi une maturation importante les premières semaines de vie, et acquiert petit à petit la capacité de traiter les informations olfactives complexes (*Maier & Zhang, 2023*). En revanche, le Tubercule Olfactif (TO) a un développement majoritairement *in utero*, et est soumis à quelques événements de maturation durant les premières semaines telles qu'une myélinisation intensive (*Martin-Lopez et al., 2019*).

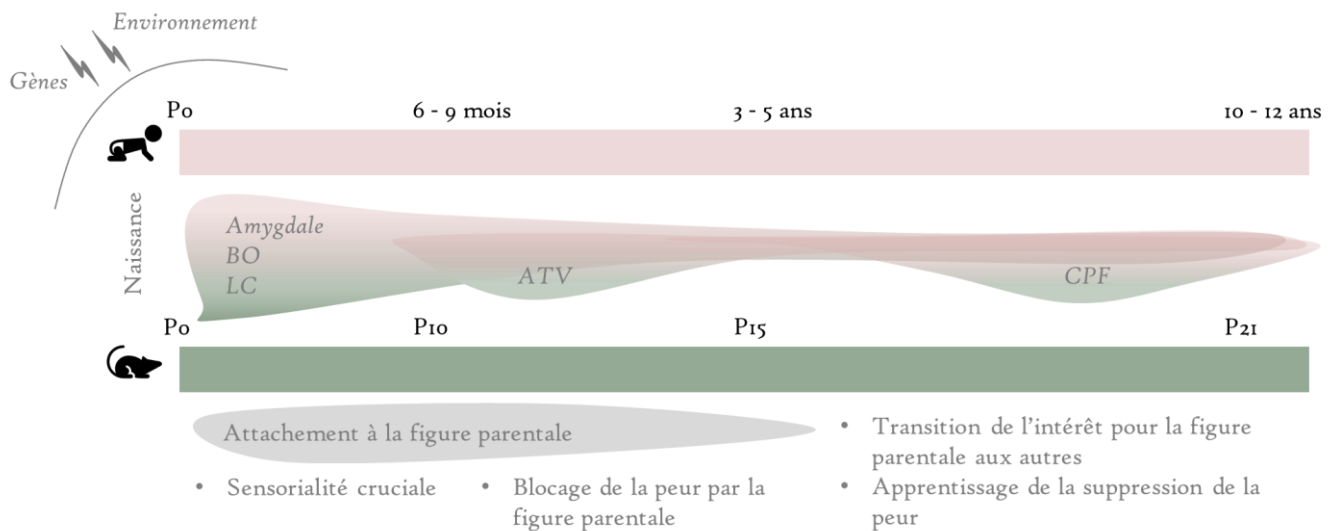


Figure 4. Illustration de la période critique pour l'humain (en haut) et la souris (en bas). Implication des structures cérébrales principales et évènements majeurs.

-ATV : Aire Tegmentale Ventrale ; BO : Bulbe Olfactif ; CPF : Cortex Préfrontal ; LC : Locus Cæruleus ; P : Postnatal Day-

-Inspirée de (Nelson et al., 2019; Opendak & Sullivan, 2019; Rincón-Cortés & Sullivan, 2016)-

En conclusion, il paraît évident que l'environnement de la vie précoce conditionne le développement cérébral global de l'individu. La période périnatale a été rapidement identifiée comme période critique, cependant l'adolescence est une fenêtre temporelle également importante, où certaines régions sont sujettes à des phénomènes de maturation et de remodelages morphologiques et fonctionnels (Andersen et al., 2008; Andersen, 2022; Caballero et al., 2016; Crews et al., 2007; Loi et al., 2017; McCormick & Mathews, 2010; Spear, 2000; Tsoory et al., 2007).

La recherche souligne de plus en plus l'importance de s'intéresser au neurodéveloppement pour mieux comprendre les troubles affectifs et les maladies psychiatriques. Leurs occurrences semblent en effet plus précoces qu'on ne le croit. La période périnatale est ainsi considérée comme une fenêtre clé quant à la vulnérabilité aux maladies psychiatriques et à l'expression d'un phénotype plus ou moins résistant ou troublé plus tard dans la vie. La perturbation de cette période critique par des facteurs comme le stress peut avoir des conséquences importantes sur la santé physique et mentale, car elle surviendrait au moment où les circuits neuronaux sous-tendant la régulation des émotions ne sont pas complètement matures (Schroeder et al., 2018).

En cas de stress durant la vie précoce, le développement neuronal est chamboulé, ce qui aboutit à plus ou moins long terme, à des comportements inadaptés et perturbés. Nous aborderons plus tard les conséquences à l'âge adulte du stress précoce, mais pour l'instant nous allons en décrire les répercussions à court terme, chez le jeune individu, de l'enfance à l'adolescence.

2.3) *Stress précoce : une vue d'ensemble sur les premières influences*

Stress précoce et comportement. Le stress précoce peut provoquer chez l'enfant : une externalisation excessive des comportements, de l'agressivité et/ou de l'hyperactivité, de la difficulté à identifier, à réguler et à exprimer les émotions, ou encore des problèmes de comportements sociaux (Lupien et al., 2009; McLaughlin et al., 2014; Smith & Pollak, 2020). Des conséquences sont visibles aussi chez l'adolescent avec une hausse des niveaux d'agressivité et des difficultés émotionnelles (Jeon & Bae, 2022; Kirsch et al., 2020; Lupien et al., 2009; McLaughlin et al., 2014), un risque accru de troubles psychiatriques tels que la dépression, l'anxiété et les comportements suicidaires, et/ou une intensification des comportements à risques, tels que l'abus de substances et l'engagement dans des activités délinquantes (Gunnar & Quevedo, 2007; McLaughlin et al., 2014). Chez les rongeurs, les petits précocement stressés sont moins affirmés, ils favorisent l'inaction et la résignation à la confrontation face à une situation stressante. L'anxiété, les symptômes de types dépressifs, l'agressivité, et ou les difficultés d'apprentissage et de mémoire sont aussi retrouvés jusqu'à l'adolescence (Bolton et al., 2018; Honeycutt et al., 2020; Manzano Nieves et al., 2020; McCormick & Mathews, 2010; Pryce et al., 2002; Rincón-Cortés & Sullivan, 2016).

Stress précoce et physiologie. Les effets néfastes du stress précoce sur différents réseaux cérébraux sont au moins partiellement médiés par l'axe HPA. Le stress précoce induit en général l'hyperactivité de cet axe, aboutissant à une perturbation des systèmes hormonaux et une hausse de la sécrétion de cortisol ou de corticostérone chez le jeune, qui agissent dans différentes régions cérébrales (Bath et al., 2016; Breton et al., 2024; Danese & McEwen, 2012; Gunnar & Quevedo, 2007; McCormick & Mathews, 2010; Tan et al., 2021). Ceci peut impacter le développement cérébral, physiologique et physique du jeune individu avec des altérations du volume de certaines régions telles que le CPF ou l'hippocampe, ou des décalages de croissance chez l'humain et l'animal : maturation précoce des gonades, retard physiologique de l'ouverture des yeux, déficits de poids du corps et du cerveau etc. (Bath et al., 2017; Burns, 2018; Czarnabay et al., 2019; Gunnar & Quevedo, 2007; Manzano-Nieves et al., 2018; McLaughlin et al., 2014).

Stress précoce et modulations épigénétiques. Le stress précoce provoque des modifications épigénétiques, c'est-à-dire des changements d'expression de gènes mais pas de la séquence d'ADN. Or la période critique correspond déjà à un moment de remaniements épigénétiques, dont la perturbation par le stress impacterait profondément l'individu et son fonctionnement (Daskalakis et al., 2013; Jaworska-Andryszewska & Rybakowski, 2019). Une étude chez l'humain a montré qu'en comparaison à des enfants élevés dans leurs familles biologiques, les enfants institutionnalisés dans des orphelinats présentaient des défauts dans les états de méthylation de l'ADN, dont certains influenceraient directement les comportements adaptatifs (dans environ 7 à 14 % des cas) (Naumova et al., 2019). Le stress précoce peut perturber les remaniements épigénétiques normaux liés à la régulation des hormones de l'axe HPA, aux enzymes modifiant les histones ou à la méthylation et déméthylation de l'ADN dans plusieurs structures cérébrales (Burns, 2018; Labonté et al., 2012). Ces changements impactent les cellules gliales (Lutz et al., 2017), les systèmes GABAergique et glutamatergique, le BDNF (*Brain Derived Neurotrophic Factor*) crucial pour la plasticité et le développement (Burns, 2018; Roth et al., 2009), les systèmes neuromodulateurs tels que la sérotonine (Jaworska-Andryszewska & Rybakowski, 2019), ou encore certains circuits comme celui de la récompense (Shepard & Nugent, 2020). Ces perturbations épigénétiques ont des effets durables, voire transgénérationnels (Kundakovic & Champagne, 2015; Schmauss et al., 2014).

Stress précoce et neuroinflammation. L'occurrence et la persistance de phénomènes neuro-inflammatoires jouent également un rôle dans les conséquences du stress précoce. Le stress pendant la période critique augmente les niveaux de cytokines pro-inflammatoires et réduit ceux des cytokines anti-inflammatoires, ce qui peut renforcer l'activité de l'axe HPA et accroître les niveaux de corticostérone (Majidi et al., 2016; Tan et al., 2021). La neuroinflammation peut impacter la balance prolifération/raffinement, la complexité dendritique, l'équilibre excitation/inhibition, les niveaux de myélinisation et les synapses (Andersen, 2022) ou encore moduler la microglie (Andersen et al., 2008; Catale et al., 2020). En conclusion, le stress au moment de la période critique instaure une neuroinflammation qui perturbe profondément l'activité et le fonctionnement des circuits en maturation, même si son effet à long terme est encore mal compris.

Stress précoce et microbiote. L'axe microbiote-intestin-cerveau fait référence à la communication bidirectionnelle entre le microbiote intestinal et le cerveau. Des études ont montré une altération du microbiote causé par le stress précoce (Anisman et al., 1998; Barouei et al., 2012; O'Mahony et al., 2009, 2014, 2020) avec une diminution de sa diversité (Moussaoui et al., 2017) ou encore une augmentation de la perméabilité intestinale (Walker et al., 2017). Récemment, il est apparu que l'axe microbiote-cerveau intervient dans la gestion des émotions et qu'une altération peut favoriser la survenue de troubles affectifs (Coley et al., 2021; Cruz-Pereira et al., 2020, 2023). De plus, ces dysfonctionnements peuvent entraîner ou renforcer la neuroinflammation impactant ainsi l'axe HPA et la circuiterie cérébrale associée. Cependant, si la piste du microbiote intestinal est intéressante pour comprendre les bases du stress précoce, elle manque cruellement d'informations notamment chez le modèle humain.

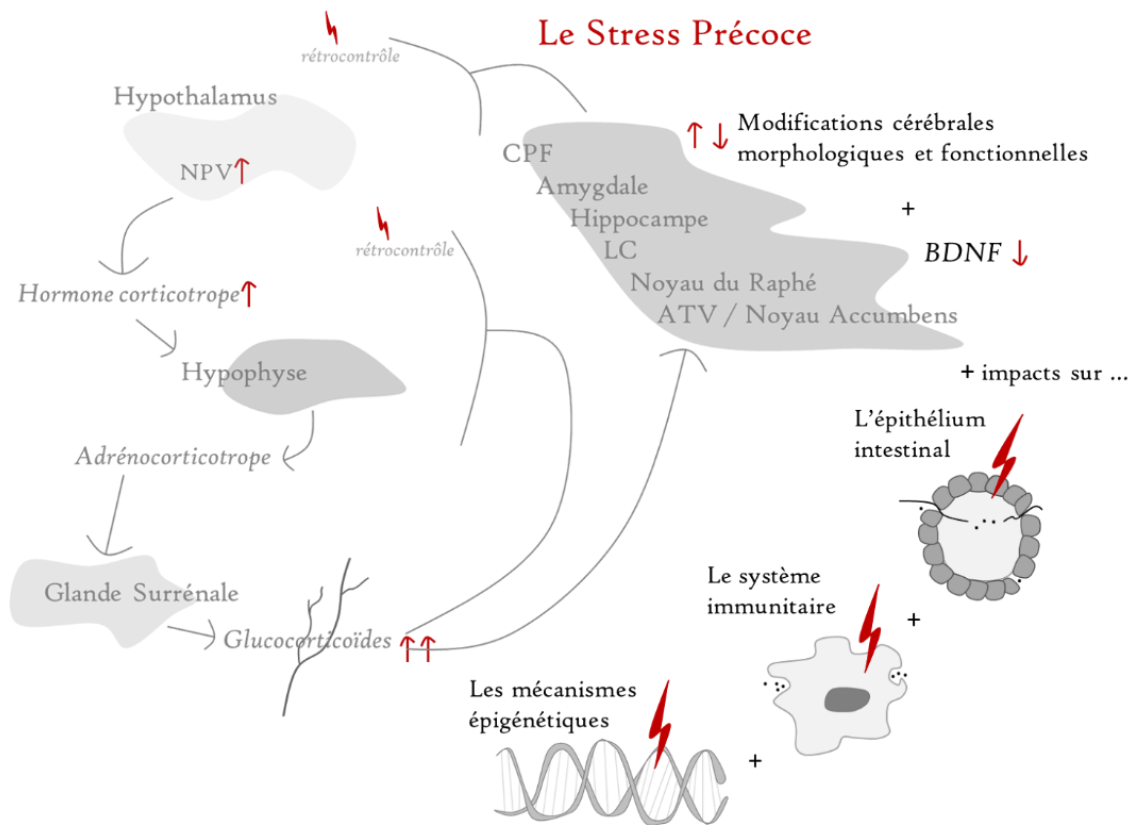


Figure 5. Les impacts du stress lors de la période critique.

-ATV : Aire Tegmentale Ventrale ; BDNF : Brain Derived Neurotrophic Factor ; CPF : Cortex Préfrontal ; LC : Locus Cœruleus ; NPV : Noyau Paraventriculaire-
-Inspirée de (Alves et al., 2022; Andersen, 2022; Burns, 2018)-

Stress précoce et troubles du fonctionnement cérébral. Le stress précoce directement, et les mécanismes mentionnés précédemment, affectent le développement de différentes structures cérébrales de **l'enfant**, ce qui induit ou module les réponses comportementales. Le volume et le fonctionnement de l'amygdale, du CPF ou encore de l'hippocampe, impliqués dans la régulation des émotions et des comportements, peuvent être modifiés (J. Bremner et al., 2003; Krugers et al., 2017; McLaughlin et al., 2014; Teicher et al., 2003, 2004, 2006, 2016). Plusieurs études d'IRM fonctionnel (fMRI) signalent en effet des perturbations de la connectivité fonctionnelle du CPF, de l'amygdale, de l'ATV, de la substance noire et de l'hippocampe chez les enfants ayant subi différentes sortes de traumatismes (Lambert et al., 2017, 2019; Malter Cohen et al., 2013; Marusak et al., 2015, 2017; Miller et al., 2022; Schreiber et al., 2017). Il est important de souligner que l'on retrouve les mêmes régions cérébrales que celles décrites auparavant, mûrissant pendant la période critique, sensibles aux glucocorticoïdes, et impliquées dans diverses réponses émotionnelles (atténuation de la peur, engagement dans les conflits, traitement des menaces). Celles-ci sont dégradées par le stress précoce en fonction du type d'adversité, de sa durée, ou bien du sexe (J. Bremner et al., 2003; Lambert et al., 2017, 2019; Malter Cohen et al., 2013; Marusak et al., 2015, 2017; McLaughlin et al., 2014; Schreiber et al., 2017; Teicher et al., 2004).

Chez le jeune animal, différents modèles décrivent les conséquences neurobiologiques à court terme du stress précoce. Il semble ainsi impacter la morphologie fine de différentes structures :

en perturbant la différenciation et la maturation dendritique dans le striatum et le CPF autour de P15, P20 (*Post-natal day* - jour après la naissance) (Farrell et al., 2016; He et al., 2021), ou en diminuant la production de synapses dans l'hippocampe affectant sa connectivité par exemple (Andersen & Teicher, 2004; Molet, Maras, et al., 2016). De manière globale, en altérant le développement et la maturation de plusieurs structures (telles que l'amygdale, le CPF) et circuits (tels que limbique, de la récompense, de l'attachement et de l'apprentissage de peur et de gestion de la menace), le stress précoce interfère dans leur fonctionnement et/ou la libération des neurotransmetteurs correspondants, ce qui *in fine* module le comportement (Babicola et al., 2021; Molet et al., 2014; Raineki et al., 2017, 2019; Robinson-Drummer et al., 2019; Sullivan & Opendak, 2021). Ainsi, le stress précoce peut induire une hyperactivité de l'hypothalamus impliqué dans la réponse au stress (Gunn et al., 2013), des déficits de la transmission glutamatergique cortico-striatale régulant la flexibilité comportementale et cognitive chez les souris mâles (De Carvalho et al., 2023), des déséquilibres de la balance excitation/inhibition dans plusieurs structures chez les rats adolescents (Andersen, 2022; Holland et al., 2014; Honeycutt et al., 2020; Manzano Nieves et al., 2020), ou encore des déséquilibres dans l'influence DAergique dans l'amygdale basolatérale sous-tendant les impacts sociaux sur le jeune rat (Opendak et al., 2021). Le stress précoce agit donc sur le développement, la structure et le fonctionnement du cerveau, à l'origine d'un grand nombre de troubles en fonction du contexte, du type de stress, de sa durée, de l'âge et du sexe.

En conclusion, à court terme, le stress précoce tronque les processus de croissance neuronale et modifie la maturation des structures cérébrales conduisant à des dérégulations du comportement émotionnel (Bath et al., 2016; Goodwill et al., 2019; Manzano Nieves et al., 2020; Pattwell & Bath, 2017). Celles-ci apparaissent tôt dans la vie et expliquent en partie les répercussions délétères du stress précoce une fois adulte. Par rapport à d'autres types de stress, le stress précoce est unique puisqu'il touche le cerveau en développement. Cependant, si une partie des jeunes exposés au stress souffrent de troubles plus tard dans la vie, une partie substantielle n'est pas concernée. Par conséquent, il peut y avoir un phénomène de résilience -que nous évoquerons à la fin de ce manuscrit. Ceci témoigne de la grande diversité des effets du stress précoce, qui perdurent et/ou évoluent une fois adulte, ce qui fait l'objet de la prochaine partie.

2.4) Le poids du stress précoce : impacts à long terme chez l'humain

Impact comportemental. Les données épidémiologiques montrent que le stress précoce chez l'humain a des impacts comportementaux durables. Il est associé aux troubles de l'humeur et de la réponse émotionnelle chez les adultes, hommes et femmes (Negele et al., 2015; Touchant & Labonté, 2022; Tretyak et al., 2020), des déficits dans les capacités de mémoire, les fonctions exécutives ou la prise de décision (Birn et al., 2017; Jaworska-Andryszewska & Rybakowski, 2019), des troubles des comportements addictifs et des difficultés dans les milieux social et professionnel (Danese & McEwen, 2012), ou encore des symptômes anhédoniques (Corral-Frías et al., 2015) avec des modifications du comportement d'anticipation et de consommation de la récompense (Ironside et al., 2018). Ces altérations peuvent aller jusqu'à la survenue de psychopathologies et de maladies psychiatriques telles que l'anxiété, la dépression, la schizophrénie, les troubles psychotiques ou le syndrome de stress post-traumatique (Bolton et al., 2017; Burns, 2018; Danese & McEwen, 2012; Jaworska-Andryszewska & Rybakowski, 2019; McLaughlin et al., 2014), et ce, en fonction du sexe dans la population clinique (Alastalo et al., 2013; Frodl et al., 2010; Zoladz et al., 2022).

Si on prend l'exemple de **la dépression**, la relation causale entre le stress de manière générale et la maladie est bien établie (Kendler et al., 2002, 2004). De manière importante, parmi les stressseurs favorisant la dépression, le traumatisme à l'enfance apparaît comme majoritaire (Anda et al., 2006; Croy

et al., 2010). Les individus ayant subi un traumatisme infantile développent une dépression dans 67 % des cas, plus tôt, avec des symptômes plus sévères, longs et récurrents, ainsi qu'une résistance plus forte aux traitements (Andersen, 2022; Jaworska-Andryszewska & Rybakowski, 2019; Kaufman & Charney, 2001).

Des altérations comportementales du même type sont observées à long terme chez le rongeur et le primate adulte après un traumatisme infantile (Tan et al., 2021). Toutefois, en raison de la diversité des modèles de stress précoce, nous ne passerons pas en revue toutes les répercussions sur le comportement de l'animal adulte.

Altération physiologique. Le fonctionnement de l'axe HPA, altéré chez l'enfant, peut rester perturbé chez l'adulte, avec une hyperactivation chronique et donc à terme une augmentation de la sécrétion de cortisol et une sensibilité accrue au stress (Gunnar & Quevedo, 2007). Ceci peut contribuer au développement de problèmes de santé physique tels que les maladies cardiovasculaires, le diabète, les troubles immunitaires ou bien l'obésité (Danese & McEwen, 2012; Jaworska-Andryszewska & Rybakowski, 2019).

Bases neurobiologiques. Les altérations structurelles et fonctionnelles de plusieurs régions cérébrales, y compris le CPF et l'hippocampe, persistent chez les adultes ayant été exposés à un stress précoce (J. Bremner et al., 2003; Danese & McEwen, 2012; Herzberg & Gunnar, 2020; Kaiser et al., 2018; Stein et al., 1997). À long-terme, il continue donc d'affecter préférentiellement les réseaux impliqués dans la gestion de la menace, le traitement de la peur, la régulation des émotions et les comportements motivés et relatifs à la récompense (Insana et al., 2016; Teicher & Samson, 2016). On retrouve des altérations de la voie mésocorticolimbique et plus précisément de la libération de DA dans les structures cibles (Arnold et al., 2020; Ironside et al., 2018; Novick et al., 2018), des défauts d'activité du striatum ventral (Hanson et al., 2016), de la connectivité entre le CPFm, l'amygdale, le striatum ventral et le noyau accumbens (Hanson et al., 2018; Ironside et al., 2018), et entre l'amygdale, le CPF et le gyrus frontal inférieur (Smith & Pollak, 2020), ou encore de la balance entre l'excitation et l'inhibition au sein de ces structures (Reh et al., 2020).

Quel que soit son type, le stress précoce est quasi-systématiquement relié à une vulnérabilité émotionnelle et comportementale, pouvant aller jusqu'au développement de troubles psychiatriques, psychologiques et affectifs à l'âge adulte. Les effets listés ci-dessus font ainsi grandement écho à ceux mis en évidence chez le jeune. Comprendre le stress précoce et ses conséquences à plus ou moins long terme représente un pan actif de la recherche, et notamment de la recherche animale qui tente de le modéliser afin d'appréhender les mécanismes neuronaux spécifiques de ses effets et de contrer ses préjudices. Ainsi nous allons maintenant expliquer l'intérêt des différents modèles animaux et le choix du modèle de notre étude.

2.5) Modélisation du stress précoce

Comme nous l'avons dit, les conséquences du stress précoce dépendent de beaucoup de facteurs, ce qui explique en partie la grande diversité de modèles animaux (Perry et al., 2021). Un traumatisme vécu seul, en présence de la figure parentale ou directement *via* le comportement de cette figure parentale, a des conséquences différentes par exemple (Opendak et al., 2019). Dans ce contexte, j'ai rédigé une revue bibliographique au début de ma thèse, parue en 2021, intitulée *Relationship between depression and olfactory sensory function: a review* (REVUE), qui dépeint la relation bidirectionnelle entre la perception olfactive et les troubles de l'humeur tels que la dépression, sujet que nous aborderons plus tard dans ce manuscrit. Plus important ici, cette revue détaille différents modèles de dépression

chez l'animal, notamment les principaux modèles de stress précoce : la séparation maternelle et la restriction des conditions d'hébergement (*Limited Bedding and Nesting* - LBN). Ces deux modèles sont loin d'être exhaustifs et comportent eux-mêmes des caractéristiques variables en fonction des études (*Köhler et al., 2019; Naninck et al., 2015; Rice et al., 2008; Touchant & Labonté, 2022; Walker et al., 2017*).

Le modèle de séparation maternelle est le plus populaire et repose sur l'isolation plus ou moins longue des petits quotidiennement par l'expérimentateur, durant les premiers jours de vie (*Nishi, 2020; Perry et al., 2021; Planchez et al., 2019*). Ses effets dépendent de la durée et de la fréquence des phases de séparation, et de la période durant laquelle la séparation est répétée (*Köhler et al., 2019*). **Le modèle LBN** consiste à placer la mère et ses petits dans une cage particulière le premier jour du protocole (P4), et de les laisser évoluer dans cet environnement pendant 7 jours (jusqu'à P11). La cage possède des conditions d'hébergement restreintes, avec une grille faisant office de sol, pas de litière, et seulement la moitié du matériel nécessaire pour construire le nid. Ces deux modèles augmentent l'anxiété maternelle (*Ivy et al., 2008; Murthy & Gould, 2018; Walker et al., 2017*) et favorisent l'expression de comportements troublés chez les petits, et les adultes, soutenant la validité translationnelle de ces approches (*Molet et al., 2014; Rainekei et al., 2012; Walker et al., 2017*). Néanmoins, dans certaines études, ces modèles n'ont induit aucun effet sur la progéniture, ce qui sera discuté à la fin de ce manuscrit (*Murthy & Gould, 2018; Rice et al., 2008; Schmidt et al., 2011*).

Ces modèles ont fait avancer la compréhension des mécanismes sous-tendant les effets du stress précoce et alimentent ainsi une littérature riche et diverse. Dans notre étude, le modèle choisi est **le modèle LBN**, dont les intérêts sont multiples. Il est premièrement plus naturaliste, car ne nécessite qu'une seule intervention extérieure de l'expérimentateur le premier jour (P4) (*Walker et al., 2017*). De plus, il se rapproche davantage du stress précoce comme on le définit chez l'humain (*Bolton et al., 2017*). En effet, le stress précoce est chronique, répété, varié, complexe et donc multifactoriel. Il comporte des éléments d'abus, de violence et de négligence à la fois, ce qui correspond au modèle LBN, où le comportement abusif de la mère, stressée par son environnement, se reporte sur les petits de diverses façons pendant 7 jours. Enfin, le modèle LBN remplit un autre critère également très important qui est l'imprédictibilité (non expérimentale) du comportement maternel. Il est donc le plus pertinent à nos yeux pour modéliser l'adversité précoce et mesurer ses effets.

Les paragraphes précédents ont abordé les effets immédiats du stress précoce chez l'humain et l'animal, ainsi que les impacts durables chez l'humain. La prochaine partie illustre ce que la littérature relate plus spécifiquement des effets à long terme du modèle LBN chez l'animal.

2.6) Les répercussions comportementales, physiologiques et neurobiologiques du modèle animal LBN

Le modèle LBN induit des phénomènes développementaux atypiques, des altérations cérébrales avec des conséquences comportementales chez le petit, atteignant un pic à l'adolescence avant d'être plus fluctuantes à l'âge adulte (*Bath et al., 2017*).

D'un point de vu comportemental, à partir de l'adolescence et jusqu'à l'âge adulte, le protocole LBN provoque fréquemment l'expression d'un phénotype anhédonique, soit la diminution du plaisir perçu vis-à-vis d'un stimulus normalement agréable, faisant écho au symptôme majeur de la dépression chez l'humain. Cette anhédonie peut être relative aux récompenses naturelles telles que le sucre ou le chocolat, aux interactions sociales, ou encore à la consommation de drogues (*Bolton et al., 2018; Molet, Heins, et al., 2016; Molet, Maras, et al., 2016; Wendel et al., 2021*). Le LBN entrave

évidemment les comportements émotionnels et motivés (*Bolton et al., 2018; Dalle Molle et al., 2012; Krugers et al., 2017; Laguna et al., 2022; Raineki et al., 2012, 2019; M. E. Wang et al., 2013*), ou encore les comportements sociaux avec une détérioration de la reconnaissance et de l'agressivité (*Kohl et al., 2015*). Ce modèle, en influençant l'apprentissage précoce de l'attachement, altère plus tard la réponse de peur et son apprentissage ainsi que les réactions face à des situations néfastes (*Arp et al., 2016; Guadagno et al., 2021; Guadagno, Kang, et al., 2018; Guadagno, Wong, et al., 2018; Malter Cohen et al., 2013; Manzano Nieves et al., 2020*). Pour faire de telles observations, les auteurs utilisent divers paradigmes expérimentaux tels que la préférence au sucre pour révéler l'anhédonie (*Bolton et al., 2018*), l'*openfield* ou la *dark/light box* pour mesurer les niveaux d'anxiété (*Pardasani et al., 2023*), ou encore les tests de *novelty induced hypophagia* ou *novelty suppressed feeding test* pour mesurer les symptômes de type dépressifs (*Dulawa & Hen, 2005; Goodwill et al., 2019*). On évoque ainsi la notion de « spectre des conséquences cognitives du stress précoce », qui relate bien la diversité des conséquences comportementales et émotionnelles du modèle LBN.

Physiologiquement, bien que les résultats soient plus fluctuant, le modèle LBN favorise l'hyperactivité de l'axe HPA chez les rats et les souris jeunes, adolescents/tes et adultes (*Bolton et al., 2017; Breton et al., 2024; Rice et al., 2008*). Il affecte la croissance et la physiologie du stress chez les deux sexes (*Demaestri et al., 2020*) provoquant une réactivité accrue au stress, une augmentation de la sécrétion de glucocorticoïdes, une perturbation des mécanismes de rétroaction de l'axe HPA, ou encore des réductions du taux de croissance et des anomalies métaboliques.

Au niveau neurobiologique, le modèle LBN impacte différentes structures cérébrales et les grandes fonctions associées. Il affecte les structures olfactives et limbiques appartenant au circuit de l'attachement décrit plus tôt, qui ont par exemple une connectivité moins robuste (*Demaestri et al., 2020; Perry et al., 2016*). Il modifie le fonctionnement et la morphologie de l'**amygdale**, à l'origine des effets sur la gestion de la peur cités ci-dessus (*Bath et al., 2017; Guadagno et al., 2020, 2021; Guadagno, Wong, et al., 2018*).

Dans l'hippocampe, les études sont nombreuses et révèlent des changements de la neurogenèse adulte (*Naninck et al., 2015*), dont des atteintes des niveaux de prolifération neuronale. De plus, des modifications de certaines caractéristiques de la microglie favorisent des changements de la connectivité de l'hippocampe à l'âge adulte, expliquant les défauts observés au niveau de la mémoire et des apprentissages (*Alves et al., 2022; Dayananda et al., 2023*). À une échelle plus petite, une étude révèle une diminution de la potentialisation à long-terme (*Ivy et al., 2010*), et des changements dans l'expression de molécule d'adhésion synaptique importante pour la stabilisation et la maturation synaptique (*Kohl et al., 2015*).

Vis-à-vis **des régions frontales**, des déficits cognitifs ont été associés à une diminution de la densité des interneurons dans le Cortex Orbitofrontal (COF) des femelles adultes ayant suivi un protocole LBN (*Goodwill et al., 2019*). Ce modèle impacte durablement la morphologie des neurones pyramidaux dans des sous-régions du CPF, impactant les performances cognitives associées (*X.-D. Yang et al., 2015*).

Enfin le modèle LBN perturbe durablement les structures des circuits limbique et de **la récompense** comme le noyau accumbens ou encore l'ATV, la connectivité entre les structures de ces systèmes et/ou au sein des systèmes eux-mêmes, favorisant l'expression de comportements motivés et/ou émotionnels abimés (*Bolton et al., 2018; Laguna et al., 2022; Wendel et al., 2021*).

-PREMIER ENTRACTE-

En conclusion, les chercheurs s'accordent sur le fait que les expériences précoces, dépendantes de l'interaction entre les petits et les figures parentales, influencent la plasticité de l'axe HPA et du cerveau en développement. Ces premières interactions jouent un rôle crucial dans la mise en place des mécanismes de réponse au stress et dans la bonne formation des structures cérébrales. Une expérience précoce négative influence le comportement, la physiologie et le fonctionnement cérébral de manière immédiate et durable, pouvant aller de comportements inadaptés à la pathologie. J'espère avoir clarifié ce que l'on sait du lien entre le stress précoce et les troubles affectifs. Les conclusions tirées des modèles animaux se rapprochent des observations faites chez l'humain, confirmant la pertinence et l'intérêt de la modélisation animale. Lorsque nous avons abordé la notion de période critique, nous avons évoqué la théorie de l'attachement, qui repose en partie sur le système olfactif, marquant la première véritable apparition de l'olfaction dans l'introduction de ce manuscrit. Il s'agit maintenant de recentrer ce manuscrit autour de cette thématique. En effet, il existe un lien étroit entre certains troubles affectifs et la modalité sensorielle olfactive, qui sera détaillé ultérieurement. Pour le moment, le troisième chapitre a pour objectif de présenter le sens olfactif, le système cérébral associé, son anatomie, son fonctionnement et ses mécanismes.

-Troisième Chapitre- L'Olfaction

L'olfaction et le monde. Nous arrivons ainsi à la star de cette thèse, l'odorat, l'olfaction, le nez, le sentir et au fur et à mesure, son lien avec le ressentir. Historiquement, l'olfaction est une notion présente dès l'Antiquité, notamment dans les écrits philosophiques de la Grèce antique (*Tran Ba Huy, 2000*). Les Romains utilisaient les parfums et encens pour des raisons sociétales, hygiéniques ou encore religieuses. D'autres civilisations, byzantines, orientales, égyptiennes, indiennes ou chinoises, avaient des pratiques liées aux parfums et aux encens, souvent relatives à des rites religieux et médicaux (*Caseau & Neri, 2021*). Au Moyen Âge, l'olfaction avait une connotation plus ambivalente. Les mauvaises odeurs étaient associées à la maladie et la pauvreté, tandis que les bonnes étaient perçues comme purifiantes, protectrices, gages de bonne santé (*Caseau & Neri, 2021*). Les médecins médiévaux, influencés par les théories humorales d'Hippocrate, croyaient que les odeurs pouvaient moduler la santé, et prescrivaient des substances odorantes pour équilibrer les humeurs du corps (*Javier, 2014*). Avec l'avènement de la distillation pendant la Renaissance, la production d'essences et de parfums était importante. Paracelse et d'autres alchimistes ont alors exploré les propriétés curatives des plantes et des odeurs (*Plançq, 2021*). Au XVIII^e et XIX^e siècles, de nouvelles perspectives scientifiques sur l'odorat ont été apportées, faisant que ce sens était de plus en plus compris et de moins en moins mystique (*Philpott et al., 2008*).

Au niveau artistique, les odeurs ont une place importante dans la littérature, l'art visuel, la musique ou le cinéma, notamment depuis les années 90 et l'apparition de l'art olfactif contemporain (*Wicky, 2018*). Le roman *Le Parfum* de Patrick Süskind (1985) en est un exemple fort, puisque plusieurs fois adapté au cinéma et reconnu pour la façon dont sont décrites les odeurs. En peinture, les références à l'odorat sont plus anciennes mais moins directes. Certaines œuvres telle que *L'Allégorie de l'odorat*, de la série de *L'Allégorie des cinq sens* de Jan Brueghel et Pierre Paul Rubens (1617) représentent la

personnification féminine de l'odeur dans l'environnement. L'illustration de l'odorat dans la peinture est souvent relative aux femmes, aux fleurs, au fait de se parfumer et aux sentiments. Globalement l'odeur a toujours été utilisée pour illustrer un souvenir nostalgique d'un amour perdu ou une envie pressante de revoir un amour naissant avec des notions d'entêtement, de séduction, de capture, de possession « Il se répand autour de la femme que l'on aime un parfum céleste, ce n'est plus de l'air, c'est de l'amour que l'on respire » (*Alphonse Karr 1839*). À travers cette représentation que l'on pourrait considérer comme pauvre, le lien fort existant entre l'odorat et le sentiment d'amour et de bonheur s'enracine dans la pensée collective. Dans la musique, les odeurs ne peuvent être représentées directement, mais certaines compositions évoquant là encore la nature ou l'amour, placent les odeurs dans le décor. Cependant, dans certaines musiques plus modernes (de rap par exemple), l'odorat fait aussi référence au fait de flairer, un danger, le feu, les balles, l'arnaque, ou encore l'argent. Ici, l'olfaction est dépeinte indirectement, comme un indicateur, un système de détection plutôt qu'un sentiment.

L'odorat est donc étudié et illustré par bien des façons, rendant compte à la fois de son importance et de la richesse de sa nature. Son essence est cependant plus dure à capturer, que ce soit à travers les émotions qu'il procure (bonheur, amour, bien-être) ou les actions qu'il initie (flairage de danger, fuite, méfiance, prise de décision). Ces aspects relatent la complexité de l'odorat, ses conséquences émotionnelles et/ou comportementales, que nous allons évoquer dans de futures parties. Il convient maintenant de rappeler que les odeurs font partie intégrante de notre environnement où elles jouent clairement un rôle sur notre perception du monde, nos émotions et nos actions.



L'Allégorie de l'odorat (1617)
Brueghel et Rubens

Figure 6. Illustration visuelle du sens olfactif à travers la peinture.

L'olfaction et l'environnement : le sens olfactif parmi les sens. Les odeurs font partie de notre environnement direct et quotidien, de manière continue et permanente. Dans les environnements domestiques et nos maisons, les odeurs transparaissent le confort et le bien-être. Les parfums d'intérieur, les bougies parfumées, les désodorisants et les huiles essentielles créent des atmosphères agréables et accueillantes, et sont choisis afin d'évoquer des souvenirs et/ou influencer l'humeur. Dans les environnements urbains et publics, les odeurs peuvent être des marqueurs puissants de différents

espaces. Les marchés, les restaurants, les parcs et même les transports en commun ont des signatures olfactives distinctes, pouvant influencer la perception d'un lieu, notre comportement et nos habitudes. Cette emprise des odeurs en a fait une discipline sociétale, puisque le marketing olfactif est une pratique courante dans le commerce pour créer encourager les achats.

L'olfaction est donc un sens fondamental et les odeurs ont une présence significative dans notre environnement quotidien, agissent directement sur nos états émotionnels et nos comportements. Pour ce faire, nous disposons d'un système olfactif capable de détecter les odeurs et de traiter de différentes façons les informations qu'elles portent. Vous imaginerez facilement que l'histoire ne s'arrête pas là. Ces capacités servent de plus grandes fonctions, comme la reproduction, la prise de décision, les interactions sociales ou l'alimentation. Tout ceci est ensuite modulé par l'expérience, les apprentissages, ou encore nos états. Pour commencer à entrevoir tous ces rôles olfactifs, la prochaine partie a pour but de présenter le système olfactif, de son entrée -la cavité nasale- aux centres olfactifs supérieurs et les structures cérébrales associées.

3.1) Anatomie du système olfactif

3.1.1) La porte d'entrée : l'épithélium olfactif dans la cavité nasale

La perception olfactive démarre soit lors de la respiration naturelle ou forcée, lorsque nous inspirons et que les molécules olfactives volatiles entrent directement dans la cavité nasale pour rejoindre l'épithélium olfactif (voie orthonasale), soit lorsque nous ingérons des aliments et que des molécules olfactives libérées dans la cavité buccale lors de la mastication passent en arrière du palais pour rejoindre l'épithélium olfactif (voie rétronasale) (Murphy *et al.*, 1977). Dans les deux cas, le début de la perception olfactive se passe donc au niveau de la cavité nasale, dont la partie postéro-dorsale est tapissée par une muqueuse dont fait partie **l'épithélium olfactif**. L'épithélium est la première structure du système olfactif et est composé entre autres de cellules de soutien, de cellules souches et de neurones olfactifs sensoriels (Morrison & Costanzo, 1992). Ces neurones olfactifs possèdent des cils sur leurs prolongements dendritiques apicales uniques, qui sont exposés dans la cavité et recouverts de récepteurs olfactifs (Buck & Axel, 1991). Ainsi, les molécules olfactives qui entrent dans la cavité nasale se lient à ces récepteurs. La capacité du système olfactif à détecter et à distinguer un large spectre de molécules odorantes avec une très bonne sensibilité repose en partie sur la diversité de ces récepteurs. En effet, le génome de souris comporte environ 1200 gènes codant pour les récepteurs olfactifs contre 400 chez l'humain (Mombaerts *et al.*, 1996). Ces récepteurs sont transmembranaires et couplés à des protéines G, dont la liaison avec la molécule odorante active la voie de l'AMP cyclique, ce qui *in fine* dépolarise de la cellule (Imai & Sakano, 2008). Si cette dépolarisation dépasse le seuil d'excitation du neurone, un train de potentiels d'action est généré. Cette dépolarisation se transmet le long des prolongements axonaux des neurones sensoriels constituant les nerfs olfactifs, qui traversent la lame criblée de l'os ethmoïde séparant la cavité nasale de la boîte crânienne, pour rejoindre le BO, premier relai cortical de l'information olfactive (Figure 7).

Avant de passer à la description du BO, une notion importante, celle **du code olfactif combinatoire**, mérite d'être évoquée. Ce code permet au cerveau humain de discriminer plus d'un milliard d'odeurs avec seulement quelques centaines de récepteurs (Bushdid *et al.*, 2014). Une molécule odorante va se lier à une combinaison unique de récepteurs (avec des affinités variables qui dépendent des propriétés physico-chimiques de molécules comme leur poids, leur volume, leur liaison etc). Ceci va permettre la perception caractéristique de l'odeur. De plus, un récepteur peut lier différentes molécules odorantes, là encore avec des affinités variables. Ainsi chaque odorant induit l'activation d'un

pattern spécifique de récepteurs olfactifs. Ce pattern est transmis au BO, où est évoqué un pattern spatial et temporel unique dans ses différentes populations (Kontaris *et al.*, 2020), que nous allons décrire dans la partie suivante.

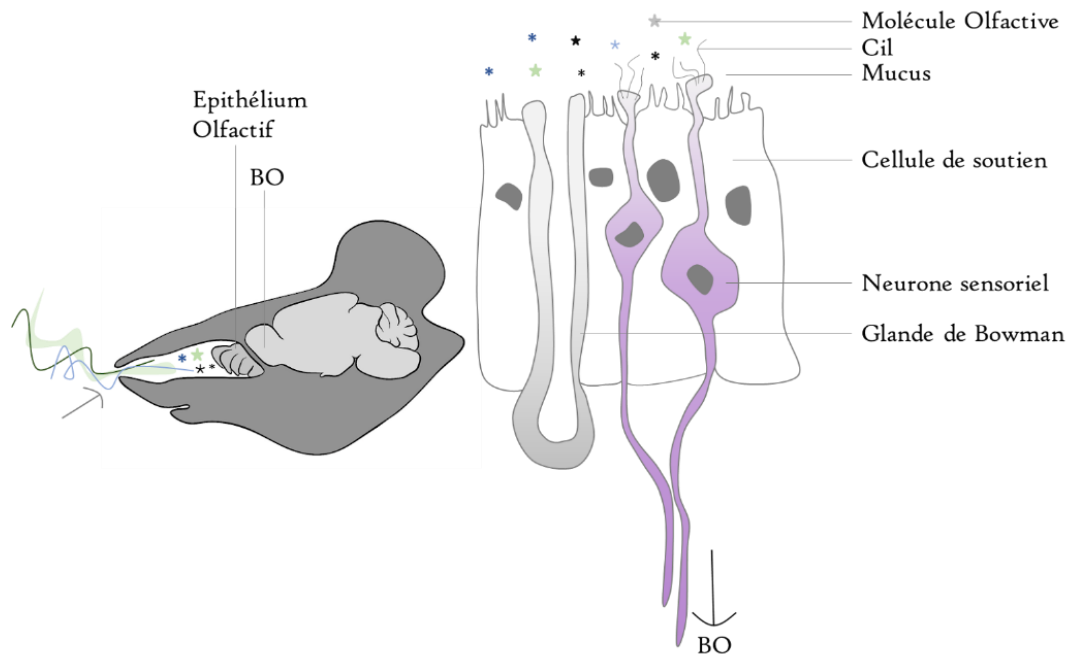


Figure 7. Organisation de l'épithélium olfactif chez la souris. Coupe sagittale d'une tête de rongeur (gauche) montrant la localisation de l'épithélium olfactif au fond de la cavité nasale et le BO. Représentation schématisée de l'épithélium en coupe transversale (droite). Les molécules odorantes (en couleurs) sont transportées par l'air inspiré dans la cavité nasale et interagissent avec les récepteurs olfactifs (non représentés) présents sur les cils des neurones sensoriels olfactifs.

-BO : Bulbe Olfactif-

-D'après (Schwob *et al.*, 2017)-

3.1.2) Le Bulbe Olfactif (BO)

3.1.2 A) Le BO et ses populations de neurones

Le BO est une structure corticale centrale, contrairement à l'épithélium olfactif qui est périphérique et externe au système nerveux central. Situé dans la partie antérieure du cerveau, le BO est constitué de six couches (Kelsch *et al.*, 2010) (Figure 8). Les axones des neurones sensoriels, ou nerfs olfactifs, établissent à leurs terminaisons des synapses excitatrices avec les dendrites apicales des cellules mitrales et à panache (neurones relais du BO) au niveau des glomérules du BO, formant **la couche glomérulaire** ou couche d'entrée (Ennis *et al.*, 2001; Harvey & Heinbockel, 2018). Les neurones sensoriels exprimant le même type de récepteurs convergent vers un nombre restreint de glomérules (Cleland *et al.*, 2007). La couche glomérulaire est composée de 1600 à 1800 glomérules chez la souris, eux-mêmes entourés et régulés par des interneurons GABAergiques et DAergiques de **la couche péri-glomérulaire**, possédant des rôles dans la modulation et le décodage du message d'entrée de l'information olfactive (Linster & Cleland, 2009). Bien que les interneurons DAergiques

ne constituent que 5 % des cellules du BO, ils font de cette structure la principale source de DA dans la partie antérieure du cerveau (Cave & Baker, 2009; Harvey & Heinbockel, 2018).

Les cellules mitrales et à panache, sont les cellules excitatrices de projection du BO et constituent le support de la sortie du message de cette structure. Elles libèrent du glutamate à leurs terminaisons axonales, au niveau des régions cibles où elles projettent. Les cellules mitrales et à panache initient des voies de traitement parallèles dans le système olfactif. Bien que ces cellules reçoivent des signaux similaires, elles répondent différemment, sont impliquées dans des fonctions distinctes et ne projettent pas sur les mêmes structures (Chon et al., 2020; Igarashi et al., 2012; Nagayama et al., 2004, 2010). Dans la suite de ce travail, nous allons nous concentrer sur les mitrales. Deux cellules mitrales voisines se connectent souvent aux mêmes glomérules, établissant des jonctions gap intraglomérulaires en plus des contacts glutamatergiques (Kosaka et al., 2005). L'activité des cellules mitrales est régulée par les interneurons périglomérulaires mais aussi par les cellules granulaires, situées dans la couche granulaire du BO (Harvey & Heinbockel, 2018; H. Takahashi et al., 2018). Enfin, les axones des celles-ci sont fortement ramifiés, permettant à une seule cellule de transmettre l'information à de nombreuses structures corticales olfactives (Nagayama et al., 2010).

La couche granulaire du BO constitue la principale population de neurones inhibiteurs régulateurs du BO, modulant le message de sortie porté par les mitrales vers les centres corticaux supérieurs. Les cellules granulaires sont la population neuronale majoritaire du BO et sont cent fois plus nombreuses que les cellules excitatrices (Parrish-Aungst et al., 2007). Elles sont dépourvus d'axones, possèdent plusieurs dendrites basales plus ou moins ramifiées (Figure 9e), ainsi qu'une unique dendrite apicale qui se ramifie et établit des contacts dendro-dendritiques réciproques avec les dendrites latérales des cellules mitrales, qui les dépolarisent avec le glutamate libéré. En retour, les cellules granulaires inhibent les cellules mitrales par libération de GABA (Shepherd, 1972; Shepherd et al., 2007). La morphologie de l'arborisation dendritique des cellules granulaires chez le rongeur est hétérogène, elle dépend de leurs localisation dans la couche (Nagayama et al., 2014) et de différents facteurs tels que les niveaux de BDNF par exemple (McDole et al., 2015).

C'est principalement au niveau de leurs dendrites basales que les cellules granulaires reçoivent des contacts synaptiques de fibres centrifuges provenant de régions telles que le cortex piriforme (contacts glutamatergiques) ainsi que des systèmes neuromodulateurs, incluant les systèmes noradrénergique, cholinergique et sérotoninergique. Ces contacts permettent de moduler l'activité des interneurons et donc à terme celle du BO (Fletcher & Chen, 2010; Linster & Escanilla, 2019; Nagayama et al., 2010). Le détail des retours centrifuges sur l'ensemble des populations du BO sera décrit dans une prochaine section (3.1.2.C).

Les cellules granulaires sont extrêmement **plastiques**, capables d'adaptations morphologiques et dynamiques, en réponse à une grande variété de stimuli, impactant donc également leur connectivité. Elles jouent un rôle essentiel dans le codage de la perception olfactive en raffinant le message olfactif avant la transmission aux structures corticales secondaires. Les cellules granulaires modulent l'activité du BO de manière spatiale et temporelle via la régulation de l'activité et de la synchronisation des cellules mitrales en fonction du contexte et de notre état interne (Kontaris et al., 2020; Lazarini & Lledo, 2011). En lien avec la notion de code olfactif mentionnée précédemment, ces interneurons amplifient le contraste entre les schémas/patterns pour clarifier l'information (Cleland & Linster, 2005; Mandairon, Didier, et al., 2008). Enfin, cette population régulatrice inhibitrice possède une caractéristique supplémentaire conférant au BO une particularité : elle est la cible d'une neurogenèse continue, qui fait l'objet du paragraphe suivant.

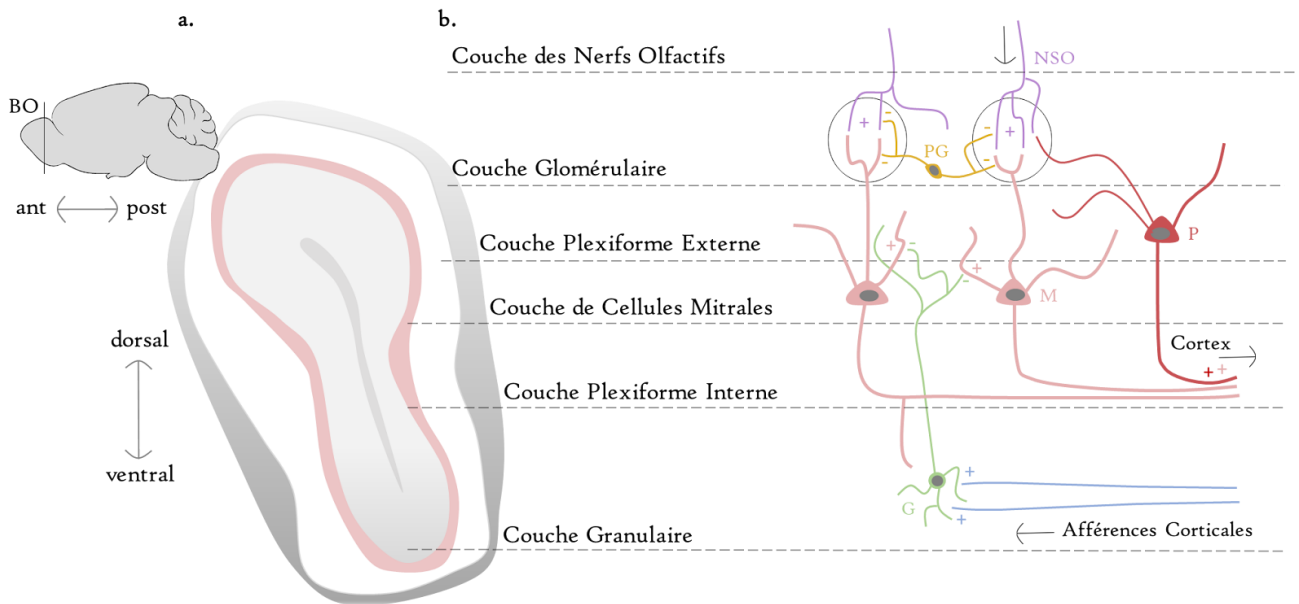


Figure 8. Le BO, son organisation et ses populations de neurones. Représentation schématique des couches du BO en coupe transversale (gauche). Détails de l'organisation cellulaire des couches du BO (droite). Les axones des neurones sensoriels olfactifs (NSO) forment des contacts glutamatergiques excitateurs avec les dendrites apicales des cellules mitrales (M) et à panache (P) au sein d'un glomérule, ainsi qu'avec les dendrites des cellules péri-glomérulaires (PG). Les cellules péri-glomérulaires (PG) modulent l'information à l'entrée du BO, tandis que les cellules granulaires (G) modulent la sortie de l'information en établissant des contacts dendro-dendritiques réciproques avec les dendrites latérales des cellules M.
-BO : Bulbe Olfactif-

3.1.2 B) Le BO, cible d'une neurogenèse postnatale et adulte

La neurogenèse bulbaire se réfère à la capacité du BO à incorporer de nouveaux neurones dès la naissance et tout au long de la vie, lui conférant une grande plasticité chez de nombreux mammifères (Alonso et al., 2024; Imayoshi et al., 2008; Lledo et al., 2006). Ce processus est rendu possible grâce à la Zone Sous-Ventriculaire (ZSV) bordant les ventricules latéraux et abritant des cellules souches. Ces cellules souches donnent naissance à des progéniteurs neuronaux, lesquels se différencient ensuite en divers types cellulaires spécifiques selon les régions de la ZSV dont ils sont issus (Lledo et al., 2008; H. Takahashi et al., 2016, 2018; Tsuboi, 2024). En effet, les cellules produites dans la ZSV, encore immatures -ou neuroblastes- migrent vers le BO en empruntant le flux migratoire rostral pour fournir de nouveaux interneurons matures et intégrés dans les couches péri-glomérulaire et granulaire du BO (Belnoue et al., 2013; Dioli et al., 2021; Grelat et al., 2018; Lazarini et al., 2014; H. Takahashi et al., 2018).

Il est intéressant de préciser que **la période postnatale** (critique), soit les quinze premiers jours chez la souris, est une fenêtre où la neurogenèse est particulièrement intense, puisque 75 % des cellules granulaires du BO sont alors produites. Ce phénomène repose sur une forte prolifération cellulaire et une quasi-absence de mort cellulaire (Lemasson, 2005; Sakamoto, Ieki, et al., 2014). Les cellules granulaires générées lors de cette vie précoce forment les éléments fondamentaux du circuit du BO et sont cruciales dans le traitement des informations olfactives innées (Dejou et al., 2024). **À l'âge adulte**, environ un mois est nécessaire pour qu'un neuroblaste récemment arrivé dans le BO se différencie en une cellule granulaire mature et fonctionnelle (Carleton et al., 2003; Kelsch et al., 2009; Tufo et al.,

2022). Cependant, parmi les cellules arrivant dans le BO, 50 % dégèrent dans des processus apoptotiques 4 à 6 semaines après leur naissance, permettant un raffinement du réseau (Lazarini & Lledo, 2011). En effet, un niveau supplémentaire de méta plasticité définit la nature des interneurons nouvellement formés selon leurs périodes de naissance. En fonction de l'âge de l'animal au moment de la naissance des cellules (stades embryonnaire, néonatal et/ou adulte), les cellules peuvent se différencier en divers sous-types de cellules granulaires (Lemasson, 2005; Sakamoto, Ieki, et al., 2014; Sakamoto, Kageyama, et al., 2014), ainsi que s'intégrer et se connecter différemment dans le BO (Bardy et al., 2010).

Les nouveaux interneurons sont très **plastiques** selon leur niveau de maturité fonctionnelle. Pendant la période d'intégration où les nouvelles cellules granulaires (nées précocement ou plus tard) sont encore immatures, elles sont très sensibles à l'activité du réseau, aux entrées olfactives ainsi qu'à la demande comportementale (Mandairon et al., 2006, 2018; Yamaguchi & Mori, 2005). Elles ont alors un niveau élevé de formation et de rétraction des filopodes sur leurs dendrites, une dynamique qui diminue à mesure qu'elles mûrissent (Breton-Provencher et al., 2014). Le câblage des nouvelles cellules dépend de leur activité et de leur capacité à se connecter de manière dynamique aux éléments présynaptiques (Lepousez et al., 2015). Néanmoins, les neurones nés à l'âge adulte adoptent une phase d'écoute active avant de participer pleinement à l'activité du réseau tandis que les interneurons formés précocement établissent directement des synapses bidirectionnelles et émettent des inputs rapidement (Figure 9d). Enfin, les systèmes neuromodulateurs qui se développent tôt dans la vie et innervent le BO, jouent un rôle important dans la survie, la maturation et l'intégration des nouvelles cellules, selon l'âge où elles ont été produites (Arenkiel et al., 2011; De La Rosa-Prieto et al., 2015; Dejoux et al., 2024; Kostka & Bitzenhofer, 2022). Une perturbation précoce de leurs connexions au BO pourrait donc altérer l'intégration des cellules granulaires formées durant cette période critique.

Pour quantifier le nombre de nouveaux interneurons formés précocement ou à l'âge adulte, une méthode classique consiste à injecter de la Bromodésoxyuridine (BrdU), analogue de la thymidine, qui prend sa place lors de la synthèse de l'ADN au moment de la division cellulaire. Les animaux peuvent ensuite être sacrifiés quelques heures ou semaines après l'injection pour mesurer respectivement le taux de prolifération des cellules souches dans la ZSV et la survie des cellules ayant migrées au sein du BO (Figure 9b).

En conclusion, les nouveaux interneurons -postnatals ou adultes- présentent une excitabilité cellulaire plus élevée (Nissant et al., 2009), une connectivité dynamique et une plasticité morphologique et fonctionnelle intenses. Ils possèdent des propriétés uniques selon leurs âges de naissance et également par rapport aux cellules préexistantes, faisant qu'ils ne sont pas impliqués dans les mêmes réponses et/ou tâches olfactives (Forest et al., 2019; Grelat et al., 2018; Hardy & Saghatelian, 2017; Lepousez et al., 2014; Sakamoto, Ieki, et al., 2014; Sakamoto, Kageyama, et al., 2014).

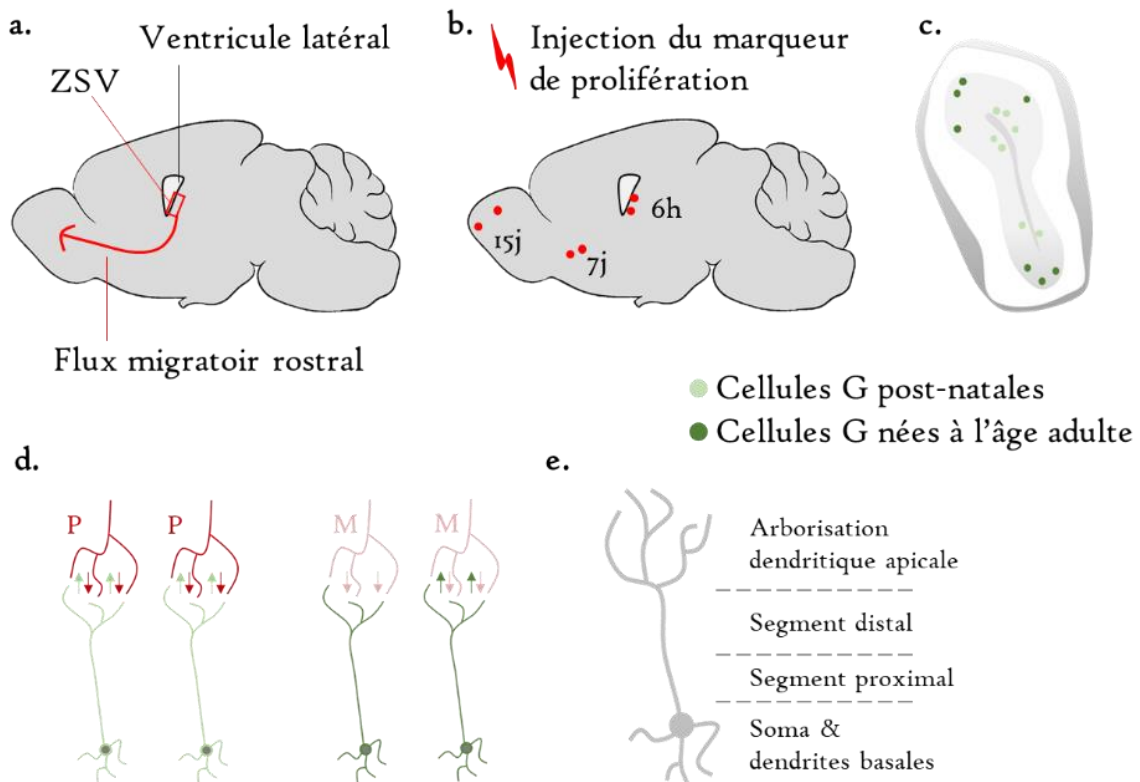


Figure 9. La production de nouvelles cellules granulaires. (a.) Schéma de la neurogenèse depuis la prolifération cellulaire dans la ZSV jusqu'à la migration par le flux migratoire rostral dans le BO. (b.) Schéma du marquage des différentes étapes de la neurogenèse avec injection d'un marqueur. (c.) Coupe transversale du BO où s'intègrent les cellules nouvellement formées selon le moment de leur naissance. Les cellules nées précocement intègrent la partie profonde de la couche granulaire tandis que les cellules nées à l'âge adulte alimentent la couche plutôt superficielle. (d.) Les cellules nées précocement sont hyperactives et établissent simultanément des synapses afférentes excitatrices et efférentes inhibitrices pour communiquer de manière bidirectionnelle avec les cellules mitrales et à panache (P) préférentiellement. Les cellules granulaires nées à l'âge adulte "écoutent avant de parler" et mettent plus de temps à développer des synapses efférentes inhibitrices qui communiquent avec les cellules à panache et les cellules mitrales (M) préférentiellement. (e.) Schéma de l'organisation structurale de la cellule granulaire.

-G : Granulaires ; ZSV : Zone Sous Ventriculaire-
-Inspirée de (Dejou et al., 2024; Lepousez et al., 2015)-

En terme de **fonctionnement**, une fois intégrés et matures, les nouveaux interneurons modulent l'activité excitatrice des cellules mitrales, et par conséquent celle du BO, en fonction des informations qu'ils reçoivent du réseau interne et des afférences centrifuges (Chow et al., 2012; Lepousez et al., 2013). Le recrutement de nouveaux neurones peut permettre le remplacement d'une population lésée, qui après une intégration fonctionnelle, promeut une restauration partielle des fonctions olfactives altérées (Lazarini et al., 2014). Au-delà des situations de lésion ou de déficit, la neurogenèse permet au BO de tirer parti de son hyper-plasticité pour adapter l'organisation de son réseau fonctionnel selon l'environnement, très imprédictible dans le monde des odeurs (Denizet et al., 2017). Les neurones issus de l'ontogenèse sont plus stables que ceux nouvellement formés, qui constituent donc une population plastique et dynamique, servant de substrat riche à l'apprentissage adaptatif. De nombreuses études ont

ainsi démontré l'implication de la neurogenèse bulbaire adulte dans divers phénomènes d'apprentissage olfactif associatif, perceptif ou de mémoire olfactive, et inversement les effets de ces apprentissages sur les caractéristiques de la neurogenèse (Forest et al., 2019; Grelat et al., 2018; Mandairon et al., 2018; Moreno et al., 2009; Sultan et al., 2010). À noter que le rôle des nouveaux interneurons varie selon la complexité de la tâche et le type d'apprentissage (Forest et al., 2019; Grelat et al., 2018; Mandairon et al., 2011; Moreno et al., 2009; Mouret et al., 2008; Sultan et al., 2010). De manière importante, les apprentissages ne sont pas les seuls à moduler la survie, la morphologie et l'intégration synaptique des nouveaux neurones (Daroles et al., 2016; Forest et al., 2019; Mandairon et al., 2018). En effet, leur sensibilité les rend vulnérables aux événements négatifs tels que le stress, pouvant donc impacter largement la plasticité du BO et de son réseau (Alonso et al., 2024).

Les nouveaux interneurons sont donc particulièrement plastiques et **vulnérables**, et l'altération de l'une des étapes de la neurogenèse peut détériorer le traitement de l'information olfactive.

Un stress chronique prolongé réduit par exemple le nombre de progéniteurs et de neuroblastes dans la ZSV et de nouveaux neurones dans le BO (Dioli et al., 2021). Un modèle d'injection chronique de corticostérone induit des déficits de la discrimination et de la mémoire olfactive associés à une réduction du taux de survie des nouveaux interneurons dans le BO (Siopi et al., 2016). Enfin, lors d'une étude parallèle à ma thèse, nous avons montré que le stress chronique induit une diminution de la densité de nouveaux neurones dans la couche granulaire du BO, ainsi qu'une réduction de la proportion de ces nouveaux neurones répondant aux odeurs plaisantes. Ces altérations semblent sous-tendre une anhédonie olfactive caractérisée par une diminution d'intérêt pour les odorants habituellement perçus comme agréables, donc attractifs (Athanassi et al., 2023).

Concernant **le stress précoce** plus précisément, la neurogenèse adulte dans l'hippocampe, une autre région cérébrale concernée par ce processus, y est sensible (Belnoue et al., 2013; Jorgensen, 2018; Loi et al., 2014, 2017; Naninck et al., 2015; Oomen et al., 2010). En revanche, l'impact du stress précoce sur la neurogenèse du BO est à ce jour bien moins compris. Des données suggèrent que la neurogenèse bulbaire pourrait être résistante face au stress prénatal (Belnoue et al., 2013), cependant, l'impact du stress précoce postnatal sur la neurogenèse bulbaire reste encore méconnu.

3.1.2.C) Le BO et ses retours centrifuges

Le BO est régulé et soumis à des neuromodulations extrinsèques des systèmes cholinergique, noradrénergique et sérotoninergique (Figure 10), tandis que l'existence de projections dopaminergiques directes reste un sujet de débat (Brunert & Rothermel, 2021; Corthell et al., 2013; Harvey & Heinbockel, 2018).

Les neurones cholinergiques en provenance de la bande diagonale de l'aire de Broca innervent principalement les couches superficielles du BO. L'activation par l'acétylcholine (ACh) des récepteurs nicotiniques et muscariniques renforce l'inhibition des cellules mitrales par les interneurons périglomérulaires, et dans une moindre mesure, par les cellules granulaires. Ceci participe à la régulation fine des mécanismes de la perception olfactive en modulant les signaux sensoriels au sein du BO (Böhm et al., 2020; Harvey & Heinbockel, 2018; Schilit Nitenson et al., 2019), en jouant sur les capacités de discrimination ainsi que sur les mécanismes d'apprentissage et de la mémoire olfactive (Brunert & Rothermel, 2021).

Le système noradrénergique module les comportements, l'humeur, l'attention, l'excitation et l'état émotionnel des individus (Devore & Linster, 2012; Linster, 2019; Linster et al., 2020). Le noyau principal de ce système est le LC, situé dans le tronc cérébral et innervant massivement le BO (Brunert & Rothermel, 2021). La NA se fixe majoritairement sur les cellules de la couche granulaire qui régulent les mitrales. Cette régulation est essentielle pour la détection, l'apprentissage et la mémorisation des stimuli olfactifs et leur stabilité -qu'ils soient perceptifs ou associatifs- ainsi que pour l'apprentissage

social des odeurs, leur discrimination et l'attribution de valeur hédonique à de nouvelles odeurs apprises (Czarnabay et al., 2019; Escanilla et al., 2010; Linster, 2019; Linster et al., 2011, 2020; Mandairon, Peace, et al., 2008; Manella et al., 2013; Moreno et al., 2012; Ramirez-Gordillo et al., 2018; Vinera et al., 2015). La NA libérée dans le BO influence sa circuiterie et les comportements motivés et guidés par les odeurs, avec des spécificités selon le caractère associatif ou non de la tâche.

La sérotonine est importante dans la régulation des humeurs, tandis que son implication dans les comportements olfactifs est moins connue. Le BO est néanmoins modulé par les prolongements sérotoninergiques venant du noyau du Raphé dorsal et médian, selon le type cellulaire contacté. Le Raphé dorsal projette sur les couches des cellules mitrales et granulaires, tandis que le Raphé médian cible plutôt la couche glomérulaire (Muzerelle et al., 2016; Petzold et al., 2009; Siopi et al., 2016). L'impact de la sérotonine sur le comportement olfactif reste ambigu, avec des effets variables notamment sur les capacités de discrimination olfactive (Carlson et al., 2016; Harvey & Heinbockel, 2018).

En conclusion, le traitement de l'information olfactive par le BO dépend de l'action de ces systèmes neuromodulateurs, qui sont en quelque sorte complémentaires. L'ACh et la NA modulent par exemple ensemble les cellules granulaires : l'ACh améliore la synchronisation des réponses odorantes et la NA optimise le rapport signal/bruit (Li et al., 2015). Ces systèmes neuromodulateurs influencent donc la précision et l'efficacité des réponses neuronales aux stimuli olfactifs en modifiant l'activité du BO ou certaines de ses caractéristiques telles que la neurogenèse (Rikani et al., 2013; Schilit Nitenson et al., 2019), jouant ainsi un rôle crucial dans le fonctionnement global du système olfactif.

Fait intéressant, des dégradations des systèmes notamment sérotoninergique et noradrénergique sont relevées dans **des troubles psychiatriques** (Blokhin et al., 2020), dont la dépression (Del Cerro et al., 2020; Du et al., 2020; Fakhfouri & Rahimian, 2022; Maturana-Quijada et al., 2024; Rajan et al., 2019). D'autre part, il est connu que le LC et le système sérotoninergique sont sensibles aux effets d'un **stress précoce** ce qui participe aux conditions psychiatriques associées (Adjimann et al., 2021; Sheppard et al., 2024). Le stress précoce pourrait, à travers une atteinte de ces systèmes régulateurs, perturber l'encodage des informations olfactives par le BO et donc le comportement olfactif associé. Ceci additionné aux effets directs du stress précoce sur les réseaux sous-tendant le comportement émotionnel pourrait expliquer les répercussions sur l'affect, l'humeur et la psychologie.

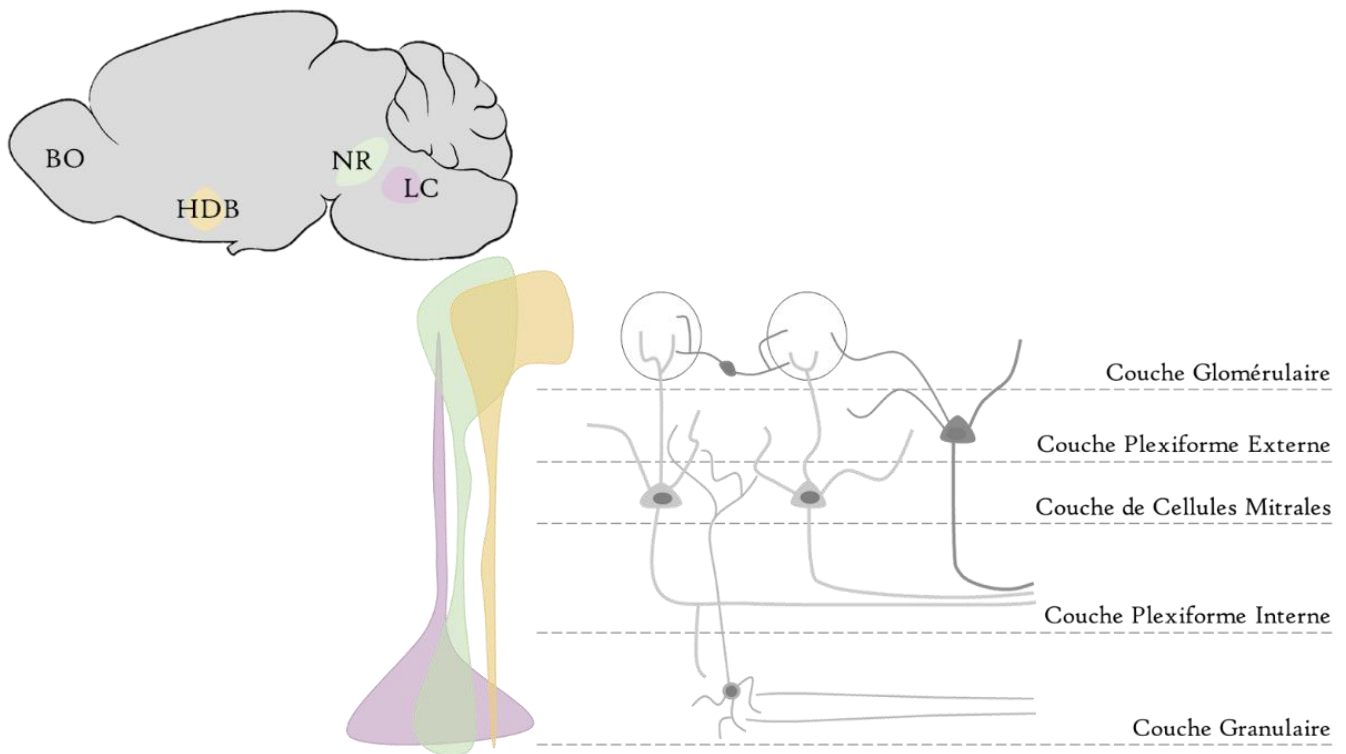


Figure 10. Le BO et ses retours centrifuges. Les formes des différentes couleurs représentent la densité de fibres innervant et modulant les couches du BO (faible épaisseur : faible innervation ; forte épaisseur : forte innervation). Les afférences noradrénergiques (en violet) viennent du LC et ciblent majoritairement la couche granulaire du BO. Les fibres sérotoninergiques (en vert) issues du NR innervent préférentiellement la couche glomérulaire. Enfin, les afférences cholinergiques (jaune) proviennent de l'HDB et projettent majoritairement dans les couches les plus superficielles du BO.

-BO : Bulbe Olfactif ; HDB : bande diagonale de l'aire de Broca ; LC : Locus Cœruleus ; NR : Noyau du Raphé-

-Inspirée de (Brunert & Rothermel, 2021)-

Le BO est le premier relai cortical dans le traitement des odeurs et une structure clé du système olfactif. Il possède des caractéristiques uniques telles que la neurogenèse adulte, le rendant très plastique et capable de modifier dynamiquement sa circuiterie selon l'environnement. Nous avons suivi le traitement de l'information olfactive à travers les différentes couches du BO, dont celle des cellules mitrales, qui relayent l'information vers des structures olfactives supérieures. Celles-ci composent la suite du système olfactif, qui s'entrelace avec celui des systèmes limbique et de la récompense, comme nous allons le voir dans la prochaine partie.

3.1.3) Systèmes olfactif, des émotions et de la récompense

L'information olfactive, contrairement aux autres modalités sensorielles, au lieu d'être préalablement intégrée au niveau thalamique, est directement envoyée aux centres corticaux faisant partie du système limbique et impliqués dans la régulation des émotions, de la mémoire et de la récompense (Landis et al., 2005; Shipley & Ennis, 1996; Soudry et al., 2011). Ceci renforce la notion selon laquelle l'olfaction est directement reliée aux émotions. Les cellules mitrales regroupent leurs

axones en un tractus olfactif latéral innervant ensuite différentes régions. Certaines structures cérébrales telles que le noyau olfactif antérieur, le cortex piriforme, le TO, le cortex entorhinal et une partie de l'amygdale, reçoivent ainsi des projections directes du BO (*Lane et al., 2020*). En retour, ces régions projettent sur le BO, communiquent entre elles et contactent d'autres structures pour poursuivre le traitement de l'information. C'est le circuit de l'information olfactive à travers les structures olfactives supérieures et appartenant au système limbique et de la récompense que nous allons maintenant détailler.

Le cortex piriforme est postérieur au BO et dorsal au TO. C'est la structure la plus importante du système olfactif en termes de taille, la plus étudiée -avec le BO- et celle qui reçoit le plus de ses projections (*Nagayama et al., 2010*). Les dendrites des cellules pyramidales du cortex piriforme établissent des synapses avec les axones des cellules mitrales, majoritairement dans sa partie antérieure, et intègrent l'information avant de la distribuer à l'ensemble du cortex via leurs axones collatéraux (*Ghosh et al., 2011; Iurilli & Datta, 2017*). La partie postérieure du cortex piriforme reçoit donc des projections de la partie antérieure, mais aussi d'autres régions cérébrales telles que le CPF, l'amygdale et le cortex entorhinal (*Johnson et al., 2000; W. Yang & Sun, 2015*) sur lesquelles elle projette également (*Chen et al., 2014*). Son activité est modulée de manière interne par des interneurons GABAergiques et par les systèmes neuromodulateurs, cholinergique, sérotoninergique, noradrénergique, et DAergique en provenance de l'ATV. Ces systèmes influencent son activité selon le type de cellules, de récepteurs, et leurs localisations au sein du cortex (*Linster & Cleland, 2016*). Au niveau **fonctionnel**, le cortex piriforme antérieur est impliqué dans la détection des odorants, le codage de leur identité chimique, de leur valence et de leur intensité (*Gottfried et al., 2006; Penker et al., 2020; Srinivasan & Stevens, 2018; Wilson & Sullivan, 2011*), tandis que sa partie postérieure joue plutôt un rôle dans des fonctions associatives telles que l'apprentissage, la mémoire et la catégorisation des odeurs, faisant écho à son hétérogénéité structurelle (*Chen et al., 2014; Choi et al., 2011; Gottfried et al., 2006; Howard et al., 2009; Johnson et al., 2000*).

Le noyau olfactif antérieur, postérieur au BO et antérieur au cortex piriforme, est très hétérogène et complexe en termes d'organisation, de morphologie cellulaire et de phénotype chimique (*Brunert et al., 2023; Brunjes et al., 2005*). C'est une région fortement interconnectée avec le BO et d'autres structures olfactives, mais aussi avec des régions non olfactives -du système limbique et/ou de la récompense- ce qui l'implique dans de nombreuses fonctions (*Brunert et al., 2023*). Le noyau olfactif antérieur était considéré par le passé comme un simple relai entre deux structures olfactives voisines, mais il serait en réalité impliqué dans des tâches bien plus complexes et diversifiées comme la communication et la reconnaissance sociale, la mémoire olfactive et contextuelle, ainsi que la modulation et le filtrage sensoriel. De plus, il jouerait un rôle dans la communication interhémisphérique (*Brunert et al., 2023*), la localisation spatiale des odeurs (*Kikuta et al., 2010; Rabell et al., 2017*), l'association d'un stimulus olfactif avec son contexte multisensoriel -soit l'intégration multimodale des signaux olfactifs (*Levinson et al., 2020*)- ou encore le codage temporel de la présentation de l'odeur (*Tsuji et al., 2019*).

Le cortex entorhinal au niveau du lobe temporal médian est connecté à l'amygdale et à l'hippocampe. Ses connexions lui confèrent un rôle dans l'encodage des souvenirs olfactifs, la mémoire et la navigation spatiale (*Knierim et al., 2014; Nilssen et al., 2019; Soudry et al., 2011*). Il est également contacté par le BO et le cortex piriforme sur lesquels il projette en retour, l'impliquant dans des fonctions telles que la discrimination olfactive (*Chapuis et al., 2013*). Au carrefour entre les systèmes olfactif et

de la mémoire, le cortex entorhinal constitue une voie du traitement mnésique de l'information olfactive (Igarashi et al., 2014).

L'une des cibles importantes des projections du BO est le **TO**, que nous allons examiner en détail. **Chez le rongeur**, le TO est postéro ventral au BO -au niveau du prosencéphale basal- et est positionné ventralement au pallidum ventral. Avec le noyau accumbens, il fait partie du striatum ventral, ce qui en fait un carrefour entre le système olfactif et de la récompense (Midroit et al., 2021; Millhouse & Heimer, 1984; Wesson & Wilson, 2011; Xiong & Wesson, 2016; Yamaguchi, 2024). Le TO a une organisation particulière et est composé de plusieurs types cellulaires. Il contient de petits amas cellulaires denses ou « Îlots de Calleja », constitués de petits interneurons granulaires GABAergiques, formés pendant la période postnatale précoce (Hsieh & Puche, 2013). Ils interviennent dans la régulation locale de l'activité du TO. La population cellulaire majeure du TO est représentée par les *medium spiny neurons* ou neurones de projections GABAergiques épineux qui ont pour marqueur spécifique DARP-32 (Cansler et al., 2020; Xiong & Wesson, 2016). Ces neurones ont la particularité d'exprimer à leurs membranes deux types de récepteurs à la DA (D1 et D2) et ont donc leur activité régulée par celle-ci (Bertran-Gonzalez et al., 2008; Le Moine & Bloch, 1995; Z. Zhang, Zhang, et al., 2017). Ils projettent sur diverses structures cérébrales telles que le striatum ventral (et *vice-versa*), le rendant important pour la régulation comportementale sur le plan motivationnel, l'ATV, qui lui envoie en retour ses projections DAergiques, ou encore le COF (Barbas, 1993; Illig, 2005; Millhouse & Heimer, 1984; Zhang, Liu, et al., 2017). Enfin, une étude récente a révélé un fait intrigant : les projections du TO vers le BO sont en réalité peu nombreuses, ce qui soulève des interrogations sur le rôle exact du premier dans la régulation du second (Zandt et al., 2019). Les cellules du BO innervent les régions du TO de manière spécifique selon leur origine, suggérant des traitements singuliers de l'information olfactive (Imamura et al., 2011; Wesson & Wilson, 2011). Le TO reçoit également des afférences de différents noyaux de l'amygdale (Usunoff et al., 2009), de l'hypothalamus (Vertes & Hoover, 2008) et de l'ATV (Midroit et al., 2021; Zhang, Liu, et al., 2017) et de façon importante, des projections directes du cortex piriforme.

Sur le plan fonctionnel, le TO -de par sa localisation et ses connexions- est impliqué dans les comportements addictifs, le traitement de l'information relative aux récompenses innées et conditionnées ainsi que dans les comportements motivés (Carlson et al., 2016; Ikemoto, 2003; Striano et al., 2014). Des travaux d'IRMf **chez l'humain** soulignent son importance dans la détermination de la source odorante ainsi que dans les processus d'attention olfactive (Zelano et al., 2005, 2007). Globalement, il joue un rôle clé dans l'encodage de la récompense ou de la punition associée à l'odeur, de la préférence olfactive et de la valeur hédonique des odeurs, que nous détaillerons plus tard (Midroit et al., 2021; Zhang, Liu, et al., 2017). Il est important de noter que les sous-régions du TO n'ont pas les mêmes implications fonctionnelles. Le TO médian semble par exemple plus impliqué dans l'addiction, la récompense et notamment celle associée aux odeurs par rapport au TO latéral (Murata et al., 2015). La place du TO et son organisation lui donne donc un rôle central dans l'intégration de composantes à la fois émotionnelles et sensorielles pour produire une réponse comportementale adaptée à un/des stimulus/i olfactif/s.

J'aimerais finir le paragraphe sur cette structure en évoquant une étude intéressante qui a été faite récemment. Même si le comportement olfactif n'a pas été étudié, (Zhang et al., 2023) ont révélé **une implication du TO dans l'occurrence de symptômes dits dépressifs chez la souris** mesurés via l'utilisation de l'*open-field*, la *light-dark box*, le test de préférence au sucre, la nage forcée et la suspension par la queue (Zhang et al., 2023). Ces symptômes étaient liés au défaut d'excitabilité et d'activation des petits interneurons granulaires du TO exprimant le récepteur D3 à la DA. Les auteurs ont ainsi présenté un modèle quant à l'implication du TO dans le trouble dépressif suivant un stress chronique (Figure 11). Cela renforce l'importance du TO dans l'entretien de la relation existant entre le système olfactif et émotionnel.

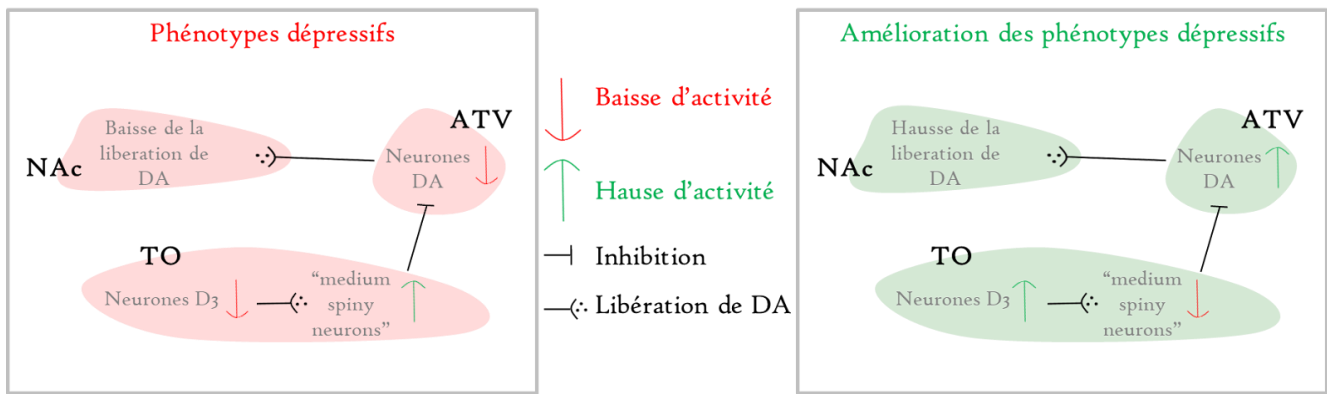


Figure 11. Modèle de -(Zhang et al., 2023)- **du circuit neuronal qui pourrait expliquer les comportements associés aux troubles affectifs chez la souris.** « Les neurones D3 du TO inhibent directement les medium spiny neurons du TO, qui, à leur tour, inhibent les neurones DAergiques de l'ATV responsables de la libération de DA dans le noyau accumbens ».

-ATV : Aire Tegmentale Ventrale ; DA : Dopamine ; NAc : Noyau Accumbens ; TO : Tubercule Olfactif-

Enfin, **l'amygdale** est située dans la partie médiale du lobe temporal antérieur et appartient au système limbique. Elle participe à la régulation des émotions et au traitement de l'information olfactive (Croy et al., 2010). L'amygdale est organisée en différents noyaux : l'amygdale basolatérale, centro-médiane et corticale. Cette dernière est la cible du BO par l'intermédiaire d'une seule synapse, supportant la saillance émotionnelle des stimuli olfactifs (Soudry et al., 2011). De manière intéressante, les projections du BO vers l'amygdale corticale semblent avoir une organisation topographique contrairement à celles envoyées vers le cortex piriforme. Ce phénomène pourrait supporter au moins en partie l'implication de l'amygdale dans les comportements innés médiés par l'odeur tandis que le cortex piriforme semble davantage recruté dans les processus d'apprentissage (Iurilli & Datta, 2017; Root et al., 2014). Il est donc établi que l'amygdale permet le traitement de la composante émotionnelle du stimulus sensoriel et participe à la régulation des émotions négatives, dont la peur, ressentie notamment après un processus de conditionnement à l'odeur (Baxter & Murray, 2002; Sevelinges et al., 2004).

Une partie du recouvrement existant entre le système olfactif, limbique et de la récompense a été détaillée (Figure 12). Nous allons maintenant aborder **ce système de la récompense** un peu plus en détails. Le sens premier d'une récompense est un stimulus qui provoque et/ou renforce le comportement d'approche (Schultz, 1997; Schultz et al., 1997). Le principal substrat de ce système et donc du traitement de récompenses naturelles et/ou conditionnées est la DA, une catécholamine produite notamment dans l'ATV, une structure composée à plus de 60 % de neurones DAergiques et localisée dans le mésencéphale (Baik, 2020; Belujon & Grace, 2017; Schultz, 2016). Les neurones de l'ATV produisant la DA sont reconnaissables grâce à la présence de la tyrosine hydroxylase (TH), une enzyme responsable de sa synthèse. Une modification dans la libération de DA peut modifier la valeur récompensante du stimulus et donc le comportement produit.

Plus précisément, les régions du système de la récompense forment deux voies principales. La première est **la voie mésolimbique** avec les projections des neurones DAergiques de l'ATV sur le striatum ventral (Fallon & Moore, 1978; Ikemoto, 2007, 2010; Swanson, 1982; Zhang, Liu, et al., 2017). **Le striatum ventral** est une région des ganglions de la base -composée du TO et du noyau accumbens-séparés anatomiquement par le pallidum ventral (Wesson & Wilson, 2011). Ces structures sont

composées des *medium spiny neurons*, reçoivent des afférences également en provenance du CPF, du TO, de l'hippocampe et de l'amygdale, et sont régulées par les systèmes neuromodulateurs cités plus en amont ainsi que par leurs propres interneurons (Mahler et al., 2014; Meredith et al., 1993; Pennartz et al., 1992; Smith et al., 2009; Wesson & Wilson, 2011).

La deuxième est **la voie méso-corticale** avec les projections des neurones DAergiques de l'ATV sur le CPF, dont le COF, sa région orbitale (Björklund & Dunnett, 2007; Fallon & Moore, 1978; Ikemoto, 2010; Swanson, 1982). Le COF est très connecté au striatum et au pallidum ventral (Haber, 2011) et reçoit également de l'information olfactive directement du cortex piriforme (Mori, 2014). Ceci fait qu'il est engagé dans de nombreux aspects de la perception olfactive comme le traitement de la valeur hédonique et du degré de familiarité des odeurs, les paradigmes d'apprentissage olfactif ou encore la mémoire olfactive (Banerjee et al., 2020; Izquierdo, 2017; Kepler et al., 2022; E. Rolls, 2008; E. T. Rolls, 2004; E. T. Rolls & Grabenhorst, 2008; Royet et al., 2001; B. A. Wang et al., 2023).

En conclusion, ces deux réseaux forment le circuit méso-cortico-limbique, plus connu sous le nom de circuit de la récompense. Ces structures sont, chez l'humain et l'animal, centrales dans le ressenti d'une sensation de plaisir (S. Becker et al., 2019). Elles sont impliquées dans l'encodage des stimuli récompensants, de la motivation, de l'addiction et de l'aversion, en passant par le traitement de la valeur hédonique, la saillance et la pertinence d'un stimulus, sa nouveauté et/ou sa familiarité ainsi que l'éveil qu'il suscite ou encore la prise de décision associée (Bromberg-Martin et al., 2010; Haber, 2011; Ikemoto, 2010; Smith et al., 2009). Les patterns d'activité de ces structures, notamment de l'ATV, peuvent être perturbés après un stress (Baik, 2020). Une perturbation à long terme de ces circuits via le stress précoce est ainsi susceptible de supporter des altérations comportementales, dont l'anhédonie, qui marque les troubles affectifs tels que la dépression (Nestler & Carlezon, 2006).

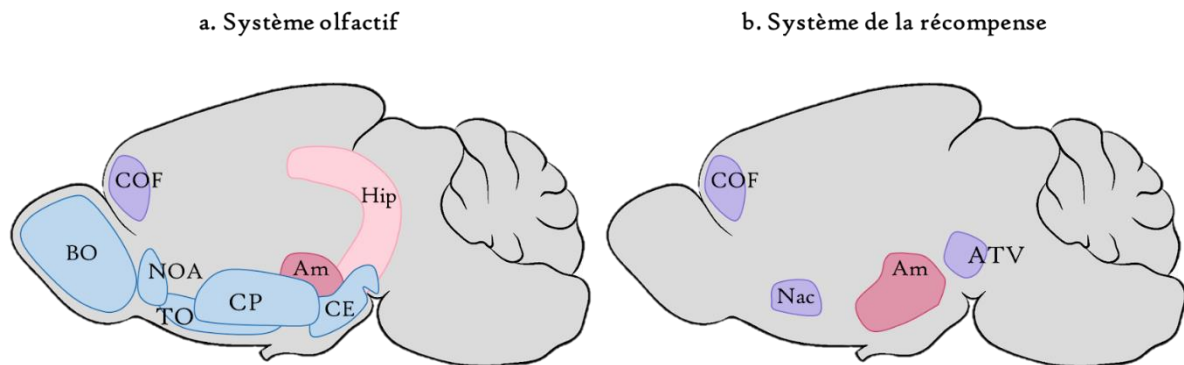


Figure 12. Représentation schématique des systèmes (a.) olfactif et (b.) de la récompense du rongeur en vue latérale. Une fois que les molécules olfactives sont entrées dans la cavité nasale et que l'information a été transmise au BO, cette information est envoyée vers les cibles primaires olfactives (bleu) via les cellules mitrales et les cellules à panache. Enfin, les cibles primaires sont connectées à d'autres structures (rose, violet) qui traitent la composante émotionnelle, mnésique, ou encore de récompense des stimuli.

-Am : Amygdale ; ATV : Aire Tegmentale Ventrale ; BO : Bulbe Olfactif ; CE : Cortex Entorhinal ; COF : Cortex Orbitofrontal ; CP : Cortex Piriforme ; Hi : Hippocampe ; Nac : Noyau Accumbens ; NOA : Noyau Olfactif Antérieur ; TO : Tubercule Olfactif-

Dans cette section, l'encodage de l'information olfactive a été décrit au-delà du BO, dans la suite du réseau olfactif, en examinant notamment les structures essentielles pour la modulation des comportements motivés et des émotions. Ce système olfactif complexe permet l'accomplissement de diverses tâches reposant sur une grande variété de fonctions olfactives. Dans ce travail de thèse, l'une d'entre elles nous intéresse particulièrement : c'est la perception de la valeur hédonique des odeurs, que nous allons décrire à présent et qui est à l'image du chapitre précédent, entre olfaction et émotion.

3.2) *Les secrets du plaisir olfactif : fonctions, enjeux et mécanismes neuraux*

La perception olfactive repose sur une multitude de fonctions distinctes qui façonnent ensemble notre expérience des odeurs. Ces fonctions sont recrutées pour générer une réponse comportementale appropriée face à un ou plusieurs stimulus/i sensoriel/s dans un contexte donné. **La détection olfactive**, en tant que première étape, correspond au seuil minimal nécessaire pour percevoir une odeur et est dépendante de l'intensité et de l'interaction entre les molécules odorantes et les récepteurs olfactifs, influencée par le ratio et l'affinité ligand/récepteur. **La discrimination olfactive** désigne la capacité de différencier deux ou plusieurs odorants, même lorsqu'ils sont perceptuellement proches. **L'identification** consiste à reconnaître et nommer les odeurs spécifiques (Marin et al., 2023). Il existe d'autres dimensions de la perception olfactive telles que l'évaluation de la comestibilité d'une substance odorante, l'appréciation de la familiarité d'une odeur, sa mémorisation ou encore l'attribution d'une valeur hédonique. Nous ne traiterons pas de l'ensemble des fonctions impliquées dans la perception olfactive mais nous nous concentrerons sur **la perception hédonique des odeurs**, un concept central dans ce travail de thèse.

Mais avant toute chose, qu'est-ce que l'hédonisme exactement ? À l'origine, c'est une philosophie qui cherche à maximiser le plaisir dans la vie humaine et repose sur l'idée que les expériences plaisantes sont intrinsèquement désirables. La quête du bien-être devient alors la principale motivation de nos actions. Dans le contexte olfactif, l'idée selon laquelle la propriété hédonique des odeurs -c'est-à-dire le plaisir qu'elles procurent- influence la pensée, la créativité ou encore la mémoire, est ancienne. Des philosophes tels que Voltaire, Descartes ou encore Rousseau (XVI^e – XVII^e siècles) croyaient fermement au lien unissant l'olfaction et les émotions. Après eux, de nombreuses figures célèbres considéraient les odeurs, ou une odeur particulière, comme une source d'inspiration pour se concentrer, imaginer, ou être créatif, soulignant la facilité avec laquelle une odeur peut être associée à un sentiment ou une émotion (Philpott et al., 2008).

En conclusion, la perception hédonique des odeurs se réfère à la manière dont les odeurs sont attirantes et provoquent du plaisir, ou au contraire sont répulsives et induisent du déplaisir (S. Becker et al., 2019). Cette fonction a bien une place centrale dans la perception olfactive puisqu'elle détermine et module un large éventail de comportements et d'états affectifs aussi bien chez l'humain que chez l'animal (Kepler et al., 2022; Kermen et al., 2021; Yeshurun & Sobel, 2010).

Chez l'humain, la valeur hédonique d'une odeur a une importance capitale (Khan et al., 2007), et est la première caractéristique utilisée pour sa description (Yeshurun & Sobel, 2010). Elle contribue à la détection de dangers tels que la fumée, un poison ou la nourriture avariée (Walliczek-Dworschak & Hummel, 2017). La perception de certains odorants peut s'apparenter purement à une expérience de plaisir associée à un contexte particulier où la valeur hédonique serait apprise. Il peut s'agir de l'odeur de gâteaux préparés par notre grand-mère lorsque nous étions petits, comme le décrivait Marcel Proust dans son livre *Du côté de chez Swann* (1975), ou encore le bien-être procuré lorsque l'on sent son bébé, son partenaire ou encore sa maison. La théorie principale suggère donc que les odeurs acquièrent leur valeur hédonique à travers les expériences émotionnelles auxquelles elles sont liées, influençant nos

préférences à long terme. Les odeurs sont initialement neutres et leur appréciation positive ou négative change selon l'expérience personnelle, nos états internes, la culture et le contexte (*Delplanque et al., 2008, 2017; Ferdenzi et al., 2020; Rouby et al., 2009*). Bien que qu'elle soit matière à débat, une autre théorie propose que nous soyons prédisposés à aimer ou détester certaines odeurs. Les odeurs pourraient ainsi avoir une valeur hédonique innée modifiable à travers certains apprentissages (*Herz, 2004; Herz et al., 2004; Mandairon et al., 2009; Qiu et al., 2021; Root et al., 2014*).

Dans un contexte expérimental, mesurer la valeur hédonique des odorants chez l'humain se fait à l'aide de questionnaire « l'odeur perçue est-elle agréable ou désagréable ? » accompagnée de l'échelle correspondante (*Ferdenzi et al., 2017; Mandairon et al., 2009*). Cette mesure peut aussi être comportementale puisque le caractère plaisant ou déplaisant d'une odeur est lié à son aspect attractif ou répulsif, se traduisant par des vitesses d'approche différentes de la source odorante ou encore des mouvements de recul (*Chalençon et al., 2022*). Des mesures des caractéristiques de sniff peuvent également être faites, ce qui a montré qu'une odeur plaisante est associée à un sniff plus important en volume et en durée par rapport à une odeur déplaisante (*Bensafi et al., 2003, 2007; Ferdenzi et al., 2015*). Enfin, lorsque perçues comme extrêmement positives ou négatives, les odeurs peuvent induire des modifications physiologiques mesurables au niveau de la peau ou du rythme cardiaque (*Bensafi et al., 2002; Richard Ortegón et al., 2022; Seet et al., 2020*).

Chez le rongeur, la perception hédonique olfactive est souvent liée aux besoins fondamentaux et à la survie. Pour de nombreuses espèces, les odeurs sont cruciales dans la recherche de nourriture, la sélection des partenaires et la reproduction ou encore la navigation dans l'environnement. C'est une composante clé de leur comportement adaptatif, leur permettant d'optimiser les expériences sensorielles en fonction de leurs besoins et de leurs environnements (*Rosen et al., 2015*). Les odeurs peuvent porter une signification biologique forte : les odeurs de prédateurs provoquent la fuite tandis que les odeurs alimentaires induisent l'approche. Ainsi, la perception hédonique des odeurs est aisément observable d'un point de vue comportemental. Toutefois, les odorants sans signification biologique particulière peuvent également moduler le comportement d'approche et de retrait (*Kermen et al., 2016; Mandairon et al., 2009*). La valeur hédonique est mesurable chez l'animal via le niveau d'exploration de l'odorant ou la vitesse d'approche, donc l'attraction de l'animal pour ce stimulus (*Kermen et al., 2016*). De manière intéressante, ce comportement est corrélé à des propriétés physico-chimiques des odorants, faisant que leurs caractères plus ou moins plaisant est robuste et conservé entre les espèces comme chez le rongeur et l'humain (*Joussain et al., 2011; Khan et al., 2007; Mandairon et al., 2009*).

L'encodage de la valeur hédonique des odeurs démarre dès les premières structures impliquées dans le traitement de l'information olfactive telles que **l'épithélium et le BO**. Cette fonction est en effet modulée par la liaison des molécules olfactives et leurs propriétés sur les récepteurs olfactifs, leurs structures et leurs polymorphismes génétiques chez les individus (*Genva et al., 2019; Kermen et al., 2011; Khan et al., 2007*). De plus, l'expression des récepteurs sur les neurones sensoriels et leurs projections axonales dans le BO suivent une organisation topographique. Une activation préférentielle d'un certain pattern au niveau d'une région de l'épithélium par une odeur plutôt plaisante ou déplaisante pourrait ainsi donner suite à une représentation topographique de cette valeur hédonique dans le BO (*Ferrero et al., 2011; Kobayakawa et al., 2007; Lapid et al., 2011*).

En dehors de cette hypothèse, un codage spatial de la valeur hédonique des odorants a bien été identifié dans le BO (*Kobayakawa et al., 2007*) y compris pour les odorants sans signification biologique particulière chez le rongeur (*Kermen et al., 2016, 2021*). Dans ces études réalisées au sein de notre équipe, la partie antérieure du BO code préférentiellement l'information relative aux odorants plaisants, tandis que la partie postérieure code davantage l'information véhiculée par les odorants déplaisants. Cette représentation est retrouvée au niveau des glomérules et de la couche granulaire du BO. De plus,

l'inhibition optogénétique de ces schémas d'activation inverse la valeur hédonique des odorants : l'inhibition des cellules granulaires du BO postérieur induit une attraction pour les odorants déplaisants, alors que l'inhibition des cellules granulaires du BO antérieur provoque une répulsion pour les odorants initialement plaisants. Cette étude conclut donc que la valeur hédonique innée des odorants sans signification biologique particulière, est codée spatialement selon l'axe antéro-postérieur du BO. De plus, des études récentes suggèrent que cet encodage, en partie médié par les cellules granulaires du BO, pourrait différer en fonction du moment de la naissance de ces cellules, point que nous aborderons un peu plus tard. Comment la perception hédonique innée est codée dans le BO manque encore d'investigations, notamment pour ce qui est des odeurs sans signification éthologique relative à la survie (*Kermen et al., 2021*), et surtout concernant les odeurs naturellement plaisantes affectant simplement le bien-être et l'expérience de plaisir.

Le cortex piriforme joue également un rôle dans le traitement de la valeur hédonique des odeurs (*Yamaguchi, 2017*). Les projections du BO sur le cortex piriforme sont diffuses, ce qui fait que l'organisation topographique identifiée en amont du circuit n'est pas conservée dans cette région (*Igarashi et al., 2012; Sosulski et al., 2011; Stettler & Axel, 2009*). Cette diffusion pourrait en partie expliquer l'hétérogénéité fonctionnelle de l'implication du cortex piriforme vis-à-vis de l'encodage de la valeur hédonique des odeurs.

Chez l'humain, (*Gottfried et al., 2002*) ont montré que la partie postérieure du cortex piriforme s'active en réponse à toutes les odeurs, tandis que sa partie antérieure s'active selon la valeur hédonique des odeurs. Cette activité serait plus intense lors de la présentation d'odeurs spontanément déplaisantes par rapport à des odeurs neutres (*Bensafi et al., 2007; Gottfried et al., 2002; Royet et al., 2003; Zelano et al., 2007*). De manière intéressante, ce résultat est retrouvé en l'absence de stimulation olfactive, lorsque les sujets s'imaginent sentir les odeurs, suggérant une robustesse de cet encodage dans des conditions d'imagerie mentale (*Bensafi et al., 2007*). Plus récemment, une étude de l'équipe a montré que les odeurs désagréables activent préférentiellement la partie antérieure du cortex piriforme gauche (*Moranges et al., 2024*), confirmant ces observations. Enfin, les odeurs plaisantes et déplaisantes modifient les schémas de connectivité du cortex piriforme droit, et ce hypothétiquement pour préparer une réponse adaptée à la valeur hédonique perçue (*Ruser et al., 2021*). Ces résultats suggèrent l'existence de schémas spécifiques d'activation pour les odeurs agréables et désagréables dans le cortex piriforme

Chez le rongeur, l'introduction d'une nouvelle odeur tôt dans la vie -différente de l'odeur maternelle- et son association aux soins induit une attraction pour la nouvelle odeur comparable à celle de l'odeur maternelle. Ce phénomène est lié à une activation du cortex piriforme, qui serait donc impliqué dans la plasticité olfactive précoce et dans la reconfiguration des circuits olfactifs encodant la valeur hédonique des odeurs (*Raineki et al., 2010; Roth & Sullivan, 2005*). Ces études et d'autres sont plutôt relatives à la représentation hédonique apprise (*Li et al., 2008; Mandairon et al., 2014; Millman & Murthy, 2020; Roesch et al., 2007*). Cependant, en ce qui concerne les odeurs inné-ment positives, les données concernant l'implication du cortex piriforme sont peu nombreuses.

Le rôle du **TO et de l'ATV** dans la perception de la valeur hédonique des odeurs **chez l'humain** est plutôt établi dans la littérature (*Araujo et al., 2005; Bensafi et al., 2012; Midroit et al., 2021; Yamaguchi, 2017*). L'entrée sensorielle olfactive directe dans le TO, combinée à la libération de DA provenant de l'ATV dans cette zone cérébrale, explique en partie ce rôle (*Murata, 2020; Murata et al., 2015, 2024*).

Chez le rongeur, la formation de préférences olfactives implique aussi le TO (*Midroit et al., 2021; Wesson & Wilson, 2011; Zhang, Liu, et al., 2017*). Premièrement, il est recruté lors de l'encodage de **la valeur hédonique apprise** des odeurs et de l'expression de comportements d'attraction ou d'évitement (*FitzGerald et al., 2014; Gadziola et al., 2015; Millman & Murthy, 2020; Murata et al.,*

2015), et ce, en fonction des récepteurs DAergiques (D1 ou D2) activés (*Gadziola et al., 2020; Murata et al., 2019*). De plus, le TO intervient dans la modulation de l'attention sélective envers des odeurs spécifiques lors de comportements dirigés vers un but (*Carlson et al., 2018*). La stimulation directe du TO médian, ou de la voie ATV-TO, modifie les préférences olfactives (conditionnées par la récompense), confirmant son implication (*FitzGerald et al., 2014; Zhang, Liu, et al., 2017*) et l'importance de l'innervation DAergique dans ce processus.

Le TO est aussi recruté dans la réponse aux odeurs possédant **une valeur hédonique innée**, notamment lorsque celle-ci porte une signification biologique forte comme l'alimentation (*Murata, 2020*) ou le lien social entre les animaux (*Agustín-Pavón et al., 2014; DiBenedictis et al., 2015*). Cependant, le traitement des odeurs sociales dépend d'un circuit particulier, le système olfactif accessoire, qui ne sera pas abordé ici. Dans l'étude de (*Zhang, Liu, et al., 2017*), des odeurs sans signification biologique spécifique pour le rongeur sont également utilisées. Ils ont montré que l'activité du TO médian varie avec les valeurs hédoniques de ces odeurs. L'activation optogénétique de l'ATV peut induire une préférence olfactive pour une odeur neutre, mais aussi stopper l'aversion pour une odeur répulsive. À l'inverse, bloquer les projections DAergiques de l'ATV sur le TO inhibe l'expression des préférences olfactives (*Zhang, Liu, et al., 2017*). Les projections DAergiques de l'ATV sur le TO médian joue donc un rôle clé dans la formation et le maintien de préférences olfactives et dans les comportements associés. Toujours dans le contexte d'odeurs ayant une valeur hédonique innée mais pas de signification biologique forte, il a été montré au sein de l'équipe que les odorants plaisants activent spécifiquement le TO médian et l'ATV, induisant une préférence de place conditionnée sous le contrôle de la transmission DAergique par rapport aux odorants déplaisants. Ces observations chez le rongeur ont été étendues à l'humain où les odorants plaisants induisent également une préférence de place conditionnée, associée à une augmentation du signal BOLD en IRMf spécifiquement dans le TO (*Midroit et al., 2021*).

Enfin, le **COF**, que nous avons précédemment mentionné, est impliqué dans le plaisir hédonique et plus précisément dans la perception de la valeur hédonique des odeurs chez l'humain (*Berridge & Kringelbach, 2015; Grabenhorst & Rolls, 2009; Kepler et al., 2022; E. T. Rolls, 2004; E. T. Rolls & Grabenhorst, 2008*). Cette structure semble être un véritable nœud -ou « Hub »- dans l'encodage de la dimension plaisante et récompensante des odeurs (*Callara et al., 2021*), et ce également chez l'animal (*Castro & Berridge, 2017*). Les odeurs possédant des valeurs hédoniques différentes modulent les patterns de connectivité entre le COF et le cortex piriforme, le cortex cingulaire antérieur, l'amygdale et le reste du CPF, régions avec lesquelles il est très connecté (*Araujo et al., 2005; Kepler et al., 2022*).

L'amygdale traite également les informations positives ou négatives relatives aux odeurs (*Jin et al., 2015; Kepler et al., 2022*). **Chez l'humain**, une étude soutient que l'amygdale peut coder la totalité de la dimension hédonique, de déplaisant à plaisant, via des variations spatio-temporelles de son activité (*Jin et al., 2015*). Son rôle dans cette fonction serait lié à l'activité du COF, du cortex piriforme et de l'insula, soulignant l'importance de la connectivité fonctionnelle du réseau sous-tendant la perception hédonique (*Patin & Pause, 2015*). **Chez l'animal**, les études s'intéressent majoritairement au rôle de l'amygdale dans la régulation des comportements en réponse à des odeurs à valence hédonique innée forte et ayant une signification biologique robuste. L'amygdale est ainsi importante chez le rongeur dans la réponse à l'odeur de prédateur et l'aversion qui en découle (*Root et al., 2014; L. K. Takahashi, 2014*). Cependant, les mécanismes sous-tendant le rôle de l'amygdale dans la réponse aux odeurs spontanément agréables, sans intérêt éthologique marqué, nécessitent encore d'être investigués.

Le striatum ventral, le noyau accumbens et le pallidum ventral, dans la lignée de ce qui a été dit précédemment, font partie du réseau traitant l'information hédonique chez l'humain et l'animal (Berridge & Kringelbach, 2015; Menegas et al., 2017, 2018; Murata, 2020; Yamaguchi, 2017). L'étude de (Pool et al., 2022) montre que dans une tâche pavlovienne chez l'humain, la région dorso-latérale du striatum ventral est impliquée dans la motivation, tandis que la région ventro-médiale est liée au plaisir sensoriel olfactif, corrélé à l'activation du COF médial. Ceci met en évidence une dissociation fonctionnelle entre motivation et plaisir dans le traitement des récompenses olfactives. Cependant, élucider comment les structures du système de la récompense encodent l'information hédonique des odeurs en tant que telle, sans conditionnement préalable, en exploitant le pouvoir récompensant uniquement de l'odeur innément agréable, requiert encore des investigations approfondies.

Malgré une base commune, il existe donc des patterns et sous-circuits distincts codant pour les stimulus olfactifs positifs ou négatifs (Callara et al., 2021; Kepler et al., 2022; Pool et al., 2022; Tiedemann et al., 2020; Yamaguchi, 2017). La perception hédonique des odeurs ne dépend pas uniquement de l'activation isolée de telles ou telles structures mais plutôt de **la connectivité fonctionnelle** et de la synchronisation entre celles qui forment le réseau telles que le cortex piriforme et le COF (Ruser et al., 2021). Les odeurs agréables et désagréables sont même capables d'influencer différemment l'activité et la connectivité cérébrale au repos (IRMF après stimulation olfactive) (Carlson et al., 2020), et donc, probablement différents processus cognitifs et comportements.

Enfin, comme décrit plus haut par rapport au BO, l'encodage de **la valeur hédonique innée** des odorants, comme l'aversion pour les odorants de prédateurs, pourrait être surtout supporté par des circuits formés dans la période critique de développement (Muthusamy et al., 2017; Qiu et al., 2021), tandis que l'encodage de **la valeur hédonique apprise** d'un odorant dépendrait des circuits adultes et de leur plasticité telle que la population de cellules granulaires nouvellement formées (Grelat et al., 2018). Dans ce contexte, une étude a cherché à comprendre comment les expériences olfactives précoces (odeur maternelle, de mâle ou encore du nid) modulent les préférences olfactives des petits et les circuits associés (Perry et al., 2016). Ils ont premièrement identifié des réseaux amygdalo-hippocampiques et corticaux préfrontaux activés différemment en fonction de la valeur hédonique des odeurs présentes lors de l'expérience olfactive précoce. De plus, modifier cette expérience précoce olfactive en faisant cohabiter par exemple les petits avec le mâle, favorise l'attraction des petits pour l'odeur de mâle. Ceci était accompagné d'une réduction de la réponse des régions amygdaliennes normalement activées par cette odeur, rapprochant ainsi les réponses comportementales des petits de celles observées vis-à-vis de l'odeur maternelle à l'origine. Cette étude permet de confirmer que la perception de la valeur hédonique des odeurs est en partie conditionnée par l'expérience précoce, et qu'interférer dans l'expérience olfactive précoce change les circuits olfactifs supportant la formation et le maintien de préférences olfactives.

Ainsi, en chamboulant l'expérience olfactive précoce, le stress durant la période critique pourrait modifier les préférences olfactives innées et donc la réponse aux odeurs, les comportements émotionnels associés et enfin les états affectifs. De plus, il est connu que le COF, le complexe ventro-striatal, certains noyaux de l'amygdale ou encore l'ATV, sont détériorées après un stress précoce et dans les troubles affectifs dont la dépression (Belujon & Grace, 2017). Ceci pourrait expliquer en partie la détérioration de l'appréciation hédonique de certains stimuli olfactifs. Ainsi, comprendre les réseaux sous-tendant l'encodage de la perception hédonique des odeurs, et notamment leurs altérations dans le contexte d'un stress précoce, est essentiel pour mieux appréhender le lien entre processus sensoriels et émotionnels.

-DEUXIÈME ENTRACTE-

Nous avons parcouru comment la valeur hédonique des odeurs est codée au sein du système olfactif, mettant en lumière le rôle central de ses connexions avec les circuits limbique et de la récompense. La nature plaisante ou déplaisante des odeurs est étroitement liée à la capacité de ressentir des émotions positives ou négatives, renforçant le lien entre la perception olfactive hédonique et les états émotionnels. Comprendre comment le stress précoce modifie la perception de la valeur hédonique innée des odeurs, décrypter les circuits neuronaux sous-jacents et appréhender la vulnérabilité face aux troubles affectifs nécessite des investigations approfondies et constitue l'objectif central de cette thèse. Avant de passer au cœur de ce projet, la prochaine partie se propose de décrire ce que l'on sait actuellement vis-à-vis du lien entre perception olfactive et troubles affectifs.

-Quatrième Chapitre- Ressentir et Sentir

4.1) Pertes olfactives, perte du bien-être

« *L'olfaction rend le monde vivant et émotionnel* » (Croy & Hummel, 2017). En effet, les odeurs provoquent des réponses émotionnelles et comportementales fortes (Gadziola et al., 2020). Impacter la perception olfactive peut influencer les émotions et les humeurs. Les dysfonctions du sens olfactif peuvent ainsi être des marqueurs de troubles affectifs et de maladies psychiatriques, aussi bien en tant que facteurs de vulnérabilité comme décrit ci-après, que de conséquences directes comme nous le verrons plus tard (El-Hage, 2022).

En tant que **facteur de vulnérabilité**, une diminution, une perturbation ou une disparition complète de la capacité à sentir peut altérer la qualité de vie, les plaisirs quotidiens et le bien-être, en impactant notamment l'alimentation ou le comportement social (Ballanger et al., 2019; Croy et al., 2014; Hummel & Nordin, 2005; Murata et al., 2024). À une échelle de gravité supérieure, les perturbations olfactives contribuent à la psychopathologie des troubles psychiatriques, précipitant ou prolongeant les états anxieux et dépressifs chez l'humain comme chez l'animal (Atanasova et al., 2008; Croy et al., 2013, 2014; Croy & Hummel, 2017; Rochet et al., 2018; Taalman et al., 2017). Dans l'étude de (Eliyan et al., 2020), la détérioration de la capacité d'identification est prédictive du trouble dépressif. Dans une autre étude, les patients souffrant de dysfonctionnements olfactifs voient leurs symptômes de dépression s'aggraver en fonction de la sévérité de la perte olfactive (Kohli et al., 2016). La perte olfactive est présente dans soixante-huit troubles neurologiques différents en dehors de la dépression (Leon & Woo, 2022) et figure souvent parmi les tous premiers symptômes. Des données épidémiologiques ont argumenté que l'exposition à des polluants atmosphériques et l'inflammation des voies respiratoires peuvent augmenter le risque de souffrir d'une maladie mentale, en partie à cause d'atteintes du système olfactif (Hasegawa et al., 2022).

Les effets des troubles olfactifs sur l'humeur et la santé mentale peuvent en partie être expliqués par **l'atteinte d'un circuit partagé**. Les altérations du système olfactif rendent difficile le traitement émotionnel de la stimulation olfactive, provoquant ou perpétuant la boucle des altérations émotionnelles liées à l'état pathologique (Marin et al., 2023). Il est suggéré que la réduction de la qualité de vie due aux perturbations olfactives soit associée à une dysrégulation des zones olfactives et limbiques telles que le TO, à la diminution de la libération d'opioïdes endogènes dans des sous-régions spécialisées, ainsi

qu'à des dysfonctionnements des récepteurs olfactifs périphériques (Murata et al., 2024). Dans le cas de la dépression, il est proposé que les effets de la perte olfactive dans les fonctions affectives seraient de deux niveaux, périphérique et central (Croy & Hummel, 2017). D'une part, la taille réduite du BO nivellerait négativement la quantité de ses projections sur les systèmes limbique et de la récompense, et de ses retours centrifuges, cristallisant la dépression. D'autre part, la dépression aurait des effets plus stables. Elle réduirait l'attention que porte l'individu à son environnement, diminuant les entrées sensorielles et le renouvellement des récepteurs olfactifs par exemple.

Les premières études s'intéressant aux effets d'une perte olfactive sur le phénotype émotionnel de l'animal ont utilisé la bulbectomie, soit le retrait chirurgical ou pharmacologique du BO (Planchez et al., 2019; Yuan & Slotnick, 2014). Le constat de l'apparition de phénotypes dépressifs suite à cette manipulation a motivé la recherche à approfondir la compréhension des effets du stress sur le sens olfactif comme nous allons à présent l'explorer. Car en effet, la relation entre perte olfactive et perte de bien-être est bidirectionnelle.

Une perte olfactive peut ainsi amener à des bouleversements de l'affect plus ou moins durables et favoriser l'occurrence d'un état pathologique, illustrant le premier pan de la relation unissant olfaction et émotion. À l'inverse, des bouleversements de l'humeur se répercutent sur le sens olfactif et peuvent aussi le détériorer profondément.

4.2) Quand le stress déforme le sens olfactif

4.2.1) Pathologie du stress et perception olfactive

Le stress est capable de dégrader la perception sensorielle. Au départ, la recherche s'intéressant à la relation entre les modalités sensorielles et les pathologies liées au stress s'est peu concentrée sur l'olfaction (Brand & Schaal, 2017). Pourtant, en raison des fortes connexions entre les voies olfactives et les réseaux régulant l'affect, comprendre les interactions entre l'olfaction, le stress et les troubles affectifs suscite de plus en plus d'intérêt. En effet, la simple réduction de la qualité de vie peut être liée à des défauts du système olfactif, notamment du TO (Murata et al., 2024).

Chez l'humain, la modélisation expérimentale d'un **stress aigu** modifie la sensibilité olfactive (Pacharra et al., 2016) et réduit les capacités de détection et d'identification des odeurs (T. Takahashi et al., 2015). Un stress aigu social augmentant le cortisol salivaire (stress physiologique) est associé à de meilleures performances d'identification des odeurs et une augmentation de l'intensité perçue (Hoenen et al., 2017). En revanche, une hausse du sentiment de colère (stress perçu/émotionnel), qui augmente également le cortisol, corrèle avec une moins bonne performance d'identification des odeurs. Bien qu'un haut niveau de cortisol puisse être parfois associé à de meilleures capacités olfactives, des émotions comme la colère et l'anxiété, qui augmentent également le cortisol, réduisent ces mêmes capacités. Cela pourrait reposer sur le fait que des émotions complexes, comme la colère ou l'angoisse, activent des circuits supplémentaires ou distincts du simple stress physiologique. Dans une autre étude, l'induction expérimentale de l'anxiété altère la valeur hédonique des odeurs en entraînant une détection plus lente et une amplification de la négativité perçue des odeurs neutres (Krusemark et al., 2013), intensifie la réponse observée en IRMf dans les zones cérébrales olfactives (cortex piriforme antérieur et COF) et limbiques (cortex cingulaire antérieur) et enfin, augmente les interactions fonctionnelles entre l'amygdale et les zones corticales olfactives. La perception olfactive semble donc bien dépendante de l'état émotionnel.

Le stress chronique affecte lui aussi le système olfactif et entraîne des déficits à long terme du comportement médié par les odeurs (Masuo et al., 2021). Toutes les fonctions olfactives peuvent ainsi

être touchées dans un large éventail de maladies liées au stress (*Taalman et al., 2017; Vermetten, 2003; Wilson et al., 2014*). Fait intéressant, les déficits olfactifs surviennent alors que les autres fonctions sensorielles sont souvent maintenues. Ceci étant dit, ces déficits sont dépendants du type de stress, de sa nature et de son origine, de la perturbation psychiatrique et/ou psychologique qui en découle, de sa durée, de l'âge au moment de la survenue du trouble ou encore du sexe. Par exemple, les patients souffrants d'anxiété généralisée peuvent avoir des diminutions des capacités de détection, d'identification et de discrimination (*B. Chen et al., 2021; Clepce et al., 2010, 2012*). Dans le cas du **trouble majeur de la dépression**, la sensibilité olfactive est réduite, ce qui n'est pas le cas de la dépression saisonnière ou bipolaire (*Brand & Schaal, 2017*).

Le trouble majeur de la dépression est en effet souvent accompagné d'altérations de la détection, de la discrimination et de l'identification (*Kohli et al., 2016*), associées à des changements morphologiques de l'épithélium olfactif ou encore des diminutions du volume du BO (*Athanassi et al., 2021; Negoias et al., 2016; Pause et al., 2001*). Concernant la perception de la valeur hédonique des odeurs, elle peut également être détériorée dans ce trouble (*Atanasova et al., 2008, 2010; Clepce et al., 2010; Lombion-Pouthier et al., 2006; Naudin et al., 2012; Pause et al., 2001*). Dans la majorité des cas, les odorants désagréables sont encore plus désagréables -alliesthésie négative- et les odorants agréables sont moins agréables -anhédonie olfactive- (*Atanasova et al., 2010*). Cependant, l'interaction entre le stress et l'appréciation hédonique des odeurs dépend de l'odeur, de la nature du trouble et de l'accumulation de pathologies comorbides complexifiant les investigations (*Lombion-Pouthier et al., 2006; Pause et al., 2001; Rochet et al., 2018*).

Chez l'animal, le stress détériore aussi la perception olfactive (*Bombail, 2019*). Un stress léger peut agir sur le BO, altérant la formation de souvenirs olfactifs (*Manella et al., 2013*). Un **stress aigu** peut empêcher le maintien et la manipulation en mémoire de plusieurs informations olfactives simultanément chez le rat, mettant en évidence une réduction des capacités de mémoire olfactive et de flexibilité cognitive (*Davies et al., 2013*). Fait intéressant, cette tâche semble dépendre du noyau accumbens et de l'expression de récepteurs D1 et D2 dans le complexe striato-pallidum ventral (*Onofrychuk, Glass, et al., 2024; Onofrychuk, Heidt, et al., 2024*).

Dans un contexte pathologique, le modèle de **stress chronique imprédictible** altère la perception olfactive telle que l'appréciation d'odorants plaisants (*Yang et al., 2011*), associé à une diminution de la neurogenèse dans le BO (*Athanassi et al., 2023*). En effet, durant mes recherches, nous avons montré que l'anhédonie olfactive induite par ce modèle de stress chez la souris est associée à une diminution de la survie et de l'activité des cellules granulaires du BO nées à l'âge adulte (*Athanassi et al., 2023*). Dans une autre étude, la détection olfactive est réduite chez des rats présentant des symptômes dits dépressifs suite à un stress chronique (*Raynaud et al., 2015*). Cette perte de fonction semble supportée par des pertes par apoptose de neurones sensoriels de l'épithélium olfactif. Le stress chronique provoque aussi une incapacité à détecter et à discriminer les odeurs ainsi qu'à éviter instinctivement les odeurs de prédateurs (*Vaz et al., 2018*). Ces effets sont couplés à une altération morphologique des neurones glutamatergiques pyramidaux du noyau du tractus olfactif latéral, structure le long des fibres de projections du BO et connecté à l'amygdale. Enfin, un modèle d'injection chronique de corticostérone induit des déficits dans les capacités mnésiques olfactives et celles de discrimination fine de deux odorants perceptuellement proches (*Siopi et al., 2016*).

Dans des modèles de **stress post-traumatique** utilisant des odeurs de prédateurs directement en tant que stressseurs (*Albrechet-Souza et al., 2021; Albrechet-Souza & Gilpin, 2019; Kondoh et al., 2016; Ornelas & Besheer, 2024*), les comportements d'approche de l'odeur sont altérés. Ceci est associé à des augmentations des niveaux de CRH et d'hormone corticotrope, ainsi que des modifications de l'activation du BO, du noyau accumbens et de l'amygdale, notamment de l'aire de transition amygdalo-

piriforme, à la jonction entre l'amygdale et le cortex piriforme. Il est cependant difficile d'interpréter les effets d'un stress sur la perception olfactive quand le stressleur est lui-même olfactif.

En conclusion, il est clairement établi que le stress influence la perception olfactive de multiples façons. Beaucoup reste à faire quant à la compréhension de ce phénomène et de ses mécanismes car certains modèles de stress, tels que la défaite sociale ou « l'impuissance apprise », ne semblent pas impacter la perception olfactive (Athanassi et al., 2021), alors que d'autres, comme ceux représentant le stress précoce, ne sont que peu étudiés.

4.2.2) Stress précoce et dysfonctions olfactives

Ainsi, l'impact du **stress précoce** sur la perception olfactive à l'âge adulte a été assez peu étudié.

Chez l'humain, un stress précoce aigu modifie la sensibilité olfactive en fonction de la valeur hédonique initiale des odeurs (Cortese et al., 2021). De plus, il est connu que les adultes ayant été maltraités à l'enfance ont souvent un BO d'une taille réduite de 20 %, ce qui est associé à des déficits dans la détection et l'identification des odeurs (Croy et al., 2013).

Le sens olfactif étant crucial lors de la période critique pour la survie et le développement (Opendak & Sullivan, 2019), les études **chez le rongeur** s'intéressent généralement aux effets du stress précoce à court terme, chez les petits. Ainsi, le stress gestationnel ne semble pas impacter leur mémoire olfactive ou leur capacité de discrimination ni les bases neurales associées (Belnoue et al., 2013). Cependant, le stress post natal précoce peut détériorer les capacités d'apprentissage olfactif aversif des petits (association de l'odeur de poivre avec un choc électrique), ce qui semble reposer sur un dysfonctionnement de l'axe amygdale-LC-BO, et témoigne d'une hypo-réponse au stress (Moriceau et al., 2009). Des rats ayant subi des privations maternelles présentent des déficits persistants de la mémoire olfactive. En effet, les odeurs de la mère et du nid sont normalement apprises et guident les comportements d'approche, de nutrition et d'interactions sociales des petits. Cependant chez ces rats, ces capacités sont endommagées et ce, jusqu'à l'adolescence. Ces résultats sont associés à des bouleversements du neurodéveloppement dans l'hippocampe et le BO avec notamment des patterns de différenciation et prolifération cellulaire altérés (Czarnabay et al., 2019). Une étude a de manière originale, modélisé un stress paternel, en utilisant pour la reproduction des souris mâles avec un historique de séparation maternelle (Thivisol et al., 2023). Les petits issus de ces pères présentent des déficits dans l'adaptation comportementale à la présence d'une odeur de prédateur accompagnée de perturbations de l'activité et la connectivité fonctionnelle dans le cortex cingulaire, l'hippocampe et l'amygdale basolatérale. Cette étude souligne de manière intéressante qu'un stress précoce touchant la première génération a des effets robustes sur les générations suivantes. Enfin, une autre étude très intéressante modélisant le stress chez le petit à travers un sevrage précoce (P14 au lieu de P21), a montré un déficit des capacités de discrimination, d'apprentissage et de mémoire olfactive chez ces souris devenues adultes, tandis que les capacités de détection sont comparables aux souris contrôles (Pardasani et al., 2023). Au niveau cellulaire, les souris stressées ont une activité réduite dans la couche plexiforme interne du BO supportée par une réduction des retours synaptiques inhibiteurs des interneurons GABA sur les cellules mitrales ainsi qu'une diminution de leur complexité dendritique.

Ainsi, quelques éléments viennent étayer la littérature quant aux répercussions du stress précoce sur la perception olfactive. Cependant, les éléments de réponse chez l'animal sont souvent relatifs aux capacités de détection ou de discrimination mais très peu à la perception hédonique des odeurs, qui est pourtant souvent altérée dans les troubles affectifs et fortement dépendante des émotions.

Au terme de cette introduction, j'espère que le lecteur de ce travail sera convaincu que la perception olfactive est étroitement liée aux émotions. Les effets d'une perte olfactive sur l'affect, et inversement les impacts du stress sur la perception olfactive, ont été décrits. Néanmoins, la littérature manque cruellement d'informations sur les effets à long terme du stress précoce et ses bases neurales. Comprendre les répercussions du stress précoce sur le sens olfactif, et plus précisément la perception de la valeur hédonique des odeurs, fonction olfactive majoritairement en relation avec les émotions, représente donc l'objectif majeur de cette thèse.

-OBJECTIFS DE LA THÈSE-

Parmi les étiologies des troubles affectifs et psychiatriques, le stress précoce est apparu comme l'un des plus robuste et sévère, impactant l'individu tout au long de sa vie. Beaucoup d'études visent à comprendre les conséquences psychologiques du stress précoce telle que la survenue de l'état dépressif et ses symptômes. Or, un lien étroit a été fait entre état dépressif -ou de manière plus globale- troubles affectifs et perturbations de la perception des odeurs. Dans ce contexte, j'ai établi un état des lieux de la littérature afin de mieux comprendre la relation bidirectionnelle existant entre le sens olfactif et la dépression, ce qui a abouti à la publication de la **REVUE** de littérature : *Relationship between depression and olfactory sensory function: a review*. Néanmoins, peu d'études se sont finalement intéressées à l'impact du stress précoce sur la perception hédonique des odeurs, qui est au cœur de la dimension émotionnelle du sens olfactif. Ainsi, l'objectif principal de ma thèse est de comprendre les retombées du stress précoce sur la perception hédonique des odeurs à l'âge adulte. Cette compréhension se veut multiple, puisqu'elle aborde des aspects comportementaux et neurobiologiques, qui font l'objet de mon étude principale, **P'ÉTUDE 1** : *Decoding alterations of hedonic perception induced by early life adversity*.

Le deuxième objectif est de nature méthodologique, puisque les investigations menées pendant ma thèse ont abouti à l'utilisation d'une approche novatrice dans notre équipe : la photométrie par fibre. Cette approche a pour but d'analyser comment le stress précoce altère en temps réel la libération de DA et de calcium dans le TO en réponse à des odorants plaisants et déplaisants. L'installation et l'utilisation d'un set-up de photométrie par fibre a ainsi permis l'enregistrement des fluctuations d'activité et de neurotransmetteurs (DA) dans le cerveau de souris en comportement. Nous avons ensuite développé une nouvelle approche pour coupler l'analyse des signaux à celle du comportement animal. Ce travail de développement méthodologique fait l'objet de **P'ÉTUDE 2** : *Automatic extraction and analysis of synchronized fiber photometry and video data: a method for studying the neural basis of exploratory behavior in freely moving animals*.

Enfin, un objectif annexe de ma thèse était d'étudier les effets d'un autre type de stress : le stress chronique imprédictible sur la perception olfactive. Ce travail a abouti à la publication de l'article **Annexe 1** : *Chronic unpredictable mild stress alters odor hedonics and adult olfactory neurogenesis in mice*.

-REVUE-

Relationship between depression and olfactory sensory function: a review

(publiée)

Review Article

Relationship between depression and olfactory sensory function: a review

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Abstract

Links between olfactory sensory function and effect have been well established. A robust literature exists in both humans and animals showing that disrupting olfaction sensory function can elicit disordered mood state, including serve as a model of depression. Despite this, considerably less is known regarding the directionality and neural basis of this relationship, e.g. whether disruptions in sensory function precede and contribute to altered mood or if altered mood state precipitates changes in olfactory perception. Further, the neural basis of altered olfactory function in depression remains unclear. In conjunction with clinical studies, animal models represent a valuable tool to understand the relationship between altered mood and olfactory sensory function. Here, we review the relevant literature assessing olfactory performance in depression in humans and in rodent models of depressive-like behavioral states. Rodents allow for detailed characterization of alterations in olfactory perception, manipulation of experiential events that elicit depressive-like phenotypes, and allow for interrogation of potential predictive markers of disease and the cellular basis of olfactory impairments associated with depressive-like phenotypes. We synthesize these findings to identify paths forward to investigate and understand the complex interplay between depression and olfactory sensory function.

Key words: olfactory perception, depression, humans, rodent models.

Introduction

Approximately 15% of the worldwide burden of disease is attributed to mental disorders (Prince et al. 2007; Atanasova et al. 2008), and 8 to 12% of the world's population will be affected by major depressive disorder (MDD) at least once in their life (Weissman et al. 1996; Andrade et al. 2003). MDD is one of the most common

neuropsychiatric disorders and represents a major public health concern. More than 264 million people of all ages are affected by MDD (WHO 2017). When focusing on epidemiological factors such as sex, research consistently reports greater prevalence of MDD in women, with female-to-male risk ratios of roughly 2:1 (Hirschfeld and Cross 1982; Kessler 2003; Gobinath et al. 2014; Kendler et al.

2020). MDD is associated with significant morbidity and mortality (Carney et al. 2002), with about 50% of depressed patients showing suicidal ideation or thought and 800,000 attempting and completing suicide every year.

MDD is a complex disorder that has at its core either a persistent depressed mood state and/or anhedonia (loss of interest and/or pleasure). Paired with one or both of these core features, a number of other symptoms must also be present, and can include effects on how an individual feels, thinks, eats, sleeps, or behaves. MDD is also associated with emotional, sensory, and physical problems (sadness, hopeless feelings, anxiety, moving/speaking more slowly, lack of energy) (WHO 2020), has multiple precipitating factors, and can have a multifaceted etiology. Identified risk factors include biological, genetic, epigenetic, and environmental variables (including hormonal fluctuations, genetic mutation, family history, and stress, to name a few) (Rochet et al. 2018; Kendler et al. 2020). Complicating the study of depression is the existence of differing subtypes and forms of pathology that include a depressive-like state (e.g. MDD, bipolar depression, seasonal affective disorder (SAD), to name a subset). Further, presentation of disease and risk factors contributing to the development of depression differ over the lifespan (Fiske et al. 2009; Schaakxs et al. 2017; Coleman et al. 2020). The diversity of causes, presentation, and evolution of depressive illness with aging has contributed to difficulty in studying the cause of disorder and the neurobiological underpinnings of pathology. Curiously, many of the more common symptoms of depression appear to either interact with or have direct effects on the sensory experience of the individual, which has the potential to provide novel insights into this complex disorder.

Several studies have highlighted a significant interaction between depression and the processing of signals in multiple sensory domains, with a primary focus on visual or auditory modalities (Kahkonen et al. 2007). The chemical senses (olfaction and taste) have also received attention in depression research and may provide some of the most interesting insights into the linkage between sensory function and mood. Olfaction is unique from other sensory modalities, in that olfactory inputs largely bypass thalamic relays and have nearly direct projections to brain centers that are implicated in emotional regulation and possibly mood state (Soudry et al. 2011). This direct access to limbic brain centers may be a vestige of the evolutionary importance of chemosensory function for the detection and response to threat, reproductive opportunities, and approach/avoidance of food sources. Thus, olfaction may be unique with regard to its direct access to neural substrates regulating vigilance and may be well situated to be involved in the regulation of mood.

Supporting a close linkage between olfaction and mood, the ablation or diminishment of olfactory sensory function can induce behavioral profiles reminiscent of depressive-like states in both rodents and humans (Atanasova et al. 2008; Croy et al. 2013; Taalman et al. 2017; Rochet et al. 2018). Further, greater severity of reported depressive symptoms has been associated with a greater severity of olfactory deficits. In those models, depressive symptoms can be partially rescued through olfactory enrichment (Lehrner et al., 2005; Brand and Schaal 2017; Rochet et al. 2018; Ballanger et al. 2019). While diminished olfactory sensory experience can promote the development of depressive-like phenotypes in model systems, questions remain as to whether the relationship between depression and olfactory sensory function is unidirectional. For example, does depressed mood state contribute to altered sensory function and drive decrement in sensory experience or does the decrement in sensory function worsen mood state? Studies assessing the impact of depression itself on olfactory function have been far less common leaving this a largely open question (Pollatos et al. 2007; Croy et al. 2014;

Khil et al. 2016; Kohli et al. 2016; Croy and Hummel 2017; Pabel et al. 2018). Further complicating these studies may be a selection bias, with individuals in the general population having (but being unaware of) a compromised sense of smell or taste or failing to present at the clinic for sensory deficits alone (Oleszkiewicz et al. 2020). Thus, the incidence of olfactory deficits in the general population may be under-reported. This has the potential to skew our perception of the degree to which olfaction and mood are interrelated. Further, olfactory disturbance is present in several neurological conditions that have increased risk for depression as a comorbid feature (including Alzheimer's, Parkinson's, and Schizophrenia) (Schiffman 1997; Kovacs et al. 2003; Luzzi et al. 2007; Taalman et al. 2017; Carnemolla et al. 2020; Eek et al. 2021; Son et al. 2021). Olfactory impairment has also been reported in neurodevelopmental diseases, including autism spectrum disorder (Koehler et al. 2018), and has dramatically increased worldwide in the last year due to the COVID-19 infection (Pierron et al. 2020). Given these complexities, it may be difficult to disentangle effects observed in these varied populations that are related to depressive pathology from those due to neurobiological effects associated with other forms of diseases. For those studies that have begun to probe links between olfaction and mood, it remains to be determined if olfactory impairments in depressed patients are traits (persistent characteristics that were present before and after symptomatic remission) or states (characteristics that are only present during the symptomatic phase and disappear after treatment of depression). The goal of the following sections is to review the literature from human and animal models and attempt to clarify ways in which depression and olfactory performance may be related. For the purpose of clarity, we have tried to restrict our discussion to work in populations with major depressive disorder (MDD).

Alterations in olfactory performance among humans diagnosed with depression

In studies that have assessed olfactory sensory function in populations of individuals suffering from MDD, methodological differences between studies have made results difficult to interpret or to relate across studies. To truly understand the effects of depression on olfactory sensory function, attention must be paid to multiple parameters. These include the assessment of olfactory threshold, discrimination, identity, familiarity, and the hedonic value of odors. Further, methodological considerations must also be taken into account, including the characteristics of the cohort (sex, disease history, precipitating factors contributing to depression, genetic risk, etc.), means of collecting olfactory measures (self-report, clinical assessment, etc.), and control of the intensity, complexity, or hedonic value of the odors being tested. Variation in the choice of the above-mentioned variables has made it difficult to arrive at a consensus with regard to the effects of depression on olfactory sensory function (Taalman et al. 2017). In the following sections, we review the data on olfactory function across a variety of these domains in patient populations suffering from depression.

Evaluation of perceptual and cognitive performance in depression

A number of studies have been undertaken to assess olfactory perceptual function in populations suffering from depression. A meta-analysis of that work, from Rochet et al in 2018, provides a summary of the results for olfactory threshold, identification, intensity, discrimination, and hedonics to evaluate their potential as marker tasks for depression. In the majority of studies looked at, no association was

found between depression (mainly MDD) and olfactory discrimination (Pause et al. 2003; Atanasova et al. 2010; Clepce et al. 2010; Negoias et al. 2010). Regarding odorant identification, most studies found that this dimension was not altered by MDD (Serby et al. 1990; Lombion-Pouthier et al. 2006; Pentzek et al. 2007; Scinska et al. 2008; Swiecicki et al. 2009; Negoias et al. 2010) with an exception (Zucco and Bollini 2011). In the study by Zucco and Bollini, depression was associated with a lower identification capacity. Further, the authors compared mild and severe cases of major depression and found that when the disease was more severe, deficit in identification was greater. For detection threshold, some studies identified an increased threshold to detect odorants in depressed patients (Pause et al. 2001; Postolache et al. 2002; Lombion-Pouthier et al. 2006; Negoias et al. 2010). In these studies, olfactory detection was assessed using four different olfactory tests, the single or two-alternative staircase detection procedure, the European Test of Olfactory Capabilities, and the “Sniffin’ Sticks” test battery. For 3 of the studies, MDD classification was based on a Beck Depression Inventory (BDI) score between 23 and 29. In the fourth study (Postolache et al. 2002), the patients suffered from seasonal affective disorder. Despite those positive results, additional studies have reported no relationship between depression and detection threshold (Scinska et al. 2008; Swiecicki et al. 2009; Croy et al. 2014). The discrepancies between studies may have arisen from a number of differences, including differences in the age or gender of the population being studied. For example, the study by Scinska et al. was comprised of older adults, and the work by Croy et al. assessed performance in only female subjects. Finally, the report by Swiecicki et al. included both patients suffering from unipolar and bipolar depressive disorder.

The aforementioned studies that failed to find an association between depression and olfactory detection threshold may represent true null results (true identification of no effects of depression on sensory function). Alternatively, difficulty in detecting group differences could have been influenced by heterogeneity in the sample being studied. Depression represents a grouping of multiple etiologies that all cluster under the umbrella of a single disorder. As there are multiple contributing factors to disease development and presentation of depression, specific disruptions to olfactory function may be unique to a given subgroup of individuals within the broader classification of the depressed population. To test this prediction, it would be important to take into account the specific profile of symptoms of depression (e.g. to attempt to control for symptom clusters, number and duration of episode(s), treatment history, age, and sex of the subjects, and family history). Additional key variables that may influence unique aspects of olfactory function include hormonal or age influences on sensitivity as well as treatment history on discrimination, among others. By sampling across different subtypes of depression, the ability to observe an effect on a specific dimension of olfactory sensory function in prior studies may have been diluted by the presence of multiple subgroups that were not affected on that dimension. Identification of unique effects on olfactory function in subgroups of depression (should they exist) may provide important insights into the neurobiological underpinnings of olfactory disturbance and its relation to depressive symptoms and risk factors for disease development. For instance, MDD can be observed both with and without melancholic symptoms which themselves may impact the hedonic perception or rating of odorants (Clepce et al. 2010; Fletcher et al. 2015).

Evaluation of hedonic responses to odors in depression

Given the prominence of anhedonia as one of the core symptoms of depression, more work has been carried out focusing on the

relationship between MDD and hedonic processing of odorants than other dimensions of olfactory sensory function. The majority of studies have found differences between patients and controls in the hedonic valuation of select odorants (Pause et al. 2001; Lombion-Pouthier et al. 2006; Atanasova et al. 2010; Clepce et al. 2010; Naudin et al. 2012), with a smaller proportion of studies finding no effects on hedonic perception (Swiecicki et al. 2009). In most studies, unpleasant odorants were perceived as more unpleasant (negative alliesthesia described by Atanasova et al. 2010) and pleasant odorants were perceived less pleasant in depressed patients (anhedonia) (Atanasova et al. 2010). Interestingly, some depressed patients rated some odorants as more pleasant (Lombion-Pouthier et al. 2006). These included odorants that have been shown to have anxiolytic/relaxing effects in control populations, such as lavender and citral (Hatano et al. 2012; Agatonovic-Kustrin et al. 2020). The positive ratings may be the result of the anxiolytic features of these odorants, diminishing negative scores through their improvement of mood, or by decreasing comorbid anxiety in depressed populations (Hatano et al. 2012; Agatonovic-Kustrin et al. 2020). The fact that hedonic scoring of odorants may be influenced by the ability of the odor to directly interact with mood, the choice of odorants in experimental settings, and measurement of comorbid pathology, such as anxiety, will be important for gaining a better understanding of the effects of depression on this dimension of olfactory function (Pause et al. 2001; Lombion-Pouthier et al. 2006; Rochet et al. 2018).

In addition to heterogeneity in the composition of populations and types of odorants chosen for testing, there is variability in the approach used to assess sensory function. For example, to test hedonic valuation of odorants in depressed patients, some studies used complex mixtures of odorants, which may be closer to physiological and natural conditions of everyday life experiences (Naudin et al. 2012). As in the previously described studies, authors found an association between depression and the hedonic perception of these complex mixtures. More specifically, depressed patients had difficulty identifying pleasant odorants in a mixture while they had no difficulty in identifying unpleasant ones (Naudin et al. 2012). This is in line with the results presented by Atanasova et al. (2010), where patients had a lower identification capacity for only the pleasant component of a complex odor during depressive episodes (Atanasova et al. 2010). Moreover, pleasant and unpleasant ratings for components of a mixed stimulus had a greater separation in their ratings for subjects without depression compared to depressed patients. This result was present despite no difference in the perception of intensity of the odorants between control subjects and those suffering from depression. Together, these results indicate that hedonic perception may be altered in multiple ways: qualitative alteration (hedonic rating), hedonic discrimination (gestalt rating of mixtures), and quantitative alteration (magnitude of unpleasantness).

Comparison of impairment pre and post antidepressant treatment

In addition to the comparison of control subjects with those with depression, some authors have focused on assessment of olfactory impairments before and after antidepressant treatments within depressed populations. In this work, successful treatment of depression was shown to rescue some of the observed deficits in olfactory function (Naudin et al. 2012; Croy et al. 2014; Yuan and Slomnick 2014; Taalman et al. 2017). Olfactory deficits (alteration of olfactory intensity, discrimination, and hedonic perception) in patients with MDD were sensitive to antidepressant treatment, or therapy, with improvement of olfactory scores after treatment and sometimes, a positive impact of the duration of the treatment (3 versus 6

weeks) on olfactory performance (Croy et al. 2014). It should also be noted that ~30% of depressed patients are resistant to medication/treatment, begging the question of whether comorbid alterations in sensory function are similarly insensitive to treatment. While correlations have been established between severity of symptoms and the degree of olfactory deficit, it will also be important to determine the impact of antidepressant treatment and the degree of symptom resolution on olfactory performance to better understand this relationship. Such studies may provide new insights into the mechanisms underlying altered sensory function as a disease endophenotype, and possible neurobiological alterations associated with MDD.

As alluded to above, differences in the subtype of depression may contribute to the disparate results observed in olfactory studies. Beyond symptom clusters, additional parameters of depression might impact olfactory function, including duration of illness. Pabel et al. (2018) found that while the severity of depression was not associated with disturbance in olfactory function, the course and duration of depressive episodes was. In fact, a greater duration of symptom expression and recurrent illness was correlated with diminished odor detection (diminished threshold) and poorer odor identification, with an interaction between course and duration (e.g. longer and more recurrent illness was associated with greater impairment) (Pabel et al. 2018).

Considerations for studying the relationship of depression and olfaction

Based on studies described in the sections above, it is clear that additional work is needed to further understand the relationship between depression and olfaction. In future work, multiple key variables will need to be taken into account, including the potential disparate effects of differing etiologies of depression on olfaction, base rates of olfactory disturbance in the general population, and the methods employed to assay the multiple aspects of olfactory functioning in humans. Furthermore, the parameters contributing to disease development, sex, age and hormonal status of subjects, the course and duration of illness, and treatment history will also be important factors to consider when studying the relationship between depression and olfaction. However, in patient populations, there is often difficulty in obtaining the level of precision required to control for many of these variables. As a consequence, work in translational animal models has the potential to identify specific deficits that can then be more carefully targeted and tested in clinical populations. Below, we highlight some of the work being carried out specifically in rodent models of depressive-like behavior and the relevance of these models for understanding disturbance in olfactory sensory processing.

Rodent models of depression

Below, we introduce several commonly used rodent models that are believed to recapitulate depressive-like phenotypes or to model genetic or environmental risk factors for depression and their impact on olfactory sensory function.

Relevance of rodent models for depression

Given the prevalence and high rates of morbidity and mortality associated with depression, numerous studies have emerged over the years attempting to better understand the neurobiological basis of disease (Krishnan and Nestler 2008; Douglas and Porter 2009; Hamon and Blier 2013; Yang et al. 2019). Indeed, work in humans present a number of variables that are difficult to control for,

including genetic variability, medication history, living conditions, age, sex, and the specific presentation of depressive symptoms used for exclusion/inclusion. Thus, to make progress in this area, many labs have turned to animal models that provide a means to examine both the neural circuitry as well as neurophysiological systems and molecular pathways underlying the pathophysiology of core symptoms of disease. In particular, rodent models enable precise control of genetic, environmental, and pharmacological variables that are often lacking in human studies, and permit approaching this important question through behavioral, neurobiological, or genetic models of risk for depressive-like behaviors.

The diagnosis of complex neuropsychiatric disorders such as depression is based on, and relies on, clinical observations and often phenomenological symptoms reported by the patient themselves. Thus, the use of rodent models has led to some skepticism with regard to their utility in modeling a complex disorder. It is indeed challenging to translate the complex diagnosis of a disorder in rodent models, partly because of the heterogeneity of depressive disorder and also due to the difficulty in assessing symptoms such as feelings of sadness and suicidal thoughts in rodents. Given this complexity, it is more common to use models with high construct validity (e.g. common genetic variant, environmental risk factors, and precipitating experiences) and to then rely upon endophenotyping approaches that model individual and biologically tractable features of the disorder, as opposed to attempting to recapitulate the full phenotype of disease.

A core example linking olfaction and depressive-like behavior in rodents was revealed after bilateral olfactory bulbectomy (Kelly et al. 1997; Song and Leonard 2005). After bulbectomy, rodents showed depressive-like behaviors, including decreased libido and deficits in long-term explicit memory (resembling loss of interest and cognitive deficits in depressive patients, respectively) and also a deficit in passive avoidance, a decrease in exploratory activity and motivational behavior (Hall and Macrides 1983; Lumia et al., 1992; Kelly et al. 1997; Antunes et al. 2016; Almeida et al. 2017). These symptoms were accompanied by physiological and neurochemical modifications such as changes in the functioning of cortical-hippocampal-amygdala circuit, 5-HT and dopamine dysfunction, reduction in the concentration of brain noradrenaline or affected neuroimmune function with an increase in circulating interleukin-1 β and tumor necrosis factor- α and a reduction in lymphocyte counts (Kelly et al. 1997; Pistovcakova et al. 2008; Almeida et al. 2017) biomarkers/symptoms that are similar to those observed in MDD (Strawbridge et al. 2015).

For many years, olfactory bulbectomy in rats has been used as an experimental model of depression to study and predict how patients will respond to a given antidepressant (Kelly et al. 1997; Cryan et al. 1999; Song and Leonard 2005). This procedure was particularly useful to identify changes in behavior associated with endocrine, immune, and neurotransmitter systems in depressed patients following the loss of the sense of smell and to then identify antidepressant treatments that might reverse alterations in mood. This model was critical in establishing a link between disturbed olfactory sensory function and resulting risk for depressed mood (e.g. disturbing olfactory sensory function can increase risk for depression). However, given that this manipulation destroys a key brain region for olfactory function, it does not allow researchers to test if depressed mood state can alter olfactory sensory function (e.g. whether depression contributes to altered olfactory performance). To be able to test this, alternate animal models of depressive-like behavior were needed. These models were developed to recapitulate genetic and

environmental disturbances that contribute to depressive-like behaviors, and importantly do not directly involve destroying regions of the brain critical for basic olfactory sensory function (as is the case in olfactory bulbectomy).

In humans, multiple environmental and genetic risk factors exist and contribute to risk for depression. Given this fact, it is important to study multiple animal model of depressive-like behavior, in which depressive-like behaviors are elicited in different ways (Soderlund and Lindskog 2018). Using a diversity of approaches has the potential to aid in our understanding of how each of these risk factors may impinge upon and contribute to altered olfactory sensory function in the context of pathology. Below, we introduce a subset of the model systems that have been developed to express depressive-like pathology, as well as introduce what is currently known about the impact of these manipulations on olfactory sensory functioning and neural structures involved in olfactory sensory processing.

Rodent models of depressive-like behavior and the impact on olfactory sensory function

Based on the complex etiology of depression, rodent models of depressive-like behavior have been developed that elicit “symptoms” either by manipulating the environment to which they are exposed (e.g. acute or chronic stressor) or through genetic or pharmacological means, including exogenous administration of glucocorticoids and introduction of genetic mutations associated with depression in human populations (McGonagle and Kessler 1990; Caspi and Moffitt 2006; Chen et al. 2006; Suris et al. 2010; Uher and McGuffin 2010). Some of the most useful models allow investigation at multiple levels of analysis, including interrogation of neurophysiological changes, alterations in neural circuits, as well as impact on molecular and genetic targets that may underlie behavioral disturbance. That being said, many models provide important insights, but also have limitations with regard to the translational potential or generalizability of their findings. Here, we provide a brief overview of some of the more commonly used rodent models of depression and their key characteristics.

Rodent models of genetic risk for depression.

Genetically engineered models provide an interesting approach to assess the genetic risk factors for MDD. Among the gene candidates involved in elevated risk for depression, BDNF is particularly interesting since it is involved in neuronal survival, differentiation, and synaptic plasticity. A methionine (Met) substitution for valine (Val) at the codon 66 of BDNF (BDNF^{Val66Met}), identified in humans, has been associated with risk for depression and a variety of other neuropsychiatric disorders (Pezawas et al. 2008). Mice have been developed to carry a synonymous single-nucleotide polymorphism in the BDNF gene (val66met) which has been shown to alter brain anatomy, memory, and increased anxiety-related behaviors that are not normalized by the antidepressant, fluoxetine (Chen et al. 2006). The Val66Met mutation in the BDNF gene disrupts activity dependent release of BDNF has been shown to impact the migration of cells through the rostral migratory stream to the olfactory bulb, ultimately diminishing the number of newly born granule cells in the adult olfactory bulb (Bath et al. 2008). The decrease in OB granule cell neurogenesis has been associated with impaired olfactory discrimination abilities in these mice in a spontaneous cross-habituation task (Bath et al. 2008). In these mice, no effects were observed on odor investigation, habituation, or olfactory detection thresholds, indicating a very specific deficit in olfactory sensory

function. However, much work remains to be done to understand whether additional deficits exist in olfactory function in these mice.

Another candidate pathway associated with risk for depression is disruption in serotonin function. Numerous genetic models of depression have been based on manipulating serotonin, including the generation of 5-hydroxy tryptophan (5-HT) receptor knockout mouse lines (Lemondé et al. 2003; Neumeister et al. 2004). Studies of these mice have highlighted an increased sensitivity to stress while others have shown that deletion of the serotonin related gene (e.g. tryptophan hydroxylase- *tpb2*) leads to increased immobility in forced swim test, indicative of a depressive-like phenotype (Savelieva et al. 2008; Mosienko et al. 2012). Loss-of-function mutation in tryptophan hydroxylase (*tpb*), the rate-limiting enzyme in 5-HT biosynthesis, also appeared to cause pro-depressive effects in mice (Zhang et al. 2005). *Tph2*^{-/-} dams have been shown to exhibit significant deficits in maternal care and pup retrieval, however, those effects did not appear to be due to general deficits in odorant detection or crude levels of odorant discrimination, based on an odor cross-habituation assay (Angoa-Perez et al. 2014). Further, *Tph*^{-/-} mice were able to learn to perform a reversal learning task where odorants were used as the guiding cue (Carlson et al. 2016).

In addition to genetic risk factors, a number of environmental factors have been identified that increase the risk for developing depressive-like symptoms and have been modeled in rodents. While these models have been heavily utilized to understand the neurobiology of depressive-like symptoms and treatment response, few have assessed the impact of these manipulations on olfactory sensory function. Below, we highlight a few of the more commonly employed rodent models of depressive-like behavior and the small amount that is known about their impact on olfactory function.

Unpredictable chronic mild stress (UCMS).

A common and well-validated model to induce depressive-like behavior is UCMS (Willner 2017). Over a period of several weeks, animals receive a series of different stressors (i.e. food and water deprivation, wet bedding, cage tilt, etc) several times a day and at unpredictable time points, causing moderate levels of stress. This manipulation produces long-lasting effects and has been established as a model that leads to a persistent and long-lasting depressive-like state in rodents. Consequently, rodents exhibiting depressive-like behavior generally show a decrease in sucrose preference, an increase of intracranial self-stimulation threshold or a loss of weight (Mineur et al. 2006), behavioral profiles that are indicative of a decrease in reward sensitivity and the development of anhedonia. Sustained and unpredictable stress applied in the UCMS model may recapitulate the varied stressful life events that significantly elevate risk for disorder and depressive-like behavior in humans. In this model, a decrease in the number of olfactory receptors has been observed (Li et al. 2015) as well as reduced neurogenesis in the OB (Yang et al. 2011), and impaired olfactory discrimination (Hu et al. 2020). However, we are not aware of any studies assessing other aspects of olfactory behavioral performance following UCMS.

Learned helplessness.

Because hopelessness is considered a central symptom of depression, the learned helplessness (LH) paradigm is commonly used as an animal model of depressive-like behavior (Seligman 1972). In this model, the rodent is placed in a closed chamber without any possibility of escaping and then receives several electric shocks applied to their feet. Animals are then placed in another chamber with a grid floor and receive multiple mild shocks, but now with the possibility

of escaping. Depressive-like behavior is measured by the likelihood and latency to escape the electrical shock in the new context (e.g. shock avoidance). “Depressed” rodents that were trained in the learned helplessness paradigm frequently exhibit a delayed escape time (Seligman and Peterson 2007), while severely depressed rodents do not escape the shock at all (Maier and Seligman 2016). LH is considered a strong model of depressive-like behavior as the unpredictability and the uncontrollability of the acute stressors create a hopeless, inescapable, and uncontrollable situation, eliciting a depressive-like response. This model is a valuable tool to illustrate the negative cognitive bias described in depression, where patients generally exhibit a negative view of events and interpret them as not controllable. Based on our review of the literature, we have not found any reports assessing the effect of LH on olfactory performance or olfactory circuitry.

Social defeat model.

The social defeat model is a variant of the LH model but employs social conflict as a stressor to create psychological and emotional stress (Toyoda 2017). This model is based on the repeated subordination of a mouse or rat by a dominant peer. The protocol consists of placing an intruder rodent in the home cage of a large dominant male. Under these conditions, the resident animal attacks the smaller intruder. After several physical attacks, and co-housing with the aggressor, the defeated rodent undergoes behavioral testing to test for increased expression of depressive-like behaviors (e.g. social avoidance, increased defensive behavior, and increased anxiety, decreased locomotor activity, sleep disturbances, alterations in body weight, impaired immune functions) (Toyoda 2017). The social defeat model produces physiological changes and behavioral symptoms that reflect the features of posttraumatic stress disorder (PTSD) and depression (Hammack et al. 2012; Conoscenti and Fanselow 2019). Indeed, inescapable acute threats used in social defeat (as well as LH) replicate exposure to traumatic events occurring in life (stressful events or situations of power imbalance like bullying and sexual harassment) which increase the risk for PTSD and depression. While many of these studies use social approach as a metric of learned fear and social avoidance, we are not aware of any studies that directly assess the impact of social defeat on olfactory sensory function, beyond willingness to approach a social signal. In some instances, subjugated mice even fail to approach a novel animal in a neutral environment. The lack of approach to a conspecific could either indicate an active avoidance of conspecifics due to increased anxiety or could reflect a diminished ability to either detect the chemical cues emitted by the conspecific or discriminate them from the odor of the aggressive animal that was encountered during social defeat.

Early life adversity model.

The early life adversity model is based on the idea that adverse events in early life may shape the behavioral and the biological phenotype of the offspring based on altered neurodevelopmental trajectories, ultimately resulting in behavioral phenotypes that approximate depression or psychosis (Syed and Nemeroff 2017). These models have provided evidence for increased risk for later life negative outcomes that may be the consequence of altered development. Forms of early life adversity have included models of early maternal separation (MS) (Demaestri et al. 2020) and limited nesting and bedding (Gallo et al. 2019; Goodwill et al. 2019), among other manipulations.

Early maternal separation (MS) is a procedure that exposes rodent pups to a 3-hour (sometimes 6-hour) daily maternal separation and isolation stress from postnatal days 4 to 11 (or 21).

Once these rodents are adults, they generally exhibit deficits in learning and memory (Thomas et al. 2016), increased anxiety-like behavior, and depressive-like behavior (Jin et al. 2018; Demaestri et al. 2020). More specifically, MS rearing has been shown to affect males and females similarly in early milestone development, yet only males showed changes in stress physiology and anxiety-like outcomes (Demaestri et al. 2020). Further, changes have been observed at the biological level, such as an increase in corticosterone levels and a decrease in brain neurotrophic factor (BDNF) (Wang et al. 2015).

The limited bedding model (LB) is different from the maternal separation model in form but not timing (see (Demaestri et al. 2020)). It consists of transferring the dam and pups to novel housing conditions several days following birth and leaving the dam and pups in housing conditions with limited access to material resources for nest building for a period of seven days (Gallo et al. 2019). LB affects the timing of early developmental milestones, somatic growth, and stress physiology in both sexes, yet only female pups reared under these conditions showed later development of depressive-like behaviors (Goodwill et al. 2019). LB females, but not males, exhibited depressive-like behaviors on both traditional assays as well as newly developed home cage monitoring measures. These effects emerged during adolescence and became more severe in adulthood, mirroring the sex bias in risk, time course, and diversity of behavior features of pathology observed in humans. Further, LB effects could be rapidly rescued by ketamine, a fast-acting antidepressant (Goodwill et al. 2019).

Together, these data suggest that early life adversity like LB and MS can have a crucial impact on behavior during adulthood, causing debilitating long-lasting consequences such as elevated risk for depressive-like behavior. Preliminary data from our lab has also found that LB rearing leads to diminished ability to acquire learned discrimination of highly similar odorants on an olfactory perceptual learning task, as well as effects on olfactory bulb (OB) neurogenesis (unpublished). Further data on the effects of these manipulations on olfactory sensory function are lacking.

Corticosterone model.

Some models also recapitulate neurobiological alterations observed in MDD such as alterations in brain areas involved in stress-processing (i.e. increased activity in the prefrontal cortex, the amygdala, the hippocampus, the nucleus accumbens, and the habenula), in the stress axis (i.e. dysregulation of the hypothalamic-pituitary-adrenal axis) and in the immune system (i.e. neuroinflammation) (Brites and Fernandes 2015; Zang et al. 2018; Ceruso et al. 2020). For instance, chronic exposure to elevated levels of corticosterone (i.e. injection of a synthetic form of the hormone corticosterone) mimics chronic stress and evokes a depressive-like state in animals. Several studies have shown that corticosterone-injected rodents exhibit behavioral changes indicative of a depressive-like phenotype, including increased immobility in the forced swim test, decreased grooming and elevated anxiety-like behavior in the Open field and Light/Dark-Box tests, as well as anhedonia with a decreased sucrose preference (Baez and Volosin 1994; Mitra and Sapolsky 2008; Dieterich et al. 2019). In this model, the authors evaluated olfactory discrimination using an automated operant conditioning procedure and revealed alterations in the fine discrimination of highly similar odorants with no effects on the discrimination of highly dissimilar odorants. In addition, pronounced deficits in olfactory acuity (ability to discriminate between highly similar odorants) and olfactory memory have been

also observed in this model and rescued by the antidepressant fluoxetine (Siopi et al. 2016).

In summary, while a number of genetic and environmental risk factors have been shown to contribute to the development of depressive-like behaviors, few have assessed the impact of these manipulations on olfactory sensory function. Given the diversity of models and factors that contribute to the development of depressive-like phenotypes, it seems that this would provide an excellent opportunity to investigate the impact of differing antecedents or risk factors for depression on risk for olfactory disturbance, and possible neural underpinnings driving those effects.

Neural basis of olfactory impairments in depression

It is assumed that the existence of a link between olfaction and depression would be due to effects on anatomical structures that are common to both olfactory and emotional processing (amygdala, hippocampus, orbitofrontal cortex, prefrontal cortex, accumbens nucleus, parahippocampal gyrus, insula, ventral striatum). The overlap in neural structures mediating stress responding and processing of olfactory signals suggests that olfactory structures could be involved in emotional disturbance, and could explain why depression is often accompanied by alterations in olfactory function (Croy and Hummel 2017). In addition, olfactory and limbic systems are considered “evolutionarily old” systems which have co-evolved together. Thus, olfaction which is likely used to sense mates, danger, and food sources could have a strong role in regulating motivation and by proxy emotional state and behavior (Croy and Hummel 2017). In the following sections, we review anatomical effects associated with depression in humans that may explain comorbid alterations in olfactory processing.

Impact of depression on neural circuits implicated in olfactory processing in humans

Odor perception is the result of odorant molecules binding to olfactory receptors located on sensory neurons in the nasal olfactory epithelium. These neurons then project to the OB, followed by projection to secondary olfactory areas including the anterior olfactory nucleus, olfactory tubercle, entorhinal cortex, piriform cortex, and cortical amygdala (Shepherd 1972; Wilson and Sullivan 2011). The signal is then further transmitted to additional brain centers including the hippocampus, thalamus, the ventral anterior insula, the orbitofrontal cortex (OFC), and the ventral tegmental area (Shepherd 1972; Wilson and Sullivan 2011; Courtiol and Wilson 2015). Thus, the olfactory system appears to have near-direct access to both cortical centers for perceptual processing as well as brain regions implicated in emotion and processing of reward.

Anatomical impairments of olfactory structures after depressive episodes have been observed in humans. It has been shown using magnetic resonance imaging (MRI) that depression leads to a decrease in OB volume and that the degree of OB volume reduction is correlated with the severity and maintenance of the disease—greater reduction is associated with greater impairment (Croy and Hummel 2017). Thus, the size of the OB could constitute a first biomarker for enhanced vulnerability to depression (Negoias et al. 2010).

A decrease in hippocampus, cingulate cortex, and habenula volumes has also been observed in depression (Campbell et al. 2004; van Tol et al. 2010). Moreover, some nuclei of the amygdala appeared to be predisposed to atrophy in depressed patients (Yao et al. 2020).

Using functional imaging in humans (fMRI), depression has also been associated with modified activity in other olfactory structures. For example, hyperactivity of the amygdala has been observed in depressed patients compared to controls (Drevets 2003). In the OFC, two subregions have been shown to be differentially engaged during depression: the ventromedial OFC is involved in anxiety and rumination and has shown to be hyperactive in depression while the dorsal OFC, which is involved in attention and working memory, has been shown to be hypoactive in depression (Rochet et al. 2018). Moreover, researches have found hyperactivity of the insula in depressed patients during resting state imaging and have shown that increased activity of this structure is associated with increased rumination in depression (Sliz and Hayley 2012). Finally, in accordance with studies of depression using animal models, human imaging studies have shown hyperactivity in the habenular region linked to symptoms of depression (Ranft et al. 2010). Different neuroimaging studies revealed changes in neural activity in these brain areas in response to odorant stimulation or autobiographical odor memory (Zatorre et al. 1992; Sobel et al. 1998; Rolls 2004; Rochet et al. 2018) suggesting that the observed alteration in activity and morphometry of these regions in depressed populations could impact olfactory function.

The hippocampus and the amygdala, which receive dense projections carrying information from the olfactory system, play a key role in the regulation of emotional learning and memory (Soudry et al. 2011). These structures have been reliably shown to be affected in depression (Yao et al. 2020) which could explain some alterations in the cognitive aspects of olfactory perception, including identification, olfactory learning, or memory during depression (Lemogne et al. 2006). Depression can also have a strong impact on sleep, leading to significant disturbance (Agargun et al. 1997) which has been associated with impairment in odor identification (Killgore and McBride 2006; Prehn-Kristensen et al. 2015), and cognitive function (including issues with memory, concentration, and arousal). Together, such effects may serve to alter odor perception.

To better understand the relationship between olfactory disturbances, and to determine if they may result from, contribute to, or are independent of other depressive symptoms, a more systematic evaluation of basic olfactory perception (detection, spontaneous discrimination, hedonic value, intensity) should be carried out and placed in the context of additional measures of cognitive functioning.

Impact of depression on neural circuits associated with olfaction in animals

As mentioned previously, to further our understanding of the neural circuits involved in depression, many have turned to animal models. Here, we summarize the data related to altered neural networks in rodent models of depressive-like behavior that may contribute to disturbance in olfactory sensory function.

The UCMS model in rats has been linked with a reduction in volume of the OB, a disturbance in presynaptic function, and a reduction in the number of olfactory receptor neurons in olfactory epithelium (Li et al. 2015). In two regions of the brain, there are continued high levels of neurogenesis in adulthood, the OB and the hippocampus (Ming and Song 2011). While numerous studies have shown an association between reduced hippocampal neurogenesis and increased risk for depressive-like symptoms (Warner-Schmidt and Duman 2006; Eisch and Petrik 2012), fewer studies have tested the relationship between MDD and OB neurogenesis (Siopi et al. 2016). The reduction in levels of neurogenesis and OB volume in the UCMS rat model indicate that the same stress that contributes

to the expression of depressive-like behavior can impact OB neurogenesis. While there is debate about the existence of adult OB neurogenesis in humans (Curtis et al. 2011; Sanai et al. 2011; Bergmann et al. 2015; Lim and Alvarez-Buylla 2016), numerous studies have revealed reduced OB volume and olfactory sensitivity in patients with MDD (Pause et al. 2001; Negoias et al. 2010) and the presence of neuronal turnover in the olfactory epithelium. Thus, it is possible that stress could contribute to impairments in olfactory perception in MDD patients, however, these linkages must be formally tested. While specific olfactory impairments have not yet been highlighted in animal models of depression, it would be relevant to test specific parameters, including olfactory acuity and sensitivity as well as discrimination ability in animal models of depression in which reduced OB neurogenesis has been observed. Importantly, studies showing decreased neurogenesis in animal model of depression have been able to restore levels of neurogenesis in the hippocampus (Banar and Duman 2007; Rochet et al. 2018) as well as in the OB (Siopi et al. 2016) with treatment with antidepressants (fluoxetine), consistent with improvement in olfactory deficits following treatment.

To better understand the brain regions involved in olfactory deficits in models of depressive-like behavior, Croy and Hummel (2017) posit that olfactory receptor turnover rate in the olfactory epithelium is decreased in depression. This is a potentially interesting potential mechanism for olfactory disturbance in MDD and could explain impaired olfactory threshold, identification or discrimination, in patient populations (Croy and Hummel 2017). Other studies have shown that the habenula (involved in the transfer of olfactory information to other brain areas) might also be affected in depression. Models of bilateral bulbectomy in rodents revealed a higher level of apoptosis in the habenula during depression, which could contribute to its role in olfactory disturbance in depression (Brand and Schaal 2017).

Numerous studies using the LH rat model of depressive-like behavior have shown important metabolic changes in regions of frontal cortex and hippocampus as well as a reduction in BDNF in the medial prefrontal cortex and dentate gyrus, disturbance in lipid metabolism, glutamatergic metabolism, and neurotransmission (Shirayama et al. 2015; Dwivedi and Zhang 2016; Liu et al. 2018). LH was also observed to have significant impacts on the habenula, amygdala, insular and cingulate cortex (Shumake and Gonzalez-Lima 2003; Shumake et al. 2004). Interestingly, these regions are known to be involved in attention, emotional, and cognitive processes and also in olfactory processing (Kesner et al. 2002; Pouliot and Jones-Gotman 2008; Veldhuizen et al. 2010; Brand and Schaal 2017). In this context, alterations of these regions in LH rats may significantly diminish olfactory sensory function, however, olfactory performance has not yet been thoroughly tested in this model. Possible effects of disturbance in the function of these regions could include diminished olfactory sensitivity or altered hedonic perception of odors, which have not yet been evaluated in LH rats.

Numerous studies have also analyzed the consequences of repeated exposure to social defeat in mice on neural activity in the prefrontal cortex, cingulate cortex, hippocampal formation, amygdala, and hypothalamic nuclei (Sheline et al. 2002; Videbech and Ravnkilde 2004; Bourne et al. 2013). As described above, these brain regions form important limbic structures implicated in a wide variety of emotional, cognitive, and behavioral control processes (Kumari et al. 2003; Surguladze et al. 2005; Sheline 2011), and have also been implicated in the development of PTSD. These regions have also been implicated in the processing of olfactory information. As one example, social defeat is associated with increased activity in the

amygdala which could impact the processing of olfactory signals, influencing the perceived hedonic value, the discriminability, or emotional significance of olfactory stimuli. Hamilton & Gotlib showed in 2008 that depressed patients exhibited higher amygdala activity in response to negative (but not positive) emotional stimuli that they had memorized (Hamilton and Gotlib 2008). Based on that work, it seems relevant to test the effects of social defeat on hedonic perception and response to odors.

A recent study by Czarnabay et al. (2019) found that rodents that experienced maternal separation (MS) exhibited a significant delay in the proliferation and differentiation of neurons in the hippocampus and OB (Czarnabay et al. 2019). Further, MS pups took more time to identify odors in an olfactory learning task. Thus, stress during a key period in early development appears to have negative and long-lasting effects on olfactory function in the offspring. While the primary results indicate deficits in olfactory memory of rodents raised in MS conditions, further experiments are needed to test for broader effects of MS on olfactory sensory function and possible mechanisms linking delayed development of major olfactory structures with deficit in olfactory memory.

In summary, studies using animal models of depressive-like behavior have commonly shown a reduced volume of crucial olfactory and limbic structures, including the OB and hippocampus, along with diminished neurogenesis. Numerous studies have also highlighted dysfunction of major neurotransmitter systems associated with metabolic changes including impacts on the serotonergic and dopaminergic systems in regions that are implicated in the sensing and perception of olfactory signals. While some studies have found links between depressive-like behavior and alterations in olfactory identification, few studies have tested the effects of models of depressive-like behavior on other parameters of olfactory perception. While depression has been linked with modified hedonic perception of odors in humans, the linkage between depressive-like phenotypes and hedonic perception of odors in rodent models remains largely unstudied. Studies focusing on olfactory parameters like odorant sensitivity, discrimination, and hedonic perception of odors in animal models of depression are required to further our understanding of the links between olfaction and depression and to connect results observed in animal models with results found in depressed patients. In aggregate, these results illustrate the need for additional work and more thorough analysis of olfactory perception (in particular, hedonic processing of odors) in the different animal models of depressive-like behavior. Specifically, there is a lack of important information with regard to results from animal models of depression and olfactory perceptual capacities, including effects on olfactory threshold, discrimination, identification, or hedonic perception that will be needed to draw clearer conclusions regarding the impact of depression on olfaction and its neural basis.

Conclusion

With regard to the existing bidirectional relationship between olfaction and depression, it seems that the quality of life of depressed patients is more affected when alterations of the olfactory processing are observed (Kohli et al. 2016). Quality of life is a complex concept, influenced by the physical health of the subject, their psychological state, their level of independence, their social relationships, and their perception of their environment (Rochet et al. 2018). However, since odors provide critical information for survival (e.g. food safety and enjoyment for example) and guide our social relationships, behaviors, and mood (e.g. fear and happiness, motivational behaviors,

food intake, reproduction, communication, transmission of anxiety etc.) (Brand and Schaal 2017; Croy and Hummel 2017), the impact of depression on olfactory function has the potential to impact or worsen the quality of life in the context of broader pathology. Studying the relationship between depression and olfactory sensory function has the potential to provide unique insights into the possible neurobiological basis of disease, symptom expression, and possible novel targets for treatments and interventions. However, in executing such studies, key variables must be taken into account when choosing the sample population, model system, and measures to assess these critical questions.

Future Research

In the current review, we have discussed a number of topics related to our current understanding of the relationship between depression and olfactory sensory function in both human clinical populations and animals models of this debilitating disease. Based on our assessment of the literature, a number of issues remain to be tackled in this field. In clinical populations, there is a great deal of variability in both methods being used to test olfactory sensory function, dimensions of olfactory function being tested (sensitivity, discrimination, hedonic valuation), and heterogeneity in selection criteria for inclusion of individuals with varied forms of depression. In future work, it will be important to take care to assess multiple aspects of olfactory sensory function as well as control for the composition of the population (e.g. controlling for stressful life experiences, prior treatment, and genetic risk factors for depression, and rates of olfactory disturbance in the broader population). We have also highlighted the potential importance of using animal models to further probe these links. Specifically, more work is needed to assess sensory function at the neural and behavioral level in models of genetic and environmental risk for behavioral profiles associated with depressive-like behavior. The use of animal models may provide the level of control that is often difficult to attain in human samples to further assess the directionality of the relationship between depression and sensory disturbance. Further, such models provide researchers with the ability to directly probe the neural underpinnings of these effects to better understand the reason for the overlap between sensory disturbance and pathology. In summary, further studies will be critical in bridging the current gap in knowledge regarding the relationship between depression and olfactory sensory function and such work will provide greater insight into the neurobiological basis of depression.

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Conflict of interest

The authors declare no competing interests.

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-RÉSULTATS-

-ÉTUDE 1- *Decoding alterations of odor hedonic perception induced by early life adversity (en préparation)*

-ÉTUDE 2- *Automatic extraction and analysis of synchronized fiber photometry and video data: a method for studying the neural basis of exploratory behavior in freely moving animals (en préparation)*

-ÉTUDE 1-

Decoding alterations of odor hedonic perception induced by early life adversity

(en préparation)

Résumé

L'adversité durant l'enfance augmente significativement le risque de développer des pathologies affectives tout au long de la vie, y compris la dépression. De plus, les altérations émotionnelles se manifestent souvent parallèlement à des perturbations de la perception olfactive, qui est cruciale pour les comportements vitaux et la qualité de vie. En effet, une perception olfactive altérée peut réduire les plaisirs quotidiens et exacerber les symptômes dépressifs. Dans ce contexte, l'objectif de cette étude était d'examiner l'impact de l'adversité précoce (ELA) sur la perception hédonique des odeurs à l'âge adulte et d'identifier les bases neuronales des altérations observées. En utilisant un modèle murin d'ELA induit par des soins maternels fragmentés, nous avons confirmé des changements dans les phénotypes émotionnels et découvert des altérations dans la perception des odorants plaisants mais pas des odorants déplaisants. Cela a été corroboré par des modifications morphologiques des interneurons précoces du bulbe olfactif (BO), en particulier ceux répondant aux odorants agréables, ainsi que par une altération de la survie et de la plasticité des neurones générés à l'âge adulte. Ces changements morphologiques et fonctionnels ont entraîné une altération globale de l'activité neuronale de le BO en réponse aux odorants plaisants, modifiant ainsi le message émis par cette région. De plus, le tubercule olfactif (TO), une cible directe du BO et un composant clé du circuit de la récompense du cerveau, a montré une activité neuronale altérée en réponse aux odorants plaisants, mais pas aux odorants désagréables. Cela a été accompagné d'une altération de l'activité de l'aire tegmentale ventrale (ATV), une région cérébrale impliquée dans les comportements d'approche envers les odeurs agréables. En conclusion, nos résultats indiquent que l'ELA modifie les substrats neuronaux sous-tendant la perception des odorants agréables, ce qui a des implications pour comprendre la résilience à l'anhédonie et la gravité des symptômes chez les individus à risque de dépression avec un historique d'adversité précoce.

Decoding alterations of odor hedonic perception induced by early life adversity

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Abstract

Childhood adversity significantly increases the lifetime risk for affective pathology, including depression. Furthermore, emotional alterations often manifest alongside disruptions in olfactory perception, crucial for both vital behaviors and quality of life. Indeed, altered olfactory perception can diminish daily pleasures and exacerbate depression symptoms. In this context, the objective of this study was to investigate the impact of early life adversity (ELA) on odor hedonic perception in adulthood and to identify the neural bases of observed alterations. Using a murine model of ELA induced by fragmented maternal care, we confirmed changes in emotional endophenotypes and also found alteration in the perception of pleasant but not unpleasant odorants. This was supported by morphological changes in early-born olfactory bulb (OB) interneurons, specifically those responding to pleasant odorants, as well as by impairment of adult-born neuron survival and plasticity. These morphological and functional changes resulted in a global alteration in the neural activity of the OB in response to pleasant odorants, leading to a modification of its output message. Additionally, the olfactory tubercle (OT), a direct OB target and a key component of the brain's reward circuitry exhibited altered neuronal activity in response to pleasant but not unpleasant odorants. This was accompanied by ventral tegmental area (VTA) activity alteration, a brain region recruited during approach behavior to pleasant odorants. In conclusion, our findings indicate that ELA modifies the neural substrates underlying the perception of pleasant odorants with implications for understanding resilience to anhedonia and the severity of symptoms in individuals at risk of depression with a history of early adversity.

Keywords: Early Life Adversity, olfactory perception, stress disorder, reward, dendrites, mice

Introduction

Depression affects 350 million of people around the world (*Leon & Woo, 2022*). It can originate from multiple sources with early childhood adversity emerging as a significant factor amplifying vulnerability to psychiatric pathology (*Glynn et al., 2019; Glynn & Baram, 2019*). Indeed, during the early postnatal period of development and maturation, the brain is particularly sensitive to environmental influences (*Burns, 2018; Kane & Ismail, 2017*). This period occurs in early childhood in humans (before 5 years old) (*Brenhouse & Bath, 2019; Kaufman & Charney, 2001*) corresponding to the first weeks of postnatal life in mice (*Athanassi et al., 2021*). Interactions between infants and caregivers within this sensitive window are crucial for healthy nervous system development (*Opendak & Sullivan, 2019, 2019*). Various forms of adversity such as maltreatment or neglect can disrupt the caregiver-infant interaction leading to abnormal brain development, growth, and behavior, thereby precipitating or exacerbating risk for mental illness in adulthood (*Gee, 2016; Gee et al., 2013; Gee & Casey, 2015; Glynn et al., 2019; Perry et al., 2017; Schroeder et al., 2018*). More specifically, in human and non-human primates or rodents, early adversity increases emotional reactivity with evidence of depressive-like endophenotypes, decreased motivation, behavioral indicators of helplessness, anxiety, higher levels of fear, altered reward seeking, or deficits in cognitive tasks (*Demaestri et al., 2020; Gee et al., 2018; Goodwill et al., 2019; Maestripieri et al., 2006*). Interestingly, the most common symptom in rodents is anhedonia, or loss of pleasure, as evidenced by decreased consumption of sweetened rewards, echoing the major symptom of depression in humans (*Fan et al., 2021; Whitton et al., 2023*). Regarding the neurobiological bases of early adversity in humans, studies have shown structural and functional alterations in several brain regions including the frontal and prefrontal cortex, hippocampus, and corpus callosum (*Danese & McEwen, 2012*). Long-term effects of early adversity have been observed in networks involved in threat management, motivated behaviors, reward, sleep regulation, and executive functions in humans, such as the anterior cingulate cortex, dorsolateral cortex, and the orbital cortex (*Insana et al., 2016; Teicher & Samson, 2016*). In rodents, effects of early adversity have been observed in similar brain areas, i.e. cortico-limbic regions such as amygdala, hippocampus and prefrontal cortex, involved in emotional regulation (*Bath et al., 2017; Honeycutt et al., 2020; Manzano Nieves et al., 2020; Pattwell & Bath, 2017; Rincón-Cortés & Sullivan, 2016; Romeo, 2010; Romeo et al., 2009*).

Studies in humans showed that patients with depression often present deficits in olfactory perception (*Naudin et al., 2012; Pieniak et al., 2022; Yuan & Slotnick, 2014*). Odor perception results from the binding of odorant molecules to olfactory sensory neurons present in the nasal olfactory epithelium. These sensory neurons project to the olfactory bulb (OB), the first central nervous system relay of olfactory perception. There, they establish synaptic contacts with relay cells (mitral and tufted cells), regulated by inhibitory interneurons. These interneurons continue to be born and differentiate postnatally (*Lledo & Valley, 2016*). Then, relay cells project to secondary structures including the olfactory tubercle (OT) located in the ventral striatum, which receives dopaminergic projections from

the ventral tegmental area (VTA), making it part of the reward system (Murata et al., 2015, 2019; Zhang et al., 2017). These structures are closely linked to the hippocampus, thalamus, orbitofrontal cortex (Courtillot & Wilson, 2015; Shepherd, 1972; Wilson & Sullivan, 2011). Thus, the olfactory system appears unique, in that olfactory inputs have nearly direct access to limbic brain centers, implicated in emotional regulation, mood state and motivated behaviors (Landis et al., 2005; Murata, 2020; Soudry et al., 2011; Wesson & Wilson, 2011; Xiong & Wesson, 2016). The chemosensory system exhibits additional unique features: the OB of rodents undergoes dynamic renewal of its interneurons (Denizet et al., 2017; Imayoshi et al., 2008; Lledo et al., 2006). This process begins with the proliferation of new progenitors in the subventricular zone (SVZ), which produce neuroblasts that migrate along the rostral migratory stream to reach the periglomerular and granule cell layers (Dioli et al., 2021; Lazarini et al., 2014). These adult-born interneurons form functional synapses with relay cells (Lledo et al., 2008), thus participating to olfactory function (Dejou et al., 2024; Lepousez et al., 2014; Sakamoto, Ieki, et al., 2014; Sakamoto, Kageyama, et al., 2014). While the effect of stress on adult hippocampal neurogenesis has been largely described (Alonso et al., 2024; Belnoue et al., 2013; Dioli et al., 2021; Loi et al., 2014; Oomen et al., 2010), less information is available on its effect on OB neurogenesis. Thus, while stress in adults reduces the number of proliferating cells in the SVZ, and/or of adult-born neurons in the OB along with altered odor discrimination, memory, acuity and hedonic perception, (Athanasassi et al., 2023; Dioli et al., 2021; Siopi et al., 2016), the long term effects on proliferation and survival rates in the adult OB remain unexplored.

Among the different parameters of olfactory perception (identification, intensity, familiarity), olfactory hedonics (determining whether an odorant is pleasant or not) is dominant since it is spontaneously used at first to describe an odorant (Yeshurun & Sobel, 2010). It represents an essential source of information for decision-making since it drives vital behaviors such as feeding, social relationships, and danger avoidance (Kermen et al., 2021; Walliczek-Dworschak & Hummel, 2017). In addition, this olfactory dimension is of crucial importance to trigger positive emotions and therefore, promotes and maintains well-being (Kontaris et al., 2020). The neural signature of odor hedonics has been found along the anteroposterior axis of the granule cell layer of the OB (Kermen et al., 2016). Pleasant odorants increase the activity of projection neurons of the OT and the VTA in mice (Midroit et al., 2021), thereby recruiting the reward system. Studies have also revealed that hedonic encoding depends on structures such as the amygdala and the orbitofrontal cortex (Kepler et al., 2022; R. E. Perry et al., 2016), heavily involved in mood disorders (Marin et al., 2023). This overlap in neural structures mediating response to adversity and processing of olfactory hedonics could explain why depression is often accompanied by alterations in olfactory function in adults, related to detection, identification, and discrimination (Chen et al., 2021; Clepce et al., 2010; Kontaris et al., 2020) but also to odor hedonics in humans (Atanasova et al., 2010) and rodents (Belnoue et al., 2016).

Given the importance of positive hedonic odor perception in emotional well-being, we asked if early adversity impacts odor hedonic perception and the neural basis of observed alterations. To answer these questions, we used the limited bedding and nesting protocol (LBN). This manipulation restricts access to nesting resources from P4 to P11 and leads to a fragmentation in maternal care as well as triggering increased risk for neglect and physical abuse (rough handling of pups, kicking, biting, isolation), building a chaotic environment for developing pups (*Bath et al., 2016; Brenhouse & Bath, 2019; Goodwill et al., 2019; Manzano Nieves et al., 2020*). This naturalistic protocol (*Rice et al., 2008; Walker et al., 2017*) triggers various long-term consequences on both sexes (*Bath, 2020; Bath et al., 2017; Goodwill et al., 2019*). Using this protocol of early life adversity (ELA), we observed impairment in emotional phenotype and in the perception of odorants with positive hedonic value in adult male mice. Echoing this alteration in perception, we found, through GFP plasmid injection and electroporation, coupled with labeling of c-Fos expression, that the maturation of OB neurons appeared to be sensitive to ELA, as evidenced by alterations of the fine morphology of early-born interneurons responding specifically to pleasant odorants. We also revealed that adult-born neurons, despite being born long after the period of stress, exhibited increased survival and altered activity. These morphological and functional changes led to a global alteration of OB neural activity in response to pleasant odorants. Further, two target brain regions i.e. the OT and the VTA, implicated in the encoding of the hedonic value of odorants, exhibited altered activity in response to pleasant odorants, as indicated by a measure of c-Fos expression.

Material and methods

1. Animals

194 C57BL/6N mice (Charles River Laboratories, France) were bred in house, had *ad libitum* access to food and water, and were housed on a 12 h:12 h light cycle, in a temperature and humidity-controlled environment kept at a constant temperature of $22^{\circ}\text{C} \pm 2$. All experiments were done in accordance with the European Community Council Directive of 22nd September 2010 (2010/63/UE) and the National Ethics Committee (Agreement APAFIS#20702_2019072614086203_v2). Throughout these experiments, all efforts were made to minimize pain and discomfort of the mice. Mice were obtained from multiple independent litters over several months.

2. Experimental designs

Of the 194 mice, 97 mice (55 males and 42 females) experienced ELA between P4 and P11 and 97 mice (57 males and 40 females) remained under control rearing conditions (Figure 1). Cellular analysis was conducted in males, previously divided in three subgroups. In the first experiment, 31 control males and 30 ELA males were allocated for the analysis of the morphology of interneurons, as well as the activity

of the OB, OT, and VTA. Half of each group were exposed to a pleasant odor before sacrifice, while the other half were exposed to an unpleasant odor. In a second experiment, 15 control animals and 15 ELA animals were used for the analysis of the effect of ELA on adult neurogenesis.

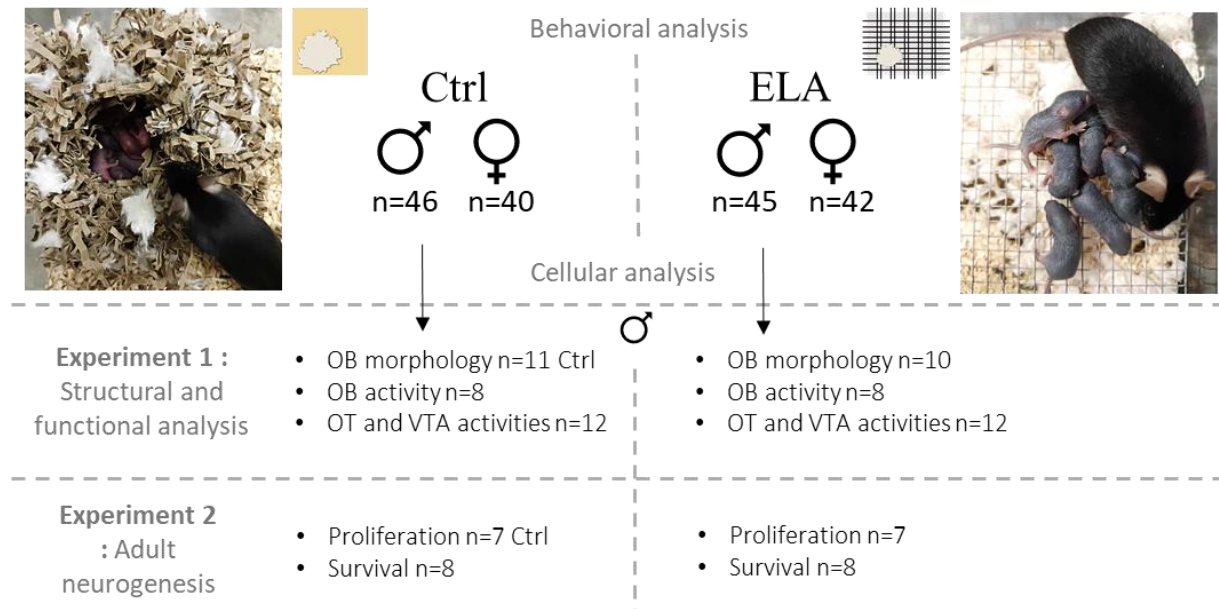


Figure 1. Experimental groups. 194 mice were divided postnatally into 2 groups, one experiencing ELA and the other remaining in control conditions. Regarding the neural bases, a first batch of mice was used to investigate the effects of ELA on the morphology of early born interneurons of the OB and activity of the OB, OT and VTA (Experiment 1). Another batch were used to assess the effect of ELA on adult neurogenesis (Experiment 2).

In “Experiment 1”, GFP plasmid injections were performed at P0-P1, prior to the ELA manipulation, (Figure 1 and 2) to allow fine morphological analysis of interneurons born during ontogenesis. In “Experiment 2”, BrdU injections were performed intraperitoneally in adult mice (50mg/kg, Sigma-Aldrich). Mice either received 3 injections, 2 h apart, on the same day and were perfused 2 h after the last BrdU injections to study cellular proliferation, or 20 days after the last BrdU injections for assessing neuronal differentiation and survival. For experimental subgroups, once mice reached adulthood, animals underwent behavioral testing from P50 to P70 on a battery of assays meant to measure olfactory function and emotional reactivity (see below). To investigate the expression of the immediate-early gene *c-Fos* based on the odor hedonic value, all mice were randomly exposed the day of sacrifice for 1 hour to either a pleasant or unpleasant odorant (Figure 1 and 2).

A. Experiment 1

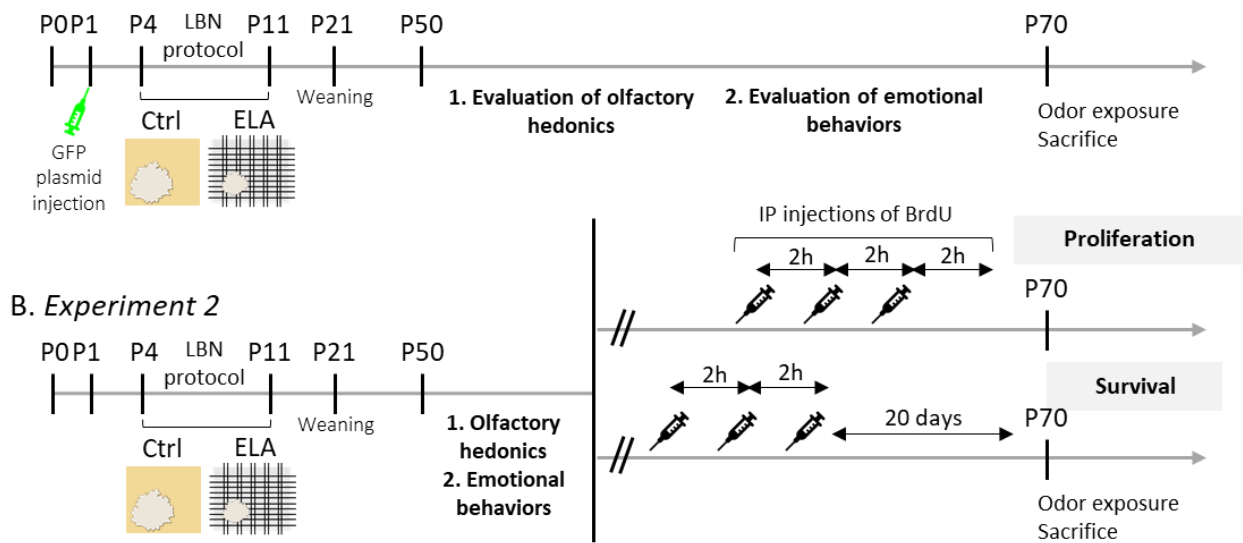


Figure 2. Experimental timelines. **A.** For analysis of neuronal structure, GFP plasmid injections were performed on subgroups of mice at P0-P1, then Ctrl or ELA condition was set up from P4 to P11 and behavioral testing occurred during adulthood. **B.** For neurogenesis investigations, BrdU injections were performed regarding a specific timing to ensure the study of proliferation or survival of adult-born neurons.

3. Limited bedding and nesting procedure

ELA was implemented through the use of the limited bedding and nesting (LBN) procedure. Following birth, dams and litter were randomly assigned to either control or ELA rearing conditions. This led to two experimental groups, i.e. controls and ELA. The LBN paradigm has been established to produce fragmented and unpredictable form of maternal care (Bath et al., 2016; Demaestri et al., 2020; Manzano Nieves et al., 2020; Manzano-Nieves et al., 2018; Rice et al., 2008). Four days following birth (P4, Figure 2), dam and pups were transferred from a standard home cage to a cage with wire mesh floor, no bedding and a small cotton nestlet (4x2 cm). Mice remained in these conditions for 7 days (until P11, see Figure 2), after which they were returned to standard housing conditions containing cob bedding and a 4 × 4 cm nestlet. Control mice remained in standard housing conditions throughout these procedures. All pups were weaned, gender segregated at 21 days of age, and housed 2 per cage.

4. Behavioral testing

All behavioral tests were performed during adulthood from P50 to P70. For experiments 1 and 2, we first assessed olfactory functions, then emotional behaviors in a random order (see below). The order of the tasks was the same between control and ELA mice.

4.1 Maternal interactions and early effects of the LBN protocol

Within “Experiment 2” (Figure 1), maternal behaviors and dams/pups interactions were analyzed during 1 hour every day, at randomly selected times of day, from P4 and P11. Male and female pups were

identified to assess sex difference in maternal care during the LBN protocol. On P4, P11, P21 and P50, animals were weighed. At the end of the ELA protocol (P11), after weighing, pups' motor behaviors were recorded during 1 min on a 50x50 cm board, and parameters that included speed, distance covered, and time spent immobile were analyzed using (A2V Volcano®).

4.2 Olfactory perception assessment

Odor Hedonic assessment. As a measure of odor hedonics, we assessed the investigation of six different odorants, (all unfamiliar to the mice and with no known biological significance). Based on prior work, three of the odorants are known to be “pleasant” and elicit significant investigation by mice (Limonene + (Lim; 5989-27-5), Citronellol (Citro; 106-22-9) and Camphor (Cam; 76-22-2) and three of the odorants are known to be “unpleasant” and are generally avoided by mice (Guaiacol (Gua; 90-051), P-cresol (Cre; 106-44-5) and Pyridine (Pyr; 110-86-1)). In order to equate the intensity of the odorants, each was diluted on the day of the test to reach similar vapor pressures (1 Pa). The test was performed on an automatic one-hole board apparatus (*Kermen et al., 2016; Mandairon et al., 2006; Midroit et al., 2021; Moreno et al., 2009*). 60 µL of 1Pa odorant was placed on a small cotton square and deposited at the bottom of a pot covered with bedding. The pot was put in the central hole of the board. Mice were placed at the bottom right corner of the board and allowed to freely explore the board for 2 minutes. Following the trial, the mouse and the pot were removed, and the board was cleaned with ethanol, and allowed to dry. The order of odor presentation was randomized between mice. For each mouse and odorant, the total time of exploration of the hole was automatically recorded based on the nose of the mouse breaking an IR beam at the entrance of the hole.

Odor detection assessment. To rule out the possibility that ELA impacts the ability to detect the olfactory stimuli, an odor detection test was performed using same odorants used for hedonic testing, at the same perceived intensity (1Pa). This experiment was performed in a standard home cage, and odorants were presented on filter paper (Whatman) in a tea ball hanging from a corner of the cage. Each mouse was tested on Lim, Citro, Cam, Gua, and Pyr in a random order. A test session consisted of three 50-second presentation of mineral oil (Hab), then one 50-second presentation of the odorant (Otest) with a 5-minute interval between phases of testing. Investigation was defined as active sniffing within 1 cm of the tea ball. Detection was indicated by a significant increase in investigation time between the Hab3 and Otest (dishabituation).

4.3 Tests of depressive-like behaviors

The Sucrose Preference test. This test was used to assess anhedonia (*Bolton et al., 2018*). Animals were placed in a cage with free access to two pipettes connected to two bottles of water: one contained only water and the other contained a 1% sucrose:water solution (bottles of 50 mL). The test was conducted over 3 days. Sucrose solution was freshly prepared every day. To avoid neophobia, sucrose at 1% was presented a week before the test to all animals, during a 24 hr period. No water or food deprivation was

applied. To prevent possible side-preference effects on drinking behavior, during the test, the bottles were switched from sides every 12 hours. Sucrose preference was calculated as the percentage of sucrose solution consumed (mL) relative to the total amount of liquid drunk during the 72-hour test (sucrose solution intake/total intake x 100).

Novelty Induced Hypophagia (NIH). The inhibition of feeding produced by novelty, termed “hyponeophagia,” provides a measure of depressive-like behavior in rodents. A significantly longer latency to approach the drinking bottle in the new cage should represent a decreased motivation to approach a sweet reward (*Goodwill et al., 2019*). The combination of willingness to approach a sweetened reward and anxiogenic new environment indexes both anxiety-like behavior and hedonic motivation to approach a sweet reward in a potentially aversive environment (*Dulawa, 2009; Dulawa & Hen, 2005*). For three consecutive days of training, mice were presented with sweetened condensed milk (Régilait®) diluted in water (1 g/mL) for 20 min in their home cage in the testing room. Milk was presented in 150 mL non-drip water bottles. The following day, the first day of test (Day 4), condensed milk was presented during 5 minutes in the same home cage and room. On day 5, animals underwent the same test but in a second room in a novel, clean cage under bright lighting with music playing and no bedding, to induce hyponeophagia. Lighting, volume and song were controlled across animals. Measures of latency to drink were compared between Day 4 (baseline) and Day 5 (novel cage) for both experimental groups.

Light/Dark Box (LDB) test. Mice were transported to a dimly lit testing room (approximately 20 lux) and allowed to acclimate for 20 minutes. The apparatus was composed of two compartments: one (2/3 of the box) was highly illuminated and the other one (1/3 of the box) was dark. The animal could freely move from one compartment to the other. All mice were placed at the left corner of the highly illuminated compartment of the box and the location and movement of the mouse was video recorded during 5 minutes and analyzed using A2V Volcano®. Time spent on the illuminated section was measured as an index of the level of anxiety-like behavior. The box was cleaned with 70% ethanol and dried between each trial.

Openfield test. Animals were placed in an open-field (50 x 50 cm). The maze was empty and designed to be large enough to elicit a decreased willingness to enter the illuminated center. All mice were placed at the top left corner. The time spent in the inner illuminated zone (4 cm from the border) and the number of entries in the inner zone were automatically recorded and analyzed as an index of anxiety-like behavior (A2V Volcano®). The open-field was cleaned with 70% ethanol and dried between trials.

4.4. Statistics

All analyses were performed using RStudio®. For behavioral analysis, we had no prior hypothesis about sex and therefore separated the analysis to investigate how stress impact each sex. We used parametric or non-parametric analysis based on the results of Shapiro and Levene tests used to respectively assess normality and homogeneity of variance. To compare two groups, bilateral two sample t-test or Mann-Whitney for unpaired data and Wilcoxon test for paired data were performed. Unilateral Two-Sample t-test were performed only when an assumption about the direction of the variation was made. To compare more than two groups in the hedonic perception analysis, 2 ways ANOVA was performed followed by Bonferroni corrected paired t-test. For olfactory detection analysis, a repeated measure ANOVA was performed followed by paired Bonferroni post hoc tests.

5. Sacrifice

At P70 (Figure 2), all mice were sacrificed 1 hour after odor stimulation using euthasol (1.25 µL/g) and transcardial perfusion of 50 ml of fixative (PFA 4%, pH = 7.4). For odor stimulation we used the same odorants as in the olfactory hedonic test, the two most attractive, Lim, Citro, and the two least attractive, Gua and Pyr. Briefly, each mouse was placed in individual clean cages for 1 hour, then, a polypropylene swab saturated with one odorant (1Pa) chosen randomly among the 2 previously cited for each category (pleasant or not). This odorant was placed in a tea ball for a further hour. Finally, mice were sacrificed 1 hour later. This 1- hour delay enables the expression of the immediate early gene c-Fos in response to odorant stimulation. The brains were then removed, post-fixed for 2 hours, cryoprotected in sucrose (20%) for 7 days, frozen rapidly at -48°C, and then stored at -20°C before serial sectioning with a cryostat (40 µm and 14 µm thick for the OB allowing respectively cellular reconstruction and c-Fos analysis, 14 µm thick for the rest of the brain, 304 µm intervals between samples).

6. Cellular analysis

6.1. Analysis of granule cell morphology

GFP Plasmid electroporation. To analyze the morphology of granule cells born during the ontogenesis of the OB, cells were labelled by electroporation of GFP plasmid at P1 (Fernández *et al.*, 2011). More specifically, a pPB(Exp)-CAGG>3xNLS/EmGFP plasmid (pPB-EmGFP plasmid, 1 µg/µL) was co-electroporated with a transposase containing plasmid pRP(Exp)-PuroCAGG>Pbase (pRP-pbase, 2 µg/µL) to allow plasmid integration. This transposase approach allows the permanent GFP labeling of the entire cell cytoplasm including its cellular body and arborization. Pups were first anesthetized for 2 minutes on ice and then placed and fixed on a custom-made support plate placed in a stereotaxic rig. The injection of 1.5 µL of the plasmid solution (containing a contrast solution, i.e. fast green (Sigma-Aldrich, St. Louis, Missouri, USA) at a final concentration of 1 %) into the lateral ventricle was carried out using a Hamilton 34G syringe at a depth of 2mm depth from the skull surface. Injected animals were then subjected to 5 pulses at 95 V of 50 ms separated by 950 ms with an Electroporator NEPA21 type

II (Nepa Gene Co., Ltd) and tweezer electrodes coated with a conductive gel (Signa gel, Parker Laboratories, Fairfield, New Jersey, USA). Animals were then warmed on a hot plate at 36.4°C and then returned to the cage with their mother.

Immunohistochemistry, confocal microscopy, 3D-reconstruction and Sholl analysis. GFP-positive neurons activated in response to pleasant or unpleasant odorants were identified using c-Fos expression. Labeling was performed on 40µm thick OB sections using rabbit anti-GFP (1/750, Chemicon/Millipore) and rat anti c-Fos antibodies (1/2000, Synaptic System). Appropriate secondary antibodies were used, goat anti-rabbit Alexa 488 (1/200, Molecular probes) and goat anti rat Alexa 546 (1/200, Molecular probes). Photos of the GFP/c-Fos-positive neurons in response to pleasant or unpleasant odorants were taken using Z stacks images (z-step of 0.8 µm) with a LSM80 confocal microscope (x20; 1024 px resolution). 3D-neuron reconstructions were then performed using NeuroLucida 360 software (MBF®) and analyzed on NeuroLucida Explorer (MBF). All measurements were done blind with regards to the identity and treatment of the mouse. The length of basal and apical dendrites of neurons was measured and compared between control and ELA mice. Then, a Sholl analysis was performed with the origin of the concentric radii set at the first branching point of the apical dendrite and ring radius increments of 10µm. Neurons were analyzed when sufficiently isolated from other neurons, with the cell soma approximately in the center of the section (*Heun-Johnson & Levitt, 2018; Rincel et al., 2018*).

Confocal microscopy, 3D-reconstruction and spine analysis. GFP-positive neurons responding to pleasant or unpleasant odorants were identified using c-Fos double-labeling, as previously described. Photos of basal and apical dendritic segments of GFP/c-Fos-positive responding to pleasant or unpleasant odorants were taken using Airy scan Z stacks image (z-step of 0.18 µm) with a Zeiss® LSM80 confocal microscope (x63, 2248 px resolution). 3D-reconstruction of dendrites and spine detection and classification was then performed using NeuroLucida 360 software (MBF) and analyzed on NeuroLucida Explorer (MBF). All measurements were done blind with regards to the identity and treatment of the mouse. First, the spine density on basal and apical segments were measured and compared between control and ELA mice. Those segments of dendrites were randomly chosen among neurons, but all placed at the same distance from the soma to avoid bias from dendrite branches order.

6.2 Cellular activity of odor hedonic processing pathway: OB, OT and VTA

Immunohistochemistry and image acquisition. To investigate the activity of OB granule cells in response to pleasant or unpleasant odorants, we analyzed sections (4±1 per animal) of 14µm-thick with c-Fos immunostaining (rat anti-c-Fos antibodies (1/2000, Synaptic System)). To assess the activity of mitral cells, we used a double-labeling using rat anti-c-Fos (1/2000, Synaptic System) and rabbit anti-Tbx21 (1/400, Synaptic System). Photos of the whole OB were taken using slide scanning workflow with

Neurolucida, then density of c-Fos-positive granule cells was measured using Neurolucida Explorer (MBF) and the percentage of activated mitral cells was counted using Qpath.

To investigate the neural activity in the OT and VTA in response to pleasant versus unpleasant odorants, a double labelling was performed using rabbit anti-DARPP-32 (1/500, AbCam) and polyclonal mouse anti-c-Fos antibodies (1/500, Synaptic System) or chicken anti-TH (1/2000, AbCam) and monoclonal rat anti-c-Fos antibodies (1/20000, Synaptic System) respectively (*Midroit et al., 2021*). Appropriate secondary antibodies were used (goat anti-rabbit Alexa 488 (1/250, Molecular probes); goat anti-mouse Alexa 546 (1/250, Molecular probes) to label DARPP32/c-Fos positive cells or goat anti-chicken Alexa 546 (1/250, Molecular probes) and goat anti-rat Alexa 488 (1/250, Molecular probes) to label TH/c-Fos positive cells). All fluorescent cell counting was done blind with regards to the identity and treatment of the mouse using AxioVision (Zeiss) software coupled to a pseudo-confocal Zeiss microscope. A mean of 90.2 (SEM=19.6) DARPP32+ cells were counted for the OT and a mean of 24.6 (SEM=7.74) TH+ cells were counted for the VTA. We calculated the percentage of TH or DARPP-32 cells expressing c-Fos-positive cells per mouse and averaged the values per group.

6.3 Analysis of adult neurogenesis

To investigate the rate of cellular proliferation within the SVZ, we performed immunohistochemistry using a mouse anti-BrdU antibody (1/100 Sigma MAB4072) on 14 μ m thick sections (4 \pm 1 per animal). To assess the survival of adult-born granule cells and their integration to the network, double-labeling was performed on 14 μ m-thick sections (4 \pm 1 per animal) of the OB using mouse anti-BrdU (1/100 Sigma MAB4072) and monoclonal rat anti-c-Fos (1/2000 Synaptic System). Appropriate secondary antibodies were used (goat anti-mouse Alexa 488 (1/200 Molecular probes) to label BrdU cells and goat anti-rat Alexa 546 (1/200 Molecular probes) to label c-Fos positive cells). Photos were acquired using a slice scanner (Zeiss Axio Scan 7) and all fluorescent counting were done blind with regards to the identity and treatment of the mouse using Neurolucida software.

6.4 Statistics

For comparisons involving additional factors related to pleasant odorant processing, such as distance from the soma, antero-posterior axis, and branch type, we applied ANOVAs. When testing only the differences between control and stress groups, t-test or Wilcoxon tests were used, depending on the data distribution. Parametric or non-parametric tests were selected based on the outcomes of the Shapiro-Wilkoxon and Levene tests, which assess normality and homogeneity of variance, respectively. Unilateral Two-Sample t-test were performed only when an assumption about the direction of the variation was made. Therefore, for Sholl analysis (Experiment 1), a 2 ways ANOVA was performed using rearing conditions and distance from the soma as factors. For spine analysis, a 2 ways ANOVA was performed with rearing conditions and branch-type (apical or basal) as factors. Bonferroni post-hoc tests with corrections were performed when possible. For cellular activity analysis in the OB (granule

and mitral cells) (Experiment 1), ANOVA was performed with position along the antero-posterior axis and rearing conditions as factors, followed by Bonferroni post-hoc tests. For neurogenesis analysis (Experiment 2), t-test were performed to compared density of BrdU+ cells between groups in both the SVZ and the OB. To compare percentage of BrdU+/c-Fos+ cells and their density, 2 ways ANOVA with rearing conditions and position along the antero-posterior axis were performed. For neural activity analysis in the OT and VTA, bilateral unpaired t-test were performed.

Results

ELA deteriorates maternal care, alters pups' growth and motor activity

We first investigated the impact of ELA on maternal care and found that dams subjected to ELA displayed altered maternal care as evidenced by an increase in latency to build the nest at P4 (unpaired bilateral t-test, $p=0.044$; Figure 3Ai) and a decrease in time spent in the nest compared to control animals (unpaired bilateral t-test, $p=0.002$; Figure 3Aii). In addition, abusive behavior was also observed in ELA mothers, as evidenced by an increase of male (unpaired unilateral Wilcoxon test, $p=0.0012$; Figure 3Bi) and females pups isolation time (unpaired unilateral Wilcoxon test, $p=0.012$; Figure 3Bi), and in the number of aggressive events (kicking and biting) toward both males (unpaired unilateral Wilcoxon test, $p=0.0022$; Figure 3Bii) and females (unpaired unilateral Wilcoxon test, $p=0.0063$; Figure 3Bii). We also investigated whether mother subjected to ELA procedure acted differently toward male or female pups and found no sex difference in degree of isolation (unpaired bilateral Wilcoxon test, $p=0.71$; Figure 3Bi) or rough treatment (unpaired bilateral Wilcoxon test, $p=0.89$; Figure 3Bii).

Regarding the impact of ELA on pup behavior, we found a decrease in the average speed (ANOVA rearing effect $F(1.94)=5.859$, $p=0.017$; Figure 3Ci), a lower distance traveled (ANOVA rearing effect $F(1.94)=8.569$, $p=0.004$; Figure 3Cii) and an increased immobility time (ANOVA rearing effect $F(1.94)=5.047$, $p=0.027$; Figure 3Ciii) compared to controls. No gender effect was found for any of these parameters (speed, $F(1.94)=2.793$, $p=0.098$, distance, $F(1.94)=0.336$, $p=0.564$ nor amount of immobility, $F(1.94)=0.422$, $p=0.518$). Altogether, these results support a direct effect of ELA on maternal behavior, as well as an alteration in male and female pup's locomotor activity.

Finally, ELA significantly slowed down the growth of pups, with more lasting effects on males (three-way mixed ANOVA, rearing effect $F(1.57)=4.653$, $p=3.50e-02$; day effect $F(1.24,70.46)=4016.33$, $p=8.43e-67$), sex effect $F(1.57)=82.975$, $p=1.03e-12$). Indeed, at P11, a lower weight was found in ELA male (Bonferroni post-hoc tests, $p=0.004$; Figure 3Dii) and female pups (Bonferroni post-hoc tests, $p=0.003$; Figure 3Dii) compared to controls. At P21, while the weight of ELA males (Bonferroni post hoc test, $p=0.050$; Figure 3Dii) was still different from controls, it returned to control values for females (Bonferroni post hoc test, $p=0.953$; Figure 3Dii). ELA reared mice of both sexes were indistinguishable from control weights by P70 (Bonferroni post hoc test, Males, $p=0.39$ and Females, $p=0.83$ Figure 3Dii).

At P70, females weighed less than males in the control (Bonferroni post hoc tests, $p=3.27e-13$; Figure 3Dii) and ELA groups (Bonferroni post hoc tests, $p=1.20e-5$; Figure 3Dii).

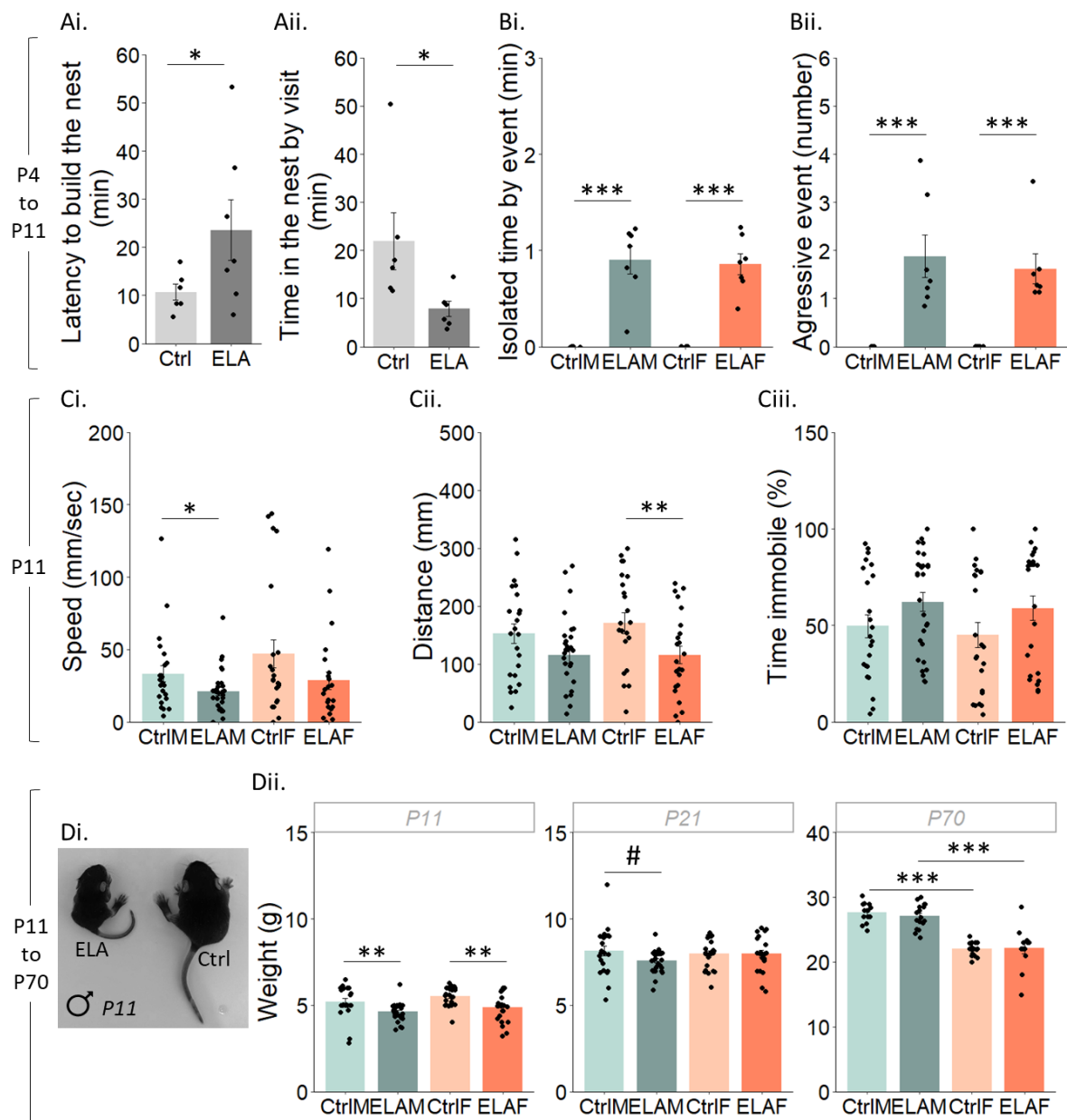


Figure 3. ELA alters maternal behavior and pup's physiology and behavior. **Ai-Aii.** The latency to build a nest was enhanced and the overall time spent on the nest was decreased in ELA mice compared to controls. **Bi-Bii.** The time spent isolated and the number of aggressive events were increased in ELA pups compared to controls with no difference between male (M) and female (F) mice. **Ci-Ciii.** Locomotor activity was altered in ELA animals compared to controls as observed by changes in speed, covered distance and immobility time. **Di.** Example of control (Ctrl) and ELA male pups at P11. **Dii.** ELA delayed the growth of male and female pups with lower weight in both sexes at P11 compared to controls but no more at P21 and adults. Points represent individual data \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, # $p = 0.05$.

ELA alters emotional behaviors in adult male but not female mice.

To examine the effects of ELA on emotional behavior of adult male and female mice several assays were used. First, to measure of anhedonia-like behavior and motivation in mice, the sucrose preference test and the NIH test were used (*Castagné et al., 2011*). Compared to male control mice, adult male ELA mice showed a reduction of sucrose consumption (unpaired unilateral Wilcoxon test, $p=0.017$; Figure 4Ai) and an increased latency to approach the sweetened milk in the novel anxiogenic environment of the NIH test (unpaired unilateral Wilcoxon test, $p=0.004$; Figure 4Bi). This indicated a significant effect of ELA on anhedonia-like behavior or the loss of motivation to consume the reward liquid, which was not observed in adult female mice (sucrose consumption, unpaired unilateral Wilcoxon test, $p=0.23$, Figure 4Aii, and NIH test, unpaired unilateral Wilcoxon test, $p=0.59$; Figure 4Bii).

As a measure of anxiety-like behavior, mice were tested in the light/dark box assay. For males, no significant difference between ELA and control male mice was observed (unpaired unilateral T test, $p=0.5$; Figure 4Ci). However, in the openfield, ELA male mice spent less time in the illuminated center compared with control mice (unpaired unilateral Wilcoxon test, $p=0.027$; Figure 4Di), suggesting moderate level of anxiety after ELA. In females, no change in the light/dark box test (unpaired unilateral T test, $p=0.08$; Figure 4Cii), nor openfield test (unpaired unilateral Wilcoxon test, $p=0.31$; Figure 4Dii) were observed. To confirm these conclusions and better understand mouse phenotype, we calculated an emotional index based on these behavioral results. A score was calculated for each mouse on each of the four tests and expressed as a percentage (from 0% to 100%). Scores of all tests were added. A significant difference in the mean index between ELA and control groups was found in adult male (unpaired bilateral Wilcoxon test, $p=0.00025$; Figure 4Ei) but not in adult female mice (unpaired bilateral Wilcoxon test, $p=0.47$; Figure Eii).

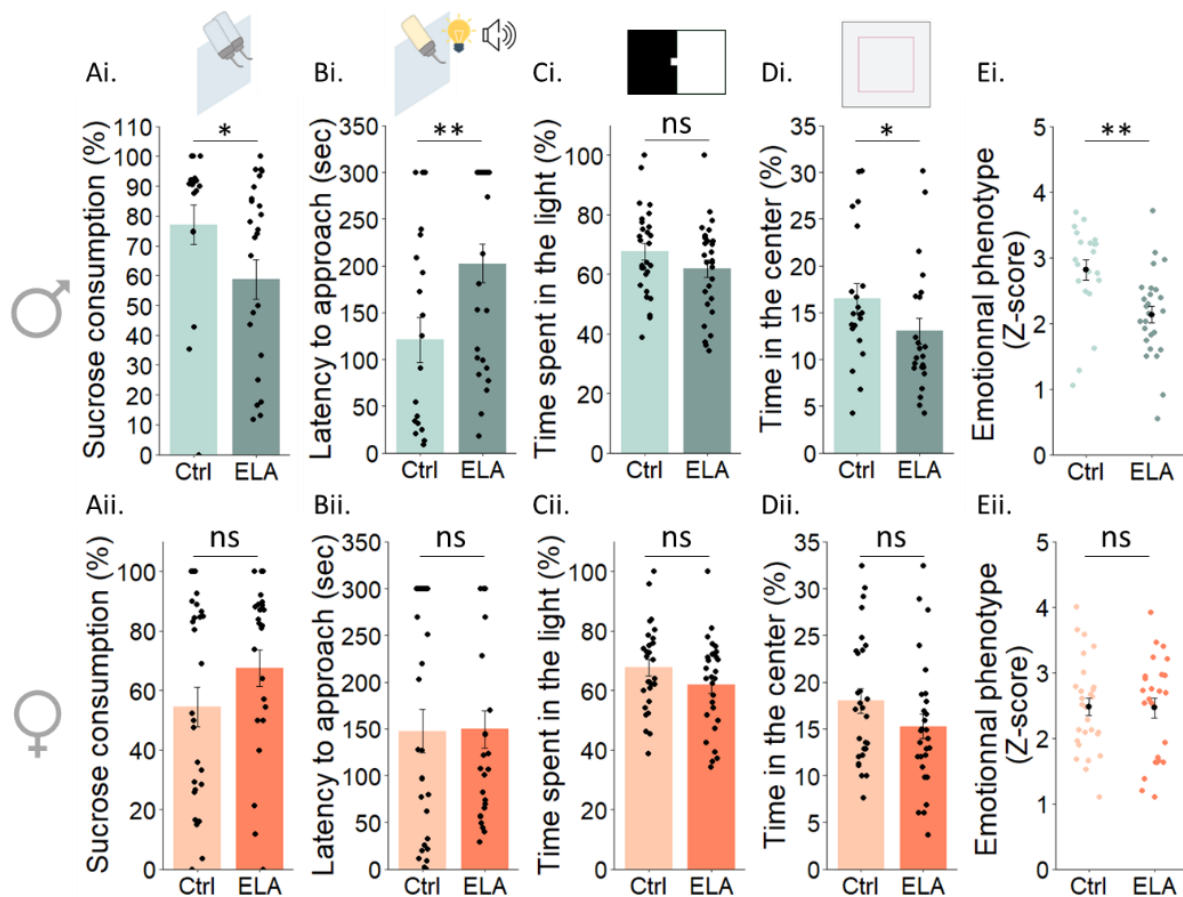


Figure 4. ELA alters emotional behaviors in adult male mice only. (Above). Experimental designs for sucrose consumption test, novelty induced hypophagia test (NIH), light/dark box test and openfield test. **A-D**. Adult ELA male mice showed evidence of a depressive-like behavior and moderate anxiety. Decreased sucrose preference or anhedonia (**A**), increased latency to approach the sweetened milk in an anxiogenic environment (**B**) and less time spent in the illuminated stressful center of the openfield apparatus (**D**) was found in ELA compared to control mice. **E**. Taken together, in males, ELA reared mice displayed an alteration of their emotional phenotype while control mice did not. **F-J**. No change was observed between ELA and control groups in adult females. $n=21$ Ctrl and 26 ELA male mice, $n=26$ Ctrl and 28 ELA females. Points represent individual values \pm SEM, * $p < 0.05$, ** $p < 0.001$.

ELA alters the perception of odorants with positive hedonic value in adult male mice

As ELA showed a significant impact on emotional phenotype of adult male but not female animals (two-way ANOVA, significant interaction between rearing and sex effect, $F(1,97)=5.457$, $p=0.022$), we focused for all subsequent analyses. Odor investigation time is reported as a measure for odor-driven attraction (Mandairon et al., 2009). We studied attraction in response to odorants known to be pleasant (Cam, Citro, Lim) and known to be unpleasant (Pyr, Gua, Cre) in male control and ELA mice (Kermen et al., 2016; Midroit et al., 2021). We first confirmed that attraction to pleasant odorants was higher than to unpleasant odorants in control mice (Bonferroni post hoc tests, $p=0.005$; Figure 5A). Remarkably, we found that the effect of ELA differed depending on the hedonic value of the odorant (two-way mixed ANOVA, significant interaction between the rearing condition and odor hedonic value

$F(1.45)=11.188$, $p=0.002$; Figure 5A). Thus, ELA mice showed significantly less interest in pleasant odors than controls (Bonferroni post hoc tests, $p=0.0009$; Figure 5A), while no difference was observed for investigation of unpleasant odors (Bonferroni post hoc tests, $p=0.43$; Figure 5A). To ensure that this observed change in hedonic perception in ELA mice was not due to an impairment in odor detection ability, we tested the ability of mice from each group to detect the odors at the same concentration as the one used in the odor preference test, by performing an odor habituation/crosshabituation test. We confirmed that ELA animals were able to detect pleasant (repeated measures ANOVA, $F(5.16)=29.293$, $p=6.71e-06$, Bonferroni post hoc tests for difference between Hab4 and OTest, $p=9.85e-06$; Figure 5Bi) and unpleasant odors (repeated measures ANOVA, $F(5.16)=18.634$, $p=0.000151$, Bonferroni post hoc tests for difference between Hab4 and OTest, $p=3.47e-05$; Figure 5Bii).

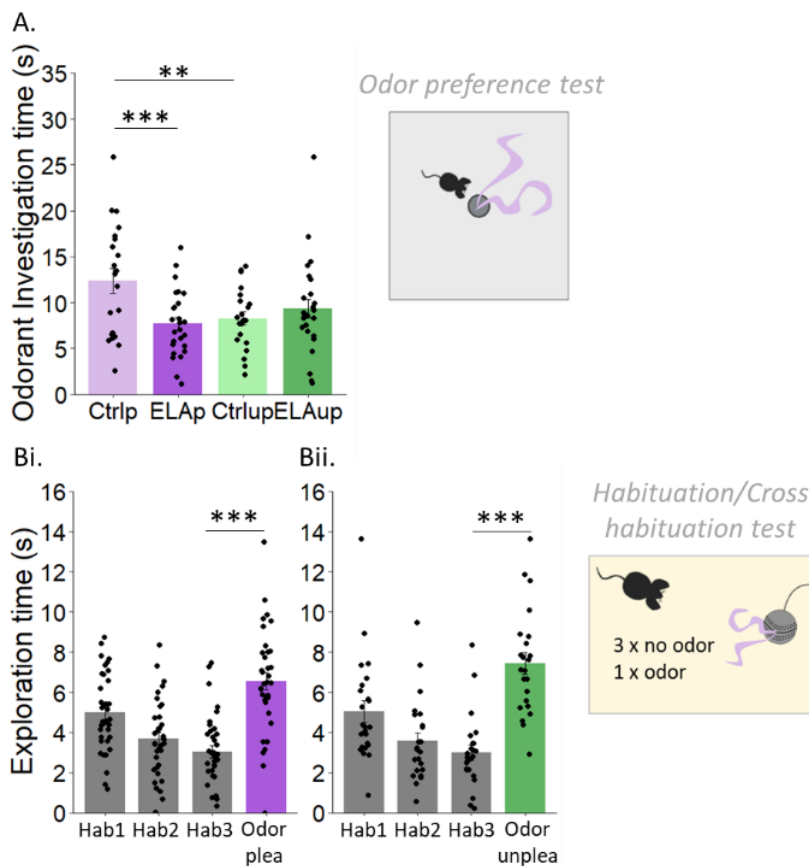


Figure 5. ELA alters the perception of pleasant odors in adult male mice. **Ai.** Odor attraction was different between pleasant (p) and unpleasant (up) odors in control mice only. ELA decreased odor attraction in response to pleasant odor in the odor preference test. **Bi-Bii.** ELA mice detected correctly both pleasant (Bi) and unpleasant odors (Bii) as observed in the Habituation/Cross habituation test with a significant increase in investigation time between the Test trial (with the odor) and Hab3 trial with no odorant. $n=21$ Ctrl and 26 ELA male mice. Points represent individual values \pm SEM, $**p<0.001$, $***p<0.0001$.

ELA affects the fine morphology of OB interneurons responding to pleasant odors.

In the OB, granule cells are critical for supporting odors hedonic perception (*Kermen et al., 2016*). Granule cells continue to be produced postnatally (P0-P1) and integrate OB neural networks during the stress procedure (P4-P11). Thus, to understand the neural basis underlying the alteration of pleasant odorant perception, we analyzed the morphology of P1 born granule cells (labeled with GFP after plasmid injection at P1 in the ventricular zone; Figure 6A and Bi) as well as their response to pleasant odors in adulthood (c-Fos-positive as a reflect of neural activity). ELA and control animals were sacrificed 70 days post-injection, after behavioral testing and exposure to odors. We analyzed dendritic arborization and spine density of the apical distal domain (main site of interactions with mitral/tufted cells) as well as the basal domain (main site of centrifugal inputs) for c-Fos-positive granule cells responsive to pleasant odors (*Mandairon et al., 2018*). We found that ELA altered dendritic arborization of c-Fos-positive cells, and that these effects were dependent on the distance from the soma (2-way mixed ANOVA, interaction between rearing and distance from the soma conditions, $F(28.211)=2.157$; $p=4.19 \times 10^{-4}$). More precisely, Sholl analysis revealed a decreased number of intersections between dendrites and radii in the basal domain of interneurons following ELA (Bonferroni *post hoc* test, $p < 0.05$ for radius 20, 40, 50, 60, 70, 80, 90; Figure 6Bii), while the apical domain remained unaffected.

We next analyzed the density of dendritic spines in the apical (Figure 6Ci) and basal (Figure 6Di) domains of granule cells responding to pleasant odors. Interestingly, we found that ELA decreased the spine density in the basal but not apical domain (2-way ANOVA, interaction between rearing and domain effect $F(1.54)=4.724$, $p=0.034$; Bonferroni *post-hoc* tests, Ctrl versus ELA basal $p=0.00364$ and Ctrl versus ELA apical $p=0.88$; Figure 6Cii and 6Dii). We further determined whether or not ELA rearing impacted spine morphology in the basal domain of the dendritic arbor. We separated spines into three subtypes (filopodia, mushroom, and thin) and quantified these across groups. We found that ELA was associated with a lower density of spines filopodia in the basal branches of granule cells responding to pleasant odors in ELA reared mice compared to controls, with no effects on other spine types (Bonferroni *post-hoc* tests, filopodia $p=0.0003$; mushroom $p=0.09$; thin bas $p=0.1$; Figure 6Cii).

In contrast to these results, dendrites of granule cells responding to unpleasant odors showed only minor changes following ELA. Thus, while a significant effect of ELA was also found on the overall dendritic complexity of interneurons responding to unpleasant odorant (two-way mixed ANOVA, interaction between rearing condition and distance from the soma, $F(29.1593)=0.737$, $p=8.44 \times 10^{-1}$), a significant difference between groups was only found at one distant radius (Bonferroni *post hoc* test, $p=0.003$ for radius 260; Figure Ei). This suggests a more modest or spurious impact of ELA on the morphology of neurons responding to unpleasant odors. Further, no effect of ELA on spine density in either the basal or apical domains were observed (2 way ANOVA, Rearing effect $F(1.48)=1.586$, $p=0.214$; domain effect $F(1.48)=2.646$, $p=0.11$; Interaction $F(1.48)=1.164$, $p=0.286$; Figure 6Eii and 6Eiii). In conclusion, ELA disrupted the fine morphology of interneurons responding to pleasant

odorants, with specific effects on the basal dendritic segment, which is typically contacted by neuromodulators involved in OB's regulation (Brunert & Rothermel, 2021; Linster et al., 2020). These interneurons are crucial for the direct modulation of mitral cell activity, and thus for shaping the OB's output signal.

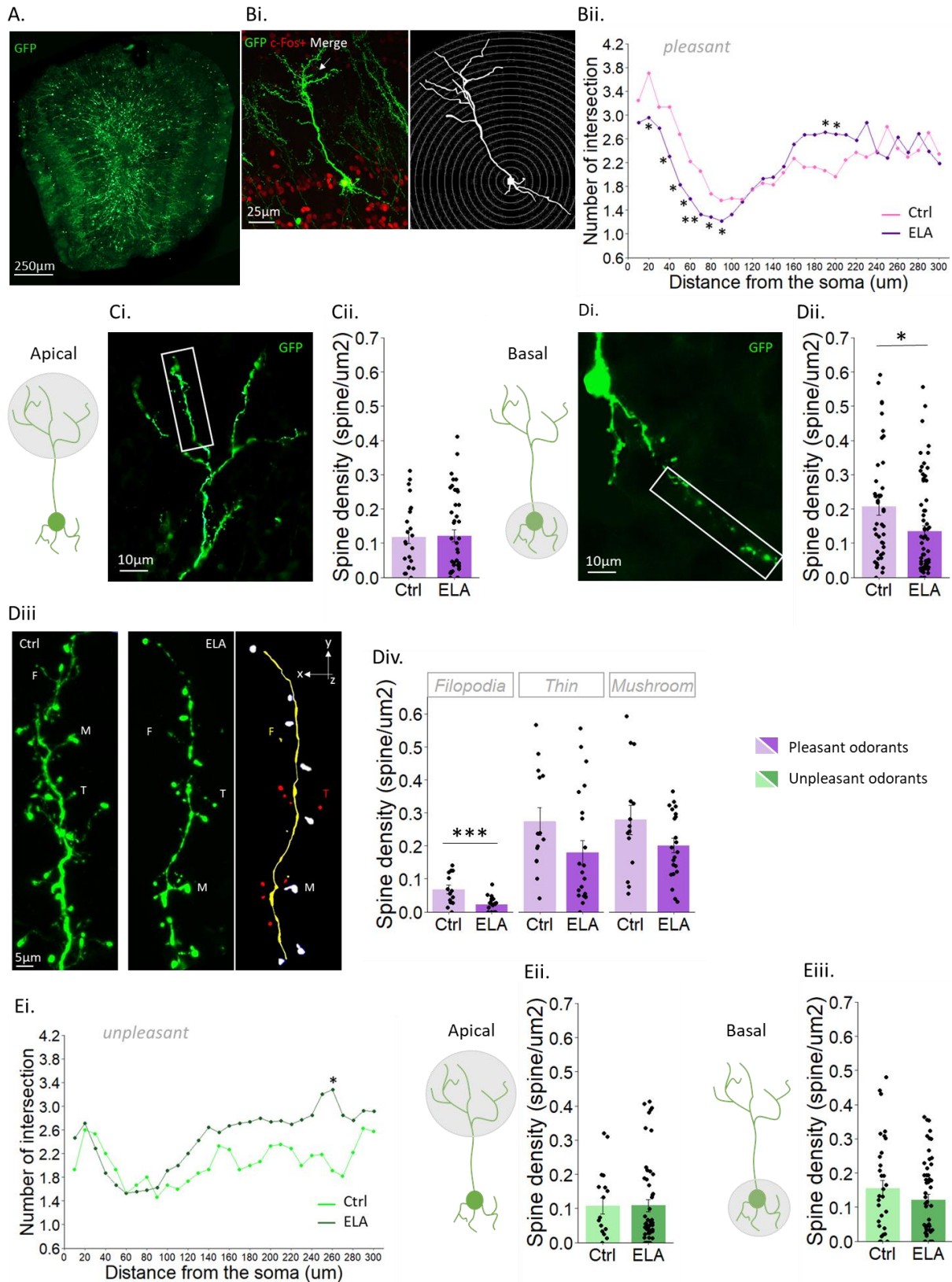


Figure 6. ELA alters the fine morphology and spine density of early born OB granule cells. **A.** Photo of the OB (Zeiss microscope tile images compiled x20). **Bi.** (Left) Maximum projection images obtained with Z-stack photos of a GFP/c-Fos+ granule cell (confocal microscope, x20, 1024p x resolution) and its 3D reconstruction before Sholl analysis (right). **Bii.** Basal dendritic complexity of granule cells responding to pleasant odorants (c-Fos+) is reduced after ELA compared to control. *Sholl analysis with origin of the concentric radii sets at the first branching point of the apical dendrite. n=42 neurons from 6 ELA mice and 37 from 6 controls for pleasant odorants (mean=7 neurons per animal ±2)* **Ci.** Maximum projection images obtained with Z-stack photos of a GFP+ granule cell in its apical domain (x20 2248px resolution). **Cii.** The synaptic density on apical dendrites of c-Fos+ granule cells was similar in ELA mice compared to controls. **Di.** Maximum projection images obtained with Z-stack photos of a GFP+ granule cell in its basal domain (x20 2248px resolution). **Dii.** The synaptic density on basal dendrites of c-Fos+ granule cells was decreased in ELA mice compared to controls. **Diii.** Maximum projection image obtained with Z-stack photos of an active GFP+ OB dendritic branche with classified spines (F: Filopodia; T: Thin; M: Mushroom; airy scan Z stacks image x63, 2248px resolution) in Ctrl animal (left), or ELA animal with its detection/reconstruction with NeuroLucida 360 (right). **Div.** The spine density of filopodia was decreased in the basal domain of interneurons in ELA animals. **Ei.** Moderate change in dendritic complexity was observed in interneurons responding to unpleasant odorants in ELA animals compared to controls. *n= 52 neurons from 5 ELA mice and 40 neurons from 4 controls for unpleasant ones (mean=10 neurons per animal ±4.* **Eii and Eiii.** ELA did not change the spine density in the apical (Eii) or basal (Eiii) branches of granule cells responding to unpleasant odorants. *n=39 neurons of Ctrl mice and n=34 neurons of ELA mice. *p < 0.05, **p<0.001.*

ELA altered activity of both OB interneurons and projecting cells.

We analyzed c-Fos gene expression in granule cells in response to pleasant odorants and found an increase of c-Fos-positive cell density in ELA compared to control animals (bilateral unpaired t-test, $p=0.008$; Figure 7Ai). Previous studies indicate that the neural signature supporting the pleasant perception of odorants tend to be localized in the anterior OB (Kermen et al., 2016). Thus, we analyzed c-Fos density based on the OB anterior/posterior axis. Interestingly, we found that the differences in neural activity in response to pleasant odorants between ELA and control animals were most robust in the anterior OB, with non-significant effects in its part (rearing effect, $F(1.15)=9.670$, $p=0.007$; OB region effect $F(1.15)=3.2$, $p=0.09$; Bonferroni *post-hoc* test Ant, Ctrl vs ELA, $p=0.0165$ and Post, Ctrl vs ELA, $p=0.660$; Figure 7Aii and Aiii).

We then analyzed how this change in the inhibitory granule cell activity after ELA affects the OB output by investigating the percentage of odor-activated mitral cells (co-expressing the specific mitral cell marker Tbx1 (Mitsui et al., 2011) and c-Fos). We showed a decrease of mitral cells activity in the anterior OB of ELA animals compared to controls (two-way ANOVA, rearing effect, $F(1.30)=11.691$, $p=0.002$; OB region effect $F(1.30)=4.465$, $p=0.043$; Bonferroni *post-hoc* test Ant, Ctrl vs ELA, $p=0.01$; and Post, Ctrl vs ELA, $p=0.66$; Figure 7Bi and Bii).

The OB is a unique brain region with a lifelong production of interneurons (Lledo et al., 2006; Lledo & Valley, 2016). We next sought to test whether ELA also impacts adult neurogenesis. To address this question, we labeled adult-born neurons using BrdU, a marker that is integrated into actively dividing cells and remains in those cells throughout their life. Effects on proliferation and integration/survival of newborn neurons were assessed by sacrificing mice 2 hours or 20 days following BrdU injection, respectively. Regarding proliferation, we found that BrdU+ cell density in the SVZ of animals with

history of ELA were similar to those observed in control mice (unpaired bilateral t-test, $p=0.15$; Figure 7Cii). In contrast, survival of adult-born neurons were affected by ELA in both the anterior and posterior OB (two-way ANOVA, rearing effect, $F(1,28)=7.190$, $p=0.012$; OB region effect $F(1,28)=0.446$, $p=0.510$; Bonferroni *post-hoc* test Ant, Ctrl vs ELA, $p=0.08$; and Post, Ctrl vs ELA, $p=0.06$; Figure 7Di and Dii). We then assessed whether ELA influenced the activity of adult-born cells and found a decreased in the percentage of BrdU-positive cell expressing c-Fos in response to pleasant odors in the granule cell layer (two-way ANOVA, rearing effect, $F(1,12)=4.633$, $p=0.05$; OB region effect $F(1,12)=0.094$, $p=0.764$; Bonferroni *post-hoc* test Ant, Ctrl vs ELA, $p=0.16$; and Post, Ctrl vs ELA, $p=0.18$; Figure 7Diii). However, the overall density of BrdU-positive cell expressing c-Fos in response to pleasant odors was similar between groups (two-way ANOVA, Rearing effect $F(1,12)=2.44$ $p=0.144$; OB region effect $F(1,12)=0.00028$, $p=0.987$; Figure 7Div). Interestingly, the change of adult-born neurons survival did not lead to a modification of the overall cellular density in the granule cell layer (DAPI-positive cells, two-way ANOVA, rearing effect, $F(1,49)=0.147$, $p=0.7$; Ctrl Ant mean= 6.863 ± 0.64 , Ctrl Post mean= 6.176 ± 0.51 , ELA Ant mean= 6.736 ± 0.74 , ELA Post mean= 6.157 ± 0.82), nor in granule cell layer area (unpaired bilateral t-test, $p=0.3$; Ctrl mean= $1.502\text{cm}\pm0.3$, Ctrl mean= 1.361 ± 0.3).

Finally, we analyzed the impact of ELA on the activity of granule cells in response to unpleasant odors and observed a similar level of c-Fos-positive cell density in ELA compared to control animals (Ctrl, mean= 0.953 ± 0.591 ; ELA, mean= 1.281 ± 0.550 ; unpaired bilateral t-test $p=0.39$; Figure 7Ei). When the OB was divided along its antero-posterior axis, we did not observed difference in c-Fos density in the anterior or posterior OB between ELA and control animals (two-way ANOVA, Rearing effect $F(1,9)=0.862$, $p=0.377$; OB region effect $F(1,9)=8.667$, $p=0.016$, Bonferroni *post-hoc* test Ant, Ctrl vs ELA, $p=0.158$ and Post, Ctrl vs ELA, $p=0.184$; Figure 7Ei). In line with this result, we did not observed a significant change in the percentage of odor-activated mitral cells (two-way ANOVA, Rearing effect $F(1,27)=0.347$ $p=0.561$; OB region effect $F(1,27)=1.210$, $p=0.281$; Figure 7Eii). Finally, the percentage of BrdU-positive cell expressing c-Fos in response to unpleasant odorant was increased in the OB of ELA compared to control animals in response to unpleasant odors (two-way ANOVA, Rearing effect $F(1,12)=9.236$, $p=0.01$; OB region effect $F(1,12)=0.004$, $p=0.95$; Bonferroni *post-hoc* test Ant, Ctrl vs ELA, $p=0.047$; and Post, Ctrl vs ELA, $p=0.11$; Figure 7Eiii). The overall density of BrdU-positive cells expressing c-Fos in response to unpleasant odors was also increased (two-way ANOVA, Rearing effect $F(1,12)=8.459$ $p=0.01$; OB region effect $F(1,12)=1.950$, $p=0.188$; Bonferroni *post-hoc* test Ant, Ctrl vs ELA, $p=0.06$; and Post, Ctrl vs ELA, $p=0.09$; Figure 7Eiv).

Here, we found that ELA altered neural processing in the OB, affecting the output signal in response to pleasant but not unpleasant odors, with effects on both neonatal and adult neurogenesis.

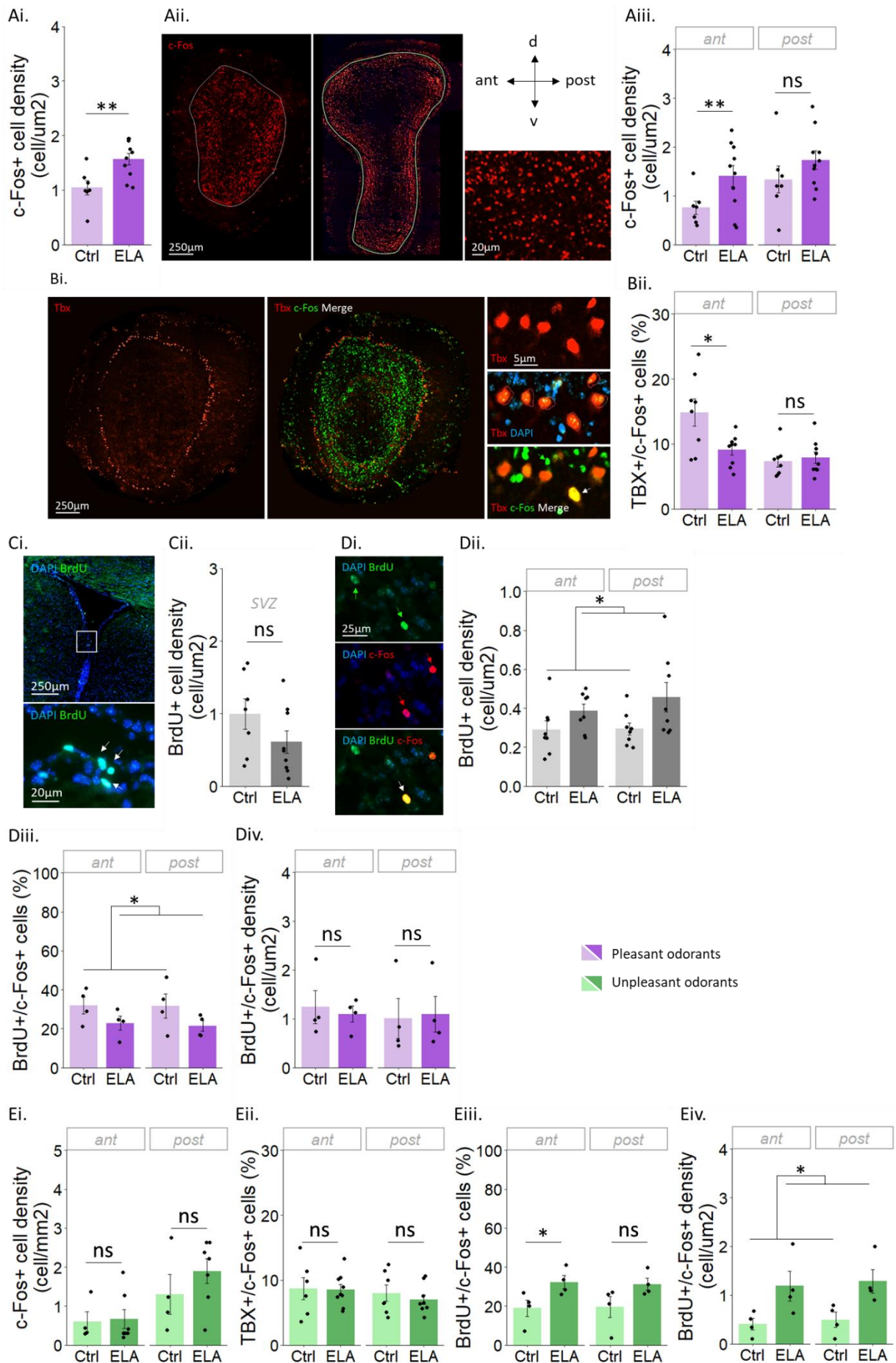


Figure 7. ELA alters OB's response to pleasant odors. **Ai.** ELA altered the density of active granule cells (c-Fos+). **Aii.** Photo of the contoured granule cell layer of the anterior (left) or posterior (right) OB (Zeiss microscope tile images compiled x20). **Aiii.** Density of granule cells responding to pleasant odors (c-Fos+) in the anterior OB was higher in ELA compared to control mice. $n=7$ controls and 10 ELA mice for pleasant odors and 4 controls and 5 ELA for unpleasant ones. **Bi.** Photo of the whole mitral cell layer in the anterior OB (left, Zeiss microscope tile images compiled x20) and of active (c-Fos+) mitral cells (Tbx+) (right, Zeiss microscope, x63). **Bii.** Percentage of mitral cells responding to pleasant odors was lower in ELA compared to control mice. **Ci.** Photo of the SVZ where cell proliferation occurs (up, Zeiss microscope, x20) with zoom on BrdU+ cells (bottom, Zeiss microscope, x40). **Cii.** ELA did not influence cell proliferation in the SVZ of adult male mice. $n=8$ controls and 8 ELA mice. **Di.** Photos of active BrdU+ cells responding to olfactory stimulation in the OB (Zeiss microscope, x40). **Dii.** ELA did not affect the survival of adult-born granule cells specifically in the anterior or posterior part of the OB. **Diii.** Adult-born granule cells of the antero and posterior OB similarly respond to pleasant odors in ELA animals compared to controls. **Div.** Similar density of active granule cells was retrieved between groups. $n=4$ controls and ELA mice. **Ei.** No difference of active granule cell density was observed between groups in response to unpleasant odors. **Eii.** Percentage of active mitral cells was similar between groups in response to unpleasant odors. **Eiii.** Higher response of adult born granule cells to unpleasant odors was retrieved in the anterior OB. **Eiv.** Higher density of adult born granule cells responding to unpleasant odors was found in the ELA group. $n=4$ mice in both groups. $ns>0.05$ * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Points represent individual values \pm SEM.

ELA impairs activity of key structures of the reward system involved in processing odorant attractiveness.

Previous studies have revealed that the encoding of pleasant odors was supported not only by the OB but also by a network including the OT and VTA (Midroit et al., 2021). The latter two structures form part of the reward circuit and are involved in motivated behavior. To focus our analysis on the neuronal activity in these two regions in ELA and control animals following presentation of pleasant or unpleasant odors, we used DARP32 and TH markers. ELA animals exhibited a lower level of c-Fos-positive cell density in response to pleasant odors compared to controls in the OT (bilateral unpaired t-test, Ctrl vs ELA, $p=0.0022$; Figure 8Aii) as well as in the VTA (bilateral unpaired t-test, Ctrl vs ELA, $p=0.0072$; Figure 8Bii). As stress can have an effect on the number of TH-positive cells (Kapor et al., 2020), which are highly represented in the VTA, we controlled that the overall density of TH-positive cells and found that the density of TH-positive cells in the VTA was similar between groups ($F(1,21)=0.882$, $p=0.358$; mean= 0.003318 ± 0.001264 in Ctrl group and mean= 0.002602 ± 0.001064 in ELA group).

Interestingly, in response to unpleasant odors, ELA animals exhibited similar level of c-Fos-positive cell density compared to controls in the OT (bilateral unpaired t-test, Ctrl vs ELA, $p=0.61$; Figure 8Ci) and the VTA (bilateral unpaired t-test, Ctrl vs ELA, $p=0.4$; Figure 8Cii). This was confirmed by a two-way ANOVA (Rearing effect $F(1,21)=6.606$, $P=0.018$ and hedonic effect $F(1,21)=11.438$, $P=0.003$). Thus, in agreement with changes observed in pleasant odor perception in ELA mice, neuronal activity is perturbed in higher olfactory structures involved in reward and motivation.

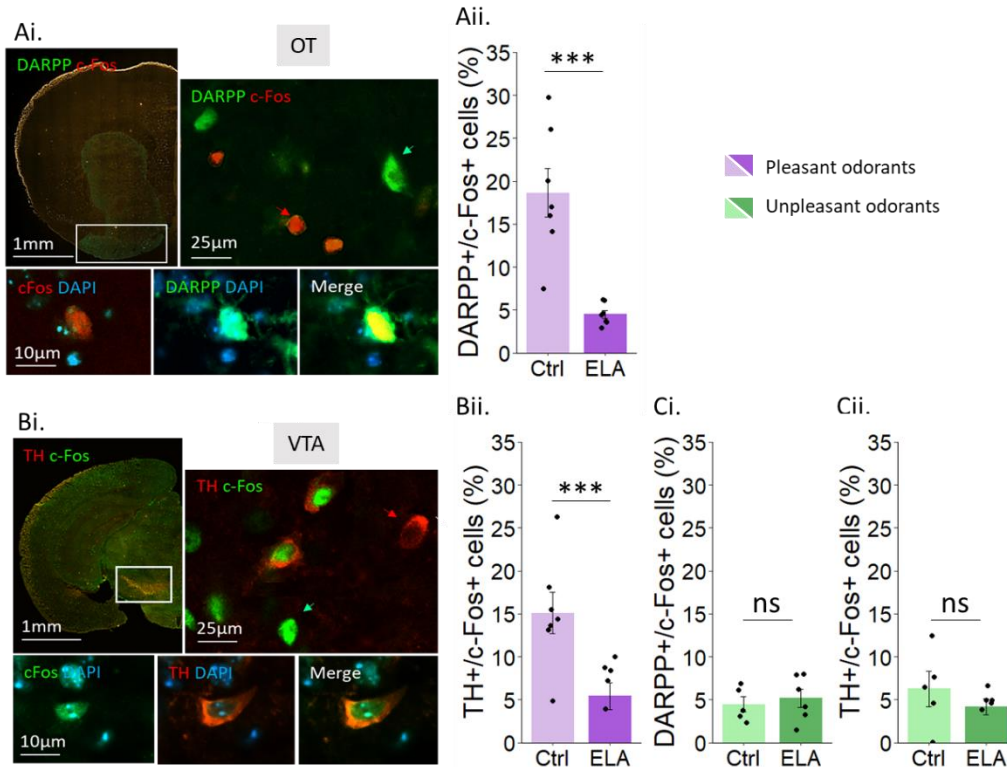


Figure 8. ELA alters neural activity in response to pleasant odors in the OT and VTA. **Ai.** Photo of section containing the OT (up left, Zeiss microscope tile images compiled x20) and its projecting DARPP32+ cells (up right). Photos of double labeled DARPP32+/c-Fos+ cells in the OT (bottom, Zeiss microscope x63). **Aii.** The percentage of DARPP32+/c-Fos+ cells was lower in response to pleasant odors in ELA mice compared to control group. **Bi.** Photo of section containing the VTA (up left, Zeiss microscope tile images compiled x20) and its TH+ cells (up right). Photos of active TH+ cells in the VTA (bottom, Zeiss microscope x63). **Bii.** Similar results were retrieved for TH+/c-Fos+ in the VTA. **Ci and Cii.** No difference was found between groups in response to unpleasant odors. $n=12$ controls and 12 ELA mice. $***p<0,001$. Points represent individual values \pm SEM.

Discussion

Effect of ELA on pups' and adults' emotional behavior is sex-specific.

First, we confirmed that LBN disrupted maternal behavior (less time in the nest) and triggered abusive behaviors towards pups (increase number of aggressions, biting, neglect/isolation). This model induced trauma, directly damaging males and female's growth and motor behaviors (in speed, distance and immobility). Interestingly, while we observed that mothers treated male and female pups similarly, physiological and behavioral differences between sexes were found at this age. This may be underpinned by developmental specificities, different sensitivities to stress, or divergent expression of a stressed phenotype (Burns, 2018; Cullity et al., 2019; Perry et al., 2021). We also observed different emotional phenotype between sexes in adulthood, highlighting the importance of sex-dependent variability in the long-term susceptibility to early stress in rodents (Bath et al., 2017; Davis et al., 2017; Ivy et al., 2010;

Naninck et al., 2015; Xu et al., 2022). Despite the prevailing notion in the literature that female sex in humans is more sensitive to stress (*Becker et al., 2007; MacMillan et al., 2001; Touchant & Labonté, 2022*), studies about stress in female rodents show more controversies (*Bittar et al., 2021; Burke et al., 2016; Loi et al., 2017*). In our study, adult females displayed behavior comparable to controls in the light/dark box, openfield, sucrose consumption, and NIH tests, unlike the male counterparts. Females may have developed resilient mechanisms or may manifest their altered emotional phenotype differently from males. Finally, we highlighted here the relevance of compiling behavioral results into an index to confirm overall altered phenotype.

ELA triggers a loss of interest for pleasant odorants in adult male mice.

As human studies have shown that stress concomitantly induced altered emotional behavior and olfactory hedonic perception (*Athanassi et al., 2021*), we chose the strategy of studying male mice that exhibited similar impairments. Indeed, ELA not only affected emotional behaviors in adult males but also impaired the perception of pleasant odorants, manifested through lower pleasant odorant investigation in the odor preference test. This could not be attributed to an alteration of odor detection since we confirmed using a habituation test that ELA animals perceived correctly the odorant at the concentration used for the odor preference test. These findings align with evidence suggesting that at least partially distinct neural networks encode pleasant and unpleasant odorants (*Kermen et al., 2016; Midroit et al., 2021; Tiedemann et al., 2020; Yamaguchi, 2017*). However, other olfactory functions have already been reported as sensitive to stress, affective disorders and depression such as discrimination or learning processes (*Athanassi et al., 2021; Belnoue et al., 2016*). In our context, we assessed attraction in response to an unfamiliar odorant, presented alone, eliminating the need for both discrimination and memory processes.

Effects of ELA on the morphology of OB interneurons.

We first focused our cellular analysis on OB granule cells as they play a crucial role in supporting the neural signature of odorant attraction (*Kermen et al., 2016*), and integrate the neural network during the stress procedure. To identify early born interneurons responding to pleasant or unpleasant odorants, we used c-Fos coupled to GFP strategy. We identified alterations in the fine morphology of early-born OB interneurons that respond to pleasant odorants in ELA animals. They exhibited decreased dendritic arborization complexity and decreased spine density in the basal domains, especially of filopodia that are immature and developing spines important for plasticity processes (*Takahashi et al., 2018*). Decreased number of spines in the basal domain can come from a direct effect of stress on granule cells morphology and/or an alteration of centrifugal pathway by stress (*Devore & Linster, 2012; Kaufman & Charney, 2001*). It is suggestive of a decreased connectivity between inputs from centrifugal projections and adult-born granule cells (*Lepousez et al., 2014; Nissant et al., 2009*), leading to an alteration of the information processing. Interneurons responding to unpleasant odorants in ELA animals exhibited low

changes in their morphology, with no significant change in the overall synaptic density and a localized (at one distance from the soma) increased complexity of their apical domains involved in dendro-dendritic contacts with mitral and tufted projecting cells (*Takahashi et al., 2018*). The morphology of neurons is activity-dependent (*Lepousez et al., 2014; McDole et al., 2015*) and neural activity relies on the integrity of a neuron's morphology. This interdependence can explain why OB processing is altered, specifically affecting the OB output (mitral cell activity), in response to pleasant odorants.

ELA deteriorates neural circuit coding pleasant odorant.

In line with morphological changes by ELA, we observed an alteration of neuronal activity in the granule cell layer of ELA animals. Indeed, surprisingly, we revealed a higher density of activated granule cells in response to pleasant odorants in ELA animals compared to controls. In the literature, early stress has been associated with increased activity in certain brain structures in humans and rodents, such as the amygdala and the hypothalamus for instance, directly linked to stress vulnerability (*Gunn et al., 2013; Malter Cohen et al., 2013; Smith & Pollak, 2020*). This can be due to a disruption of the balance between inhibition and excitation. This disruption over time could lead to long-term alterations in connectivity between brain regions (*Honeycutt et al., 2020*), promoting maladaptive behaviors.

In our case, this increased cell density comes mostly from the anterior part of the OB of ELA animals. Interestingly, we retrieved a spatial coding of odor hedonics along the anteroposterior axis of the OB that has been previously described (*Kermen et al., 2016*), with some discrepancies that may be explained by the analysis method (whole granular layer versus divided into subregions). We analyzed the impact of enhanced activity of granule cells located on mitral cells and found a lower proportion of activated mitral cells in response to pleasant odorants specifically in the anterior OB of ELA animals compared to controls. The OB projects directly to the OT which is closely connected to the VTA (*Midroit et al., 2021*). As previously described, we found in control animals an increased neural activity in response to pleasant versus unpleasant odorants in both structures. This increased activity is altered in ELA animals. Overall, the processing of pleasant odorants is damaged from the OB outputs to downstream structures involved in processing odor-driven attraction. This could explain olfactory anhedonia identified in adult mice. The altered activity in these two regions suggests that the OB might failed recruiting the reward system and/or that the functionality of the VTA was directly damaged by ELA (*Kapor et al., 2020; Marusak et al., 2017; Spycka et al., 2020*). Overall, our results confirm that at least partially different neural networks are coding odor hedonics (*Kermen et al., 2016, 2021*), and that they have different sensitivity to stress. The alteration of other structures by early stress, beyond those studied here, is certainly not to be excluded.

ELA impacts adult neurogenesis of the OB.

The granule cell layer is made up of pre-existing and adult-born neurons. The hippocampus, the other adult target of neurogenesis is vulnerable to stress (*Alonso et al., 2024; Belnoue et al., 2013; Dioli et*

al., 2021; *Loi et al.*, 2014; *Oomen et al.*, 2010; *Spyrka et al.*, 2020) with reduction of proliferation, survival and functionality. Stress also impairs OB neurogenesis (with some discrepancies between studies (*Athanassi et al.*, 2023; *Belnoue et al.*, 2013; *Dioli et al.*, 2021)). Here, while the proliferation of adult-born interneurons was not affected, their survival increased. This increased survival is in contradiction with the correlation between higher survival of adult-born neurons and improvement of mood found in the literature (*Lepousez et al.*, 2015; *Siopi et al.*, 2016)). However, their plasticity was decreased by ELA leading to similar level of adult-born cell responding to pleasant odorants between control and ELA groups. Regarding adult stress in general, studies indicated that post-traumatic stress or chronic stress can disrupt OB function by reducing neurogenesis (*Dioli et al.*, 2021; *Rahimi et al.*, 2020; *Yang et al.*, 2011)) potentially contributing to olfactory dysfunction. However, concerning early stress, there are few studies available with some suggesting a resilience of bulbar neurogenesis to early stress (*Belnoue et al.*, 2013)).

Thus, adult-born interneurons do not appear to be involved in the altered activity observed in the anterior OB of ELA animals. This can be attributed to their generation long after the ELA procedure and the difference in their plasticity and/or engagement in various olfactory functions compared to preexisting neurons (*Forest et al.*, 2019; *Kermen et al.*, 2021)). Finally, the size and global cell density of the OB in both control and ELA animals were similar, despite a higher survival rate of adult-born neurons in ELA group. These results may be explained by changes in adult-born cell turnover which further disrupt OB's balance and functioning. The higher survival rate of adult-born interneurons might also indicate a compensatory mechanism, aiming to balance the reduced recruitment of these interneurons in response to pleasant odorants in ELA animals. Regarding the response to unpleasant odorants, surprisingly, a higher proportion of adult-born granule cells was activated. However, this neural change does seem to impact the OB output as observed by the similar percentage of Tbx21/c-Fos between ELA and control animals, which is also in accordance with the similar perception of unpleasant odorants between groups.

Finally, an open question is whether olfactory anhedonia is specific or stems from the general anhedonia. In humans, high levels of self-reported perceived stress are associated with a diminished sensitivity to reward cues such as food or money (*Pizzagalli et al.*, 2008; *Treadway et al.*, 2013; *Yan et al.*, 2024)). In animal models, chronic stress lead to altered behavioral flexibility related to reward sensitivity (*De Carvalho et al.*, 2023)), as well as reduced preferences for palatable sweet tastes and odor guided behaviors (*Vaz et al.*, 2018; *Willner et al.*, 1987)) as we demonstrated here with sucrose. Interestingly, a study found that in women, higher levels of chronic perceived stress predicted lower chemosensory pleasure, mediated by an awareness of positive and negative odors. Thus, odor awareness appeared to moderate chemosensory pleasure in individuals with high levels of perceived stress (*Hu et al.*, 2024)). However, while it is unclear whether these effects are causal, related, or merely symptoms of a broad alteration in hedonic processing, the direct impact of stress on olfactory networks suggests, at least in part, a specific effect of stress on olfactory anhedonia.

Conclusion

In conclusion, this study highlights the effects of early stress on emotional and olfactory behaviors in rodents. The disrupted maternal behavior affected both male and female pups with distinct physiological and behavioral responses. In adulthood, these sex-specific differences became more pronounced. While adult females demonstrated resilience in various emotional assessments, males exhibited significant depressive-like behaviors and anxiety. This impairment of emotional behavior is accompanied in male mice by an impairment of the perception of pleasant odorant. In exploring the neural basis of impaired odor perception, we found that ELA led to morphological and functional alterations of neurons responding to pleasant odorants, from the OB to downstream structures of the reward system. This research not only sheds light on the complex interplay between early trauma and long-term behavioral outcomes but also underscores the importance of studying the effects of ELA on a broader network involved in emotional and olfactory processing.

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-ÉTUDE 2-

Automatic extraction and analysis of synchronized fiber photometry and video data: a method for studying the neural basis of exploratory behavior in freely moving animals

(en préparation)

Résumé

La photométrie par fibre permet de révéler les dynamiques neuronales qui sous-tendent les comportements. Ces enregistrements sont souvent réalisés dans le cadre de paradigmes comportementaux prédictifs et contrôlés spatialement et/ou temporellement. Toutefois, dans des conditions plus naturelles, les stimuli sensoriels et les comportements exploratoires peuvent être imprévisibles. Pour aborder cette complexité, il devient crucial d'enregistrer en continu le comportement de l'animal et de synchroniser ces observations avec les données de photométrie par fibre. Nous présentons ici une méthodologie complète, de l'implantation intracérébrale de fibres optiques chez la souris à l'acquisition de signaux de photométrie par fibre synchronisés avec les comportements. Nous introduisons également les algorithmes et les scripts conçus pour extraire, prétraiter et analyser les données. Nous avons validé l'efficacité de notre méthode en étudiant l'activité calcique et la dynamique dopaminergique dans le tubercule olfactif en réponse à des odorants plaisants et déplaisants chez des souris adultes lors d'un test comportemental de préférence olfactive. Grâce à l'utilisation des biosenseurs jRGECO1a et dLight1.2, nous avons mis en évidence une signalisation calcique et une libération de dopamine significativement plus élevées en réponse aux odorants plaisants, par rapport aux odorants déplaisants. Cette nouvelle méthode permet ainsi l'extraction et l'analyse automatiques des données synchronisées de photométrie par fibre et des enregistrements vidéo, ouvrant la voie à une meilleure compréhension des mécanismes neuronaux responsables de la perception hédonique des odeurs chez des souris. Adaptable et polyvalente, cette approche peut facilement être étendue à l'analyse d'autres formes de comportement exploratoire.

Automatic extraction and analysis of synchronized fiber photometry and video data: a method for studying the neural basis of exploratory behavior in freely moving animals

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Highlights

A method that allows automated pairing of fiber photometry data with video recordings

A method applicable to all exploratory behaviors

A precise and fast method to analyze dopaminergic dynamics in the brain during behavior

A method to analyze calcium signaling changes in response to sensory stimuli

Abstract

Background: Fiber photometry offers insight into cell-type-specific activity underlying behavior. In many cases, fiber photometry recordings are conducted in response to predictive, time-controlled behavioral paradigms. However, under more naturalistic conditions, sensory stimuli and exploratory behavior are often unpredictable. To address this, it is essential to continuously monitor the animal's behavior and synchronize it with the fiber photometry data acquisition.

New method: Here, we present a workflow starting from the intracerebral implantation of optical fibers in mice, to the acquisition of fiber photometry signals aligned with the animal's recorded behavior. Additionally, we introduce the custom algorithms and scripts designed to extract and analyze synchronized fiber photometry and behavioral data, providing a robust framework for studying the dynamic relationship between neural activity and behavior.

Results: We demonstrated the effectiveness of our method by assessing calcium activity and dopaminergic dynamics in the olfactory tubercle in response to attractive or non-attractive odors in freely moving adult C57Bl/6J mice. Using jRGECO1a and dLight1.2 biosensors, we revealed a higher level of both calcium signaling and dopamine release in response to attractive compared to unattractive odors.

Conclusion: we provide here a new method that permits the automatic extraction and analysis of synchronized fiber photometry and video data, offering deeper insights into the neural mechanisms underlying olfactory hedonics in freely moving mice. This approach is adaptable and can be extended to analyze other types of exploratory.

Keywords: fiber photometry, video recording, behavior, olfactory perception, mice

Introduction

Fiber photometry is a key technique for characterizing brain-behavior relationships *in vivo*, offering detailed insights into how specific brain regions contribute to behavior (Gunaydin et al., 2014; Guo et al., 2015; Martianova et al., 2019; Wang et al., 2021). It involves the insertion of a fiber optic probe into the brain to detect fluorescent signals from genetically encoded biosensors. Over the past two decades, a variety of genetically encoded biosensors have been developed to study different forms of signals in the central nervous system, including not only neuronal population activity but also neurotransmitter and neuropeptide signals in both superficial and deep brain structures of freely behaving animals (Bruno et al., 2021). Regarding neural activity recording, among these biosensors, calcium-sensitive sensor like GCaMPs are the most widely used (Simpson et al., 2023), but engineered genetically encoded voltage indicators (GEVIs) have also been employed (Gong et al., 2015; Kannan et al., 2018). The development of an extensive range of fluorescent sensors for biomolecules has also allowed more recently the study of neuromodulator and peptide dynamics: dLight or GrabDA for dopamine (Patriarchi et al., 2018; Sun et al., 2018), nLightG/R for noradrenaline, GACH for acetylcholine, PsychLight2 for serotonin, Mtria for oxytocin etc. (Feng et al., 2024; Simpson et al., 2023). In all cases, the fiber optic probe collects emitted light, enabling real-time quantification of neural activity or neurotransmitter release. Thus, this technique provides valuable insights into cell-type-specific activity in freely moving animals, with a temporal resolution ranging from sub-second to second timescales (Murphy et al., 2023; Patel et al., 2020; Terstege et al., 2023; Wang et al., 2021). This technique has been used to better understand brain functions, by revealing the microarchitecture of sleep (Andersen et al., 2023; Kjaerby et al., 2022), by demonstrating how chronic stress induces anxiety-like phenotypes through changes in dopaminergic neuron activity (Morel et al., 2022), by identifying a stress susceptibility signature linked to calcium transients in the nucleus accumbens (Muir et al., 2018), or by showing modulations in calcium signaling within cortico-amygdala connections during altruistic versus selfish social choices (Scheggia et al., 2022).

To facilitate the analysis of complex signal-behavior relationships, studies often employ paradigms that produce strong behavioral and brain responses. For instance, paradigms such as fear conditioning (Demaestri et al., 2024; Legaria et al., 2022; Ren et al., 2018; Verharen et al., 2020; Xu et al., 2019), chronic noise exposure as chronic stress (Peng et al., 2023), chronic social defeat stress (Muir et al., 2018), restraint stress (Klenowski et al., 2023), fear or reward learning (Sych et al., 2019), or rewarded self-stimulation (Harada et al., 2021) elicit strong behavioral changes that can be easily correlated with fiber photometry signals. This is even more evident when the brain response is triggered by external stimuli used in the conditioning phase. Some studies also benefit from focusing on specific time windows triggered by external stimuli, allowing for the observation of 'before/after' signal dynamics. In contrast, fewer studies have aligned fiber photometry signals with free exploratory behavioral events. This last method has been used in sleep cycles paired with EEG and MEG recordings (Patel et al., 2020),

social interaction tasks (Gunaydin et al., 2014; Terstege et al., 2023), maternal care observation (Dvorkin & Shea, 2022) or spatial exploration (Loewke et al., 2021), all supported by video tracking synchronized with the fiber photometry data acquisition. In this context, sensory perception is even less investigated with that methodology (Gray et al., 2021).

We therefore propose a comprehensive methodology designed for fiber photometry experiments, to facilitate the analysis of neural signals synchronized with exploratory sensory perception. The efficiency of this workflow has been demonstrated here by assessing neural activity and dopaminergic dynamics in response to olfactory stimuli. Previous research has shown that odorants with positive hedonic value compared to negative ones, can generate attraction thanks to an increased activity in the olfactory bulb (OB), the first cortical relay for olfactory information, and its target, the olfactory tubercle (OT). The OT, part of the ventral striatum, receives dopaminergic projections from the ventral tegmental area (VTA) (Midroit et al., 2021; Zhang et al., 2017). In addition, increased activity in the VTA has been observed, suggesting dopamine release in the OT in response to attractive odorants (Murata, 2020; Murata et al., 2015, 2024). Based on these findings, we thus proposed using fiber photometry to analyze neural activity and dopamine release in the OT, using respectively sensitive red protein calcium indicator jREGCO1a (Ca²⁺ biosensor) and green fluorescent genetically encoded dopamine sensor dLight1.2 (Dopa biosensor) (Patriarchi et al., 2018), in response to attractive versus unattractive odorants. Specifically, we injected a mixture of AAV viruses expressing jREGCO1a and dLight1.2, followed by the implantation of optical fibers in the OT of adult male mice. This enabled the detection of fluorescence while the mice freely explored a board containing an odorant source. Mice were video-tracked during fiber photometry recordings, and their exploration behavior was segmented and referenced into events of interest (i.e. periods spent exploring the odorant source, “*olfactory investigation events*”). With the synchronization of video data with fiber photometry signals, we were able to analyze fluctuations in dopamine and calcium biosensor activity during the events and found a higher level of calcium discharge in the OT associated with an increase of dopamine release in response to attractive odorants compared to unattractive ones.

In conclusion, we present here a method that enables automated extraction and analysis of synchronized fiber photometry data with video recordings to enhance our understanding of the neural mechanisms behind olfactory hedonic perception. It should be noted that this approach can be used not only to analyze events of interest during the task, such as olfactory investigation, but also to retrospectively examine other types of events by reviewing and re-segmenting the videos. This flexibility extends the applications of this method to the analysis of neural activity in a variety of contexts.

Material and methods

1- Animals

Six adult (2 months old) C57BL/6N mice were bred in house, had *ad libitum* access to food and water, and were housed on a 12 h:12 h light cycle. All experiments were done in accordance with the European Community Council Directive of 22nd September 2010 (2010/63/UE) and the National Ethics Committee (Agreement APAFIS 11725-2017100915448101). Mice were housed in a temperature and humidity-controlled environment kept at a constant temperature of 22°C ±2. Throughout these experiments, all efforts were made to minimize pain and discomfort of the mice.

2- Surgery

The animal was sedated using isoflurane inhalation (2.5% isoflurane 0.5% oxygen, 0.5% compressed air), and administered subcutaneous injections of Buprémorphine (0.1 mg/kg) and Metacam (20 mg/kg). Before incising the skin, subcutaneous injection of local 2% lidocaine was administered at the incision site. The animal was then placed on a stereotaxic frame (Figure 1). To minimize discomfort and avoid potential inner ear damage, ear bars were positioned against the jawbone rather than inside the ears. Once securely positioned in the stereotaxic frame, the mouse's eyes were protected with Lacrigel to prevent any ocular damage. The mouse's scalp was sanitized alternately with ethanol and Betadine two to three times. The stereotaxic coordinates (anteroposterior (AP), lateromedial (LM)) were then set in relation to the bregma, and the horizontal alignment of the skull was confirmed by ensuring that the distance between bregma and lambda measured AP = 4.2 ± 0.2 mm. A 1.5 cm incision was made from between the eyes to the back of the head using a scalpel, and the skin edges were retracted to create an optimal field of view. The skull was kept moist with periodic drops of NaCl solution.

3- Virus injection

The two viruses, pAAV hSyn-dLight1.2 (Addgene, #111068-AAV5 (4x10¹² vg/mL) and pAAV.Syn.NES-jRGECO1a.WPRE.SV40 (Addgene, #100854-AAV9 (1x10¹³ vg/mL) were mixed by going back and forth with the pipette and prepared for each animal just before brain injection (stock kept on ice). A glass pipette connected to a nanoliter 2020 injector (WPI) was filled with 200 nL of the mixture. Skull was pierced (0.5mm diameter hole) over the right medial OT (with respect to the bregma: AP, +1.7 mm; ML, -0.5 mm) using a stereotaxic microdrill (RWD®). To avoid tissue damage, the needle was lowered into the brain through the pre-pierced hole very slowly (0.1 µm/ 3 s) until it reached the coordinate of DV, -4.7 mm (Figure 1). The virus mixture was then injected at 50 nL/minute. After a 10-minute delay to ensure proper diffusion of the virus mixture in the targeted region, the needle was slowly withdrawn at the same speed (0.1 µm/3 s). To prevent blood clotting as the needle neared the scalp, the withdrawal speed was increased (0.1 µm/s). Once the pipette was removed from the brain, we sutured the skin with a resorbable suture thread (Vycril®). Between each mouse, a small amount of

the mixture (50 nL) was ejected to prevent the needle from clogging. The syringe had therefore to be filled with slightly more virus than the quantity injected.

4- Fiber implantation

The optical fibers were implanted 6 weeks after virus injection. Animals were placed on the stereotaxic frame using the same protocol as previously described. To enhance cement adhesion, minor scratches were made on the skull using the back of a scalpel. Using a cannula holder, an optical fiber (MFC_400/430-0.66_4.7mm_MF1.25_FLT, Mono Fiberoptic Cannula, Doric Lenses®) was implanted into the OT at the same coordinates as the viral infusion (with respect to the bregma: AP +1.7 mm, LM -0.5 mm, dorsoventral (DV) -4.7 mm). Given the fiber's larger diameter compared to the infusion needle, it was lowered gradually at a rate of 0.1 mm per 10 seconds. To ensure optimum tissue distribution around the fiber tip, the latter was positioned 0.1 mm below the target for 1 minute before being raised by 0.1 mm. One drop of Vetbond glue was applied to the skull around the fiber to ensure stabilization before securing it with dental cement on the scalp (Metabond) (Figure 1). Lacrigel was reapplied to the mouse's eyes. The mouse was then removed from the stereotaxic frame and placed in an individual cage under a heating lamp until fully awake. Mice were returned to their home cage the following day to prevent social isolation. For both surgeries, during the 5 post-operative days, the animals received daily injections of Metacam (20 mg/kg), and their weight was monitored.

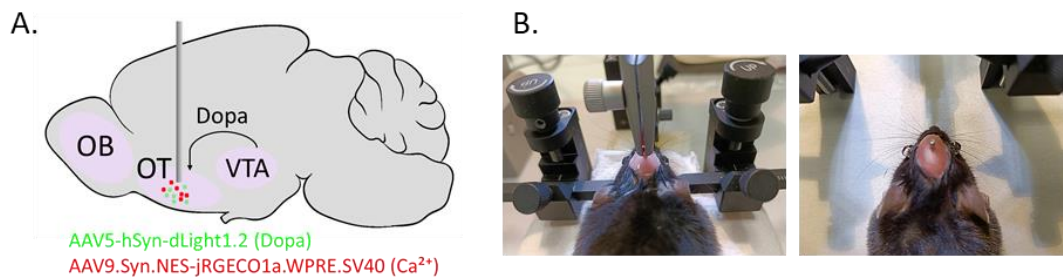


Figure 1. Viral injection and fiber implantation in the medial olfactory tubercle (mOT). **A.** Two virus, pAAV hSyn-dLight1.2 (Addgene, #111068-AAV5 (4x10¹² vg/mL) and pAAV.Syn.NES-jRGECO1a.WPRE.SV40 (Addgene, #100854-AAV9 (1x10¹³ vg/mL) were injected in the mOT (with respect to the bregma: AP, +1.7 mm; ML, -0.5 mm; DV, -4.7 mm, 200 nL at 50 nL/min). **B.** Photos showing the mouse position on the stereotaxic frame and the fiber implantation.

5- Fiber photometry experiment combined with video recordings in an olfactory preference task

Olfactory preference task.

Mice underwent a 6-week recovery period following surgery. Recordings were conducted during an olfactory preference test, where freely moving mice explored a one-hole board apparatus during a 2-

minute trial (*Kermen et al., 2016; Mandairon et al., 2009*). During the week leading up to the test, three 2-minute trials were performed in which mice explored the one-hole board apparatus without odorants (*Mandairon et al., 2009*) while connected to the fiber photometry setup for habituation. This also ensured that the setup allows for normal locomotor activity when the fiber is attached to the patch cord with a ceramic sleeve. Then, as a measure of odor hedonics, we measured the investigation time of six unfamiliar odorants with no known biological significance: Limonene (Lim; 5989-27-5), Citronellol (Citro; 106-22-9), Camphor (Cam; 76-22-2), Guaiacol (Gua; 90-05-1), p-Cresol (Cre; 106-44-5), and Pyridine (Pyr; 110-86-1). Odor preference was defined by approach and avoidance behaviors (*Kermen et al., 2016; Mandairon et al., 2009*). To ensure similar perceived intensity, all odorants were diluted to 1 Pa (similar vapor pressure). A 60 μ L of the 1 Pa odorant was applied to a small cotton square, which was placed at the bottom of a pot covered with a grid and bedding. The pot was positioned in the central hole of the board. The experiment was conducted in a dimly lit room. Before their passage, mice were kept in a separate room to prevent prior exposure to the odorant and reduce the stress associated with the experiment. Prior to each experimental trial, the mouse was connected to the setup and recorded for 2 minutes while in its home cage. This period helped minimize stress and ensured unbiased future behavioral responses by allowing the mouse to reach a steady state of sensor bleaching. It also facilitated the adaptation and balance of sensor expression, while controlling for daily variations in recordings, light power consistency, and fiber-optic connectivity (*e.g.*, avoiding gaps between the patch cord, sleeve, and rotary connector). For each experimental trial, the mouse was first placed in the bottom right corner of the board and then allowed to explore freely. After the trial, the mouse was returned to its cage, the odorant pot was removed, and the board was cleaned with ethanol and allowed to dry. The order of odor presentation was randomized across mice. Each mouse was tested for the 6 odorants. For each mouse and odorant, exploration time of the hole was measured as an index of odor hedonics.

Fiber photometry system coupled with video recording.

Fiber photometry recordings were performed using Fiber Photometry Systems from Doric Lenses® (Figure 2). Three excitation wavelengths 465 nm (Dopa-dependent signal), 405 nm (isosbestic signal), and 565 nm (Ca²⁺-dependent signal) were emitted from LEDs (465 nm: LEDC1-B_FC, 405 nm: LEDC1-405_FC, 565 nm: Doric Lenses) and controlled by programmable LED drivers (LED Driver, 4 Channel Model, Doric Lenses) and transmitted via 0.39 NA, Ø400 μ m core multimode prebleached patch cables through a Doric Dual Fluorescence MiniCube (iFMC6-G2_IE(410-420)_E1(460-490)_F1(500-540)_E2(555-570)_F2(580-680)_S Fluorescence MiniCube - 6 ports with 2 integrated photodetectors and 3 integrated LEDs, Doric Lenses). Light intensity at the tip of the patch was maintained at 10-30 μ W across sessions. dLight1.2, jREGCO1a and isosbestic fluorescence wavelengths were measured using femtowatt photoreceivers (Newport, 2151). The Synapse software controlled and modulated the excitation lights (465 nm at 209 Hz, 405 nm at 331 Hz, and 565 nm at X Hz). Video recordings were made using a camera positioned next to the rotary joint

(AFRJ_2x2_PT_400-0.57 Assisted 2x2 Fiber-optic Rotary Joint, 400 μm NA0.57 fiber, 1 m input, optimized for fiber photometry). Video recordings and fiber photometry (camera, Fiber Photometry Console, and LED Driver) were started simultaneously using the “start all devices” option in Doric Neuroscience Studio® (DNS) once the mouse has been connected in its home cage. Indeed, the latest version, Doric Neuroscience Studio® (DNS V6.3.2.0), enabled the automatization and synchronization of recordings by starting and stopping all devices simultaneously. This synchronization can also be verified afterward by examining the timescale in the Doric file (signal recordings).

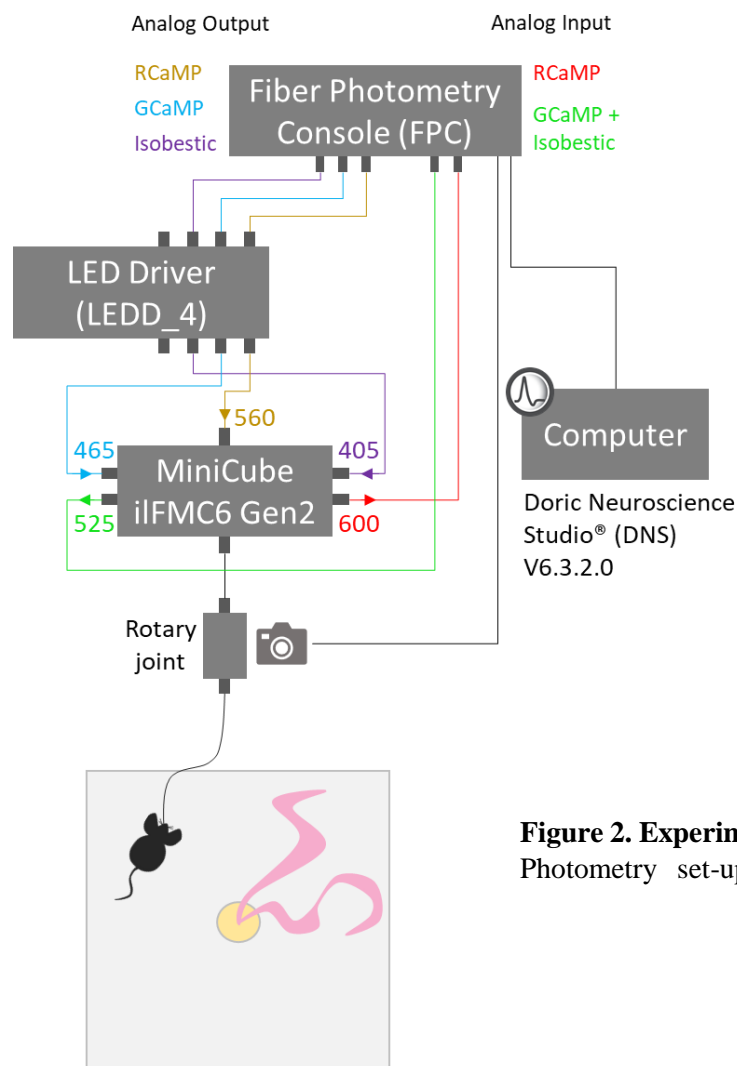


Figure 2. Experimental design. Fiber Photometry set-up during olfactory

6- Histology

A few days after recordings, mice were sacrificed using a ketamine\xylazine overdose (300 mg/kg and 20-30 mg/kg respectively) and then intracardiac perfusion of 4% formalin diluted in PBS (30 mL) at a perfusion rate of 2 mL/minute using a peristaltic pump was performed. Brains were dissected, post fixed in 4% formalin diluted in PBS for 4 hours at 4°C, and then immersed in a sucrose solution (20% in PBS)

overnight. Brains were finally frozen during 20 seconds at $-48^{\circ}\text{C} \pm 2$, and then stored at -20°C before serial sectioning with a cryostat (14 μm thick 304 μm intervals between samples). The sections were immersed in PBS for 10 minutes and cover-slipped in Vectashield (Eurobio Scientific). The presence of the viruses was analyzed on 18 sections covering the antero-posterior axis of the OT (5.42 mm), using ZEISS® microscope.

7- Data analysis

Analysis of video recordings.

Videos were analyzed using A2V Volcano®, which enabled the detection and tracking of behaving mice. Two distinct regions were defined for analysis: the hole (Figure 3Ai) and the entire board (Figure 3Aii). To ensure alignment across all videos, two reference points (P1 and P2; Figure 3Aii) were placed at the bottom corners of the board regions. Mice were detected using pixel analysis and tracked for 2 minutes while exploring the board (Figure 3B). During the 2 minutes of testing, times when the mouse entered (red lines; Figure 4) and left the odorized hole (orange lines; Figure 4) were identified and referenced as a specific event (“*olfactory investigation events*”) in an Excel spreadsheet. This allows to calculate the total duration of the odorized hole exploration. The spreadsheet is used in the MATLAB® code to analyze the fiber photometry signal corresponding to the behavior of interest (“*olfactory investigation events*”).

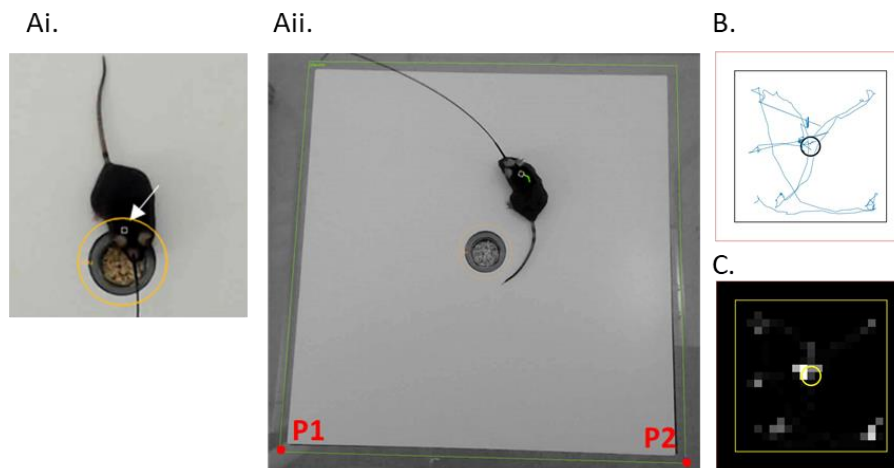


Figure 3. Video recordings and analysis. **Ai.** The mouse was detected in the hole. **Aii.** Photo of the odor preference test apparatus (hole board 30x30 cm). **B.** Mouse track map. **C.** Mouse presence

Fiber photometry data.

Signals (465 nm, 405 nm and 565 nm) were pre-processed using MATLAB® (Figure 4A). For a detailed description of the data processing with the MATLAB program, **see Annex 1**. The raw data for each mouse, collected throughout the whole recording period, were filtered using a low-pass Butterworth filter and then normalized through linear regression (Figure 4B). The $\Delta F/F$ values were calculated using the fitted isosbestic signal (405 nm) with the following formula: $(465-405)/405$ for calcium and $(565-405)/405$ for dopamine (Figure 4C). Subsequently, the Zscore was computed for the entire signal using the corresponding function in the MATLAB program (Figure 4D). To account for variability due to fiber implantation or recording issues, relative measures such as percent changes or changes in Zscore can be employed. This approach facilitates comparisons between recording sessions or across different animals.

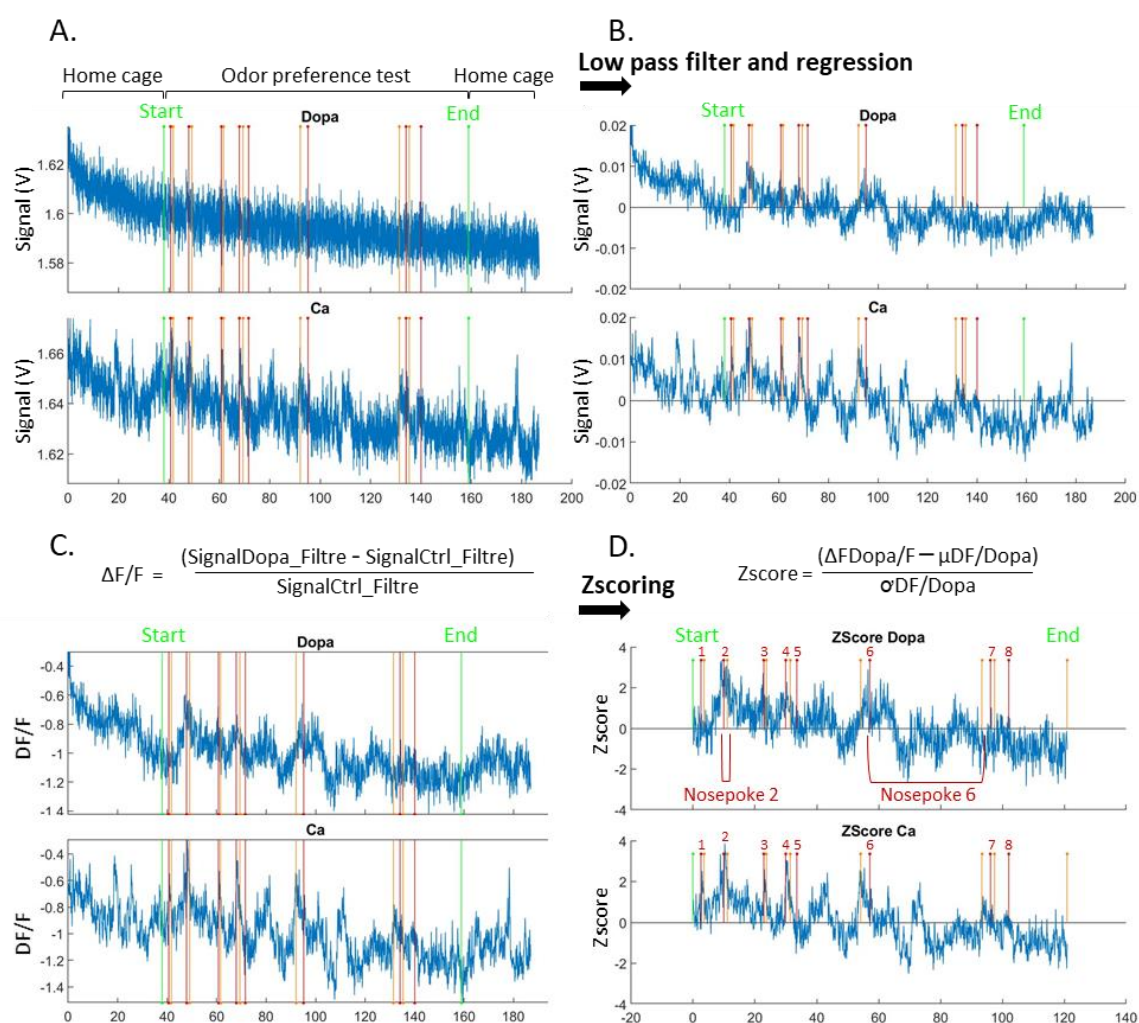


Figure 4. Fiber photometry data processing and analysis. **A.** Raw Dopa signal (up) and Ca^{2+} signal (bottom). Start and End green lines stand for the beginning and the end of the entire behavioral trial (2 minutes). Red and orange lines stand for the beginning and the end of each event (“*olfactory investigation*”) **B.** Signals after low pass filter and regression. **C.** Signals after $\Delta F/F$ calculation and **D.** After Zscoring (8 events have been observed in the trial).

To analyze an event in a trial, the Excel spreadsheet is recalled in MATLAB® to encode the start (red lines, indTpsStart0, **Annex 1**) and the end (orange lines, indTpsEnd0, **Annex 1**) of the event (“*olfactory investigation events*”) (Figure 5A). The spreadsheet contains a column name “behavior” where any event of interest is referenced by a specific name. In a trial, all Zscores of the similar event were set to zero on the time axis (red lines, indTpsStart0) and then averaged for a mouse. This was done for dLight1.2 and jRGECO1a. (Figure 5B). We thus obtained an average of the signals for each animal, centered on the start of the event and represented two seconds before and after the event. Then, we averaged the signals corresponding to the similar events between mice of the same group. This was done again for the dLight1.2 and jRGECO1a signals. Finally, we calculated the area under the curve (Auc) for the dLight1.2 and jRGECO1a signals from the onset of the event (timepoint 0; entering into the odorized hole zone) to 500 ms afterward and performed bilateral paired t-tests for statistical analysis (**Annex 1**). We also performed another analysis consisting in averaging the mean signal represented by the curve for dLight1.2 and jRGECO1a during a similar time period (0 +500 ms). Bilateral paired Wilcoxon-tests were used to compare averaged signal in the two conditions (pleasant vs unpleasant odorants) (*Morel et al., 2022*). We also generated heat maps by combining trajectory data with photometry signals (Figure 5C). These maps depict calcium and dopaminergic signals based on mouse location (in pixels; Figure Ci. and Cii) and can be further normalized by the time spent at each location (Figure Ciii. and Cv; **Annex 1**).

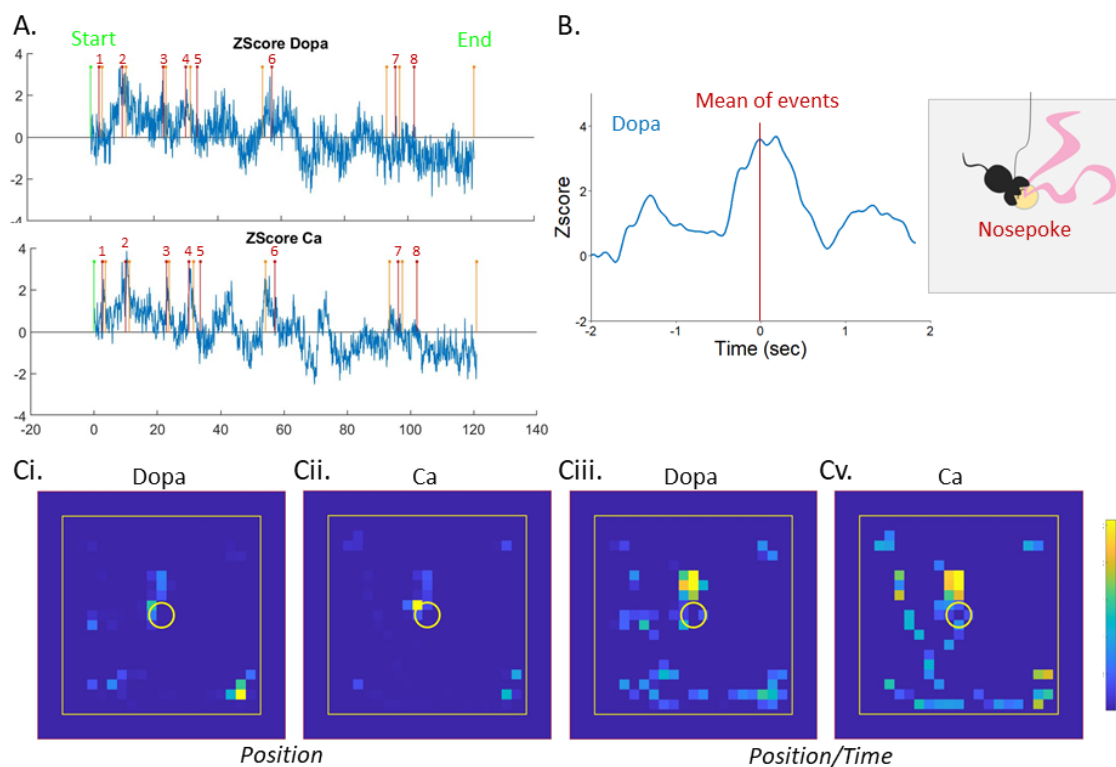


Figure 5. Merge of video and fiber photometry recordings. A. Raw Dopa signal (up) and Ca²⁺ signal (bottom). Start and End green lines stand for the beginning and the end of the entire behavioral trial. Red and orange lines stand for the beginning and the end of each event. “1” to “8” stands for 8 events of “*olfactory investigation events*” in this trial. **B.** Example of averaged Dopa signals for the “*olfactory investigation events*” of one mouse. Timepoint 0 indicates the onset of average odorant investigation. **Ci-Cii.** Dopa and Ca²⁺ signals mapped to mouse location on the board. **Ciii-Cv.** Dopa and Ca²⁺ signals mapped to mouse location, normalized by the time spent at each location.

Results

1- Dopaminergic, and calcium signals in the medial olfactory tubercle (mOT) reflected the perception of attractive *versus* unattractive odorants in mice.

First, we selected the most and least attractive odorants from the six tested for each mouse and found that mice spent significantly more time investigating the attractive odorants compared to the unattractive ones (unilateral paired Wilcoxon test, $p = 0.015$; Figure 6Aii).

For the fiber photometry data (Figure 6Bi-Bii), we observed a significant difference in the area under the curve (Auc) of the dopaminergic signal during the investigation of attractive versus unattractive odorants (bilateral paired t-test, $p = 0.02$; Figure 6Ci). However, no significant difference was found in calcium signaling between the Aucs for attractive and unattractive odorants (bilateral paired t-test, $p = 0.8$; Figure 6Cii). Interestingly, using the analysis of the mean signals, a different result was obtained for calcium data with a higher signal observed in response to pleasant compared to unpleasant odorants. For dopamine data, similar results were found with an increased dopamine signal in response to pleasant odorants compared to unpleasant ones (bilateral paired Wilcoxon test, $p < 0.0001$ for dopamine and calcium signals; Figure 6Di and Dii).

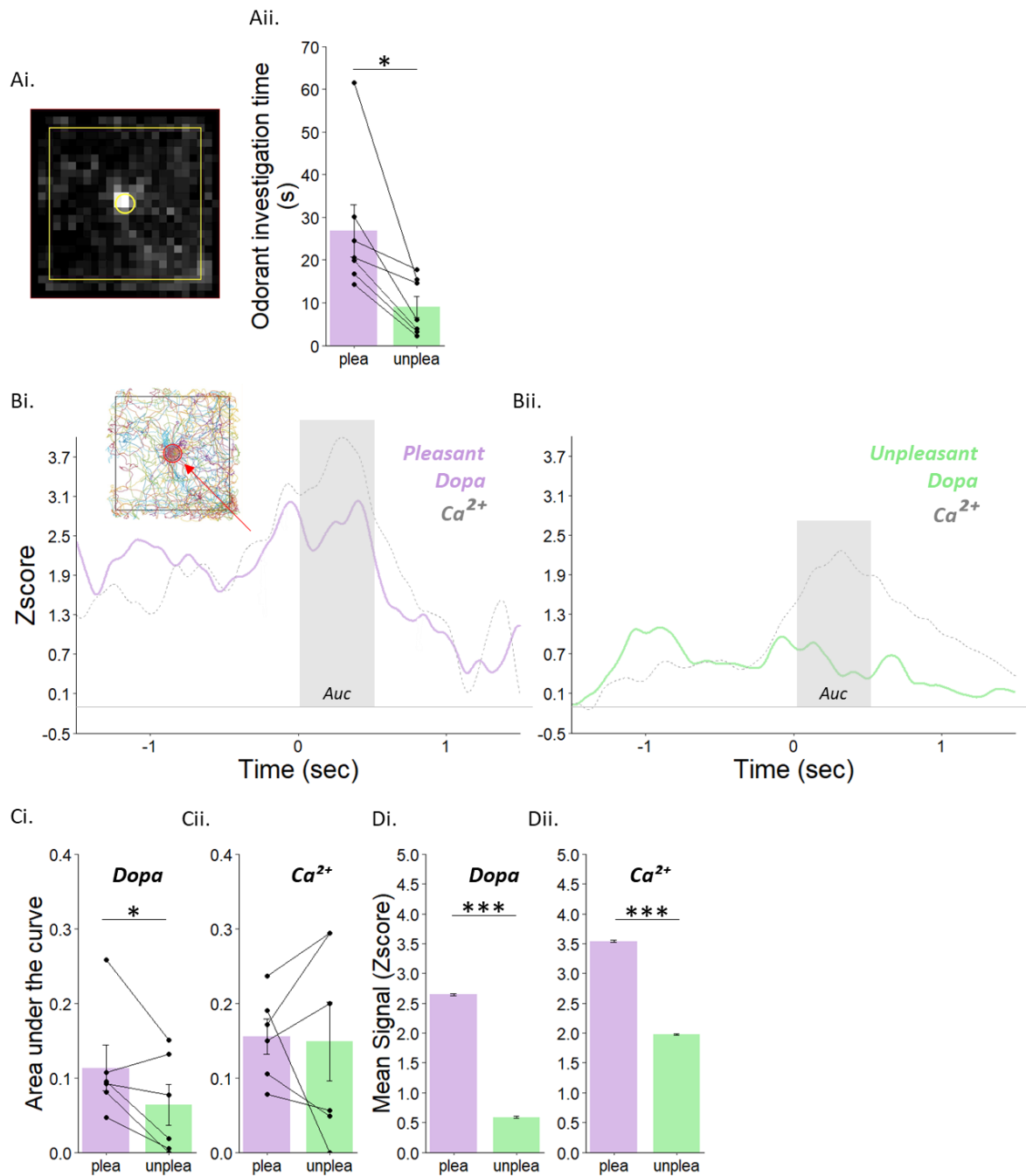


Figure 6. Dopaminergic release in the mOT of adult male mice is driven by the hedonic value of odorant. **Ai.** Heatmap showing the average exploration pattern on the one-hole board apparatus. **Aii.** Mice spent significantly more time investigating pleasant odors compared to unpleasant ones. **Bi-Bii.** Signals during the investigation of pleasant (**Bi**) and unpleasant (**Bii**) odors. **Ci.** The Area under the curve for Dopa signal was significantly higher during the investigation of pleasant odors compared to unpleasant ones. **Cii.** No significant changes were observed in Ca^{2+} signal during odorant investigations. **Di.** Averaged Dopa signal across mice within a time bin locked epoch (0 to + 500ms) centered on olfactory investigation. Mean Dopa signal was higher during the investigation of pleasant odors. **Dii.** The same result was retrieved regarding the averaged Ca^{2+} signal. *Points represent individual data \pm sem. * $p < 0.05$, *** $p < 0.001$.*

2- Analysis of dopaminergic and calcium signals in response to the escape behavior.

Using our workflow, it is possible to retroactively extract and analyze another type of event. Here, we analyzed the exploration of the edges of the board corresponding to an escape behavior (“*escape events*”) (Figure 7Ai). During the 2 minutes of testing, times when the mouse entered and left the board’s edges were identified and referenced as a specific event (“*escape events*”) in an Excel spreadsheet. This allows to calculate the total duration at the board’s edge. This new spreadsheet was used in the MATLAB code to analyze the corresponding fiber photometry signal.

The time spent investigating the edge of the board was similar to the one spent investigating the pleasant odorant (bilateral paired Wilcoxon-test, $p=0.21$; Figure 7Aii). Interestingly, despite a similar level of exploration, the Aucs of the dopaminergic and calcium signals (from 0 to 500 ms) were higher during the investigation of the pleasant odorants compared to the edges of the board (bilateral paired t-test, $p=0.0006$ and $p=0.0002$ respectively; Figure 7B and C). Similar results were obtained using the analysis of the mean signals during that same time window (bilateral Wilcoxon tests, $p<0.0001$ for both signals; Figure 7Di and Dii).

Finally, we verified the viral injection and proper implantation of the optical fibers in the animals (Figure 8). The viruses were found on 6 sections ± 1 on average, along the antero-posterior axis of the OT; corresponding to 1.604 mm (± 820).

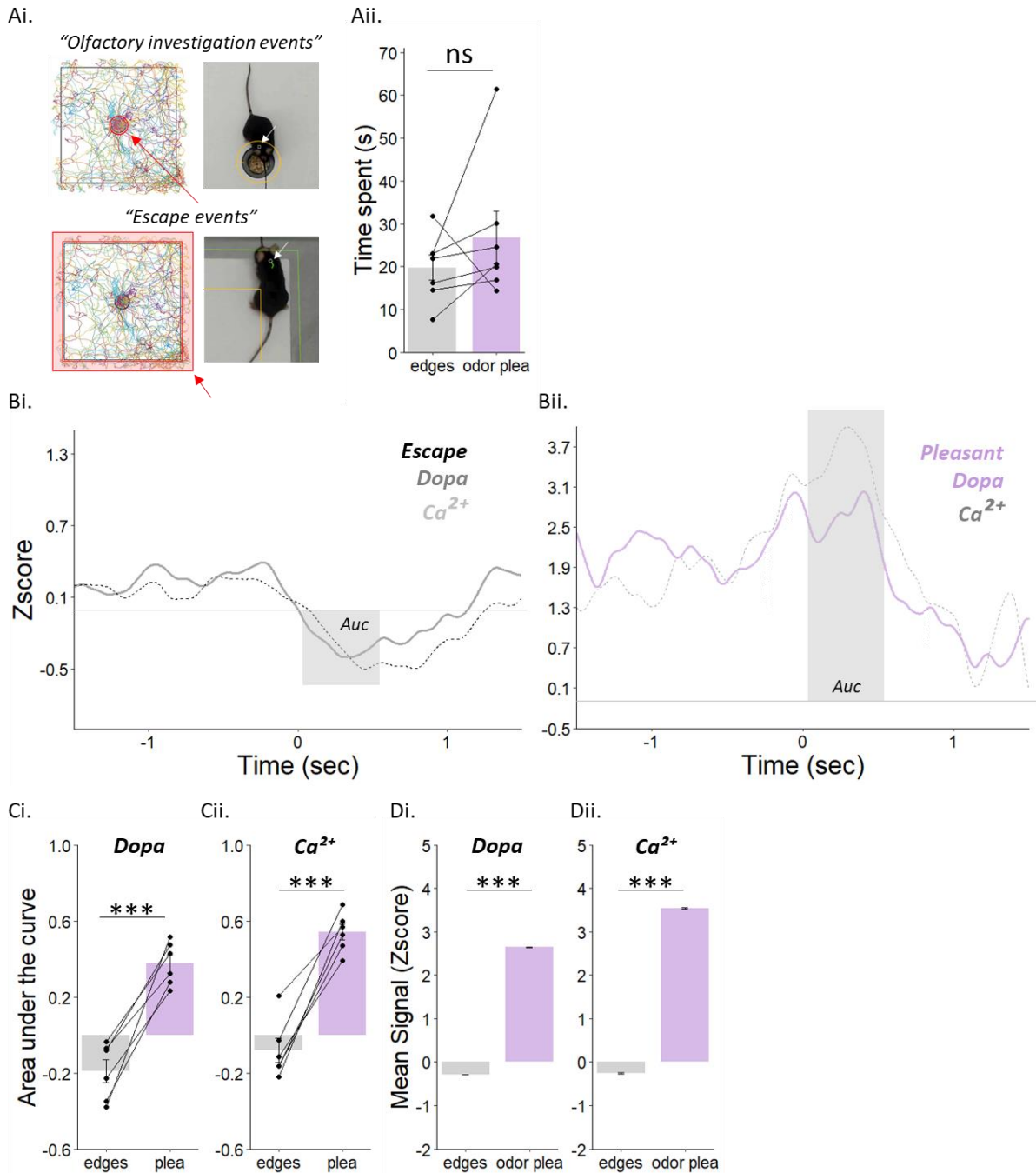


Figure 7. Signals for “escape events” did not follow the same pattern as the ones from “olfactory investigation events”. **Ai.** The mouse was detected on the hole corresponding to “olfactory investigation events” (up) or at the edges of the board corresponding to “escape events” (bottom) when the head is outside the board. **Aii.** Edges of the board were investigated as much as the hole odorized with pleasant odors. **Bi-Bii.** Signals (Zscore) during the investigation of the edges of the board (**Bi**) and the pleasant odors (**Bii**). **Ci-Cii.** Aucs corresponding to Dopa and Ca^{2+} signals were significantly higher during the investigation of pleasant odors compared to the edges of the board. **Di-Dii.** Averaged Dopa and Ca^{2+} signals across mice within a time bin locked epoch (-2 to +2 s) centered on olfactory investigation were higher during the investigation of pleasant odors. Points represent individual data \pm sem. * $p < 0.05$, *** $p < 0.001$, # $p = 0.05$.

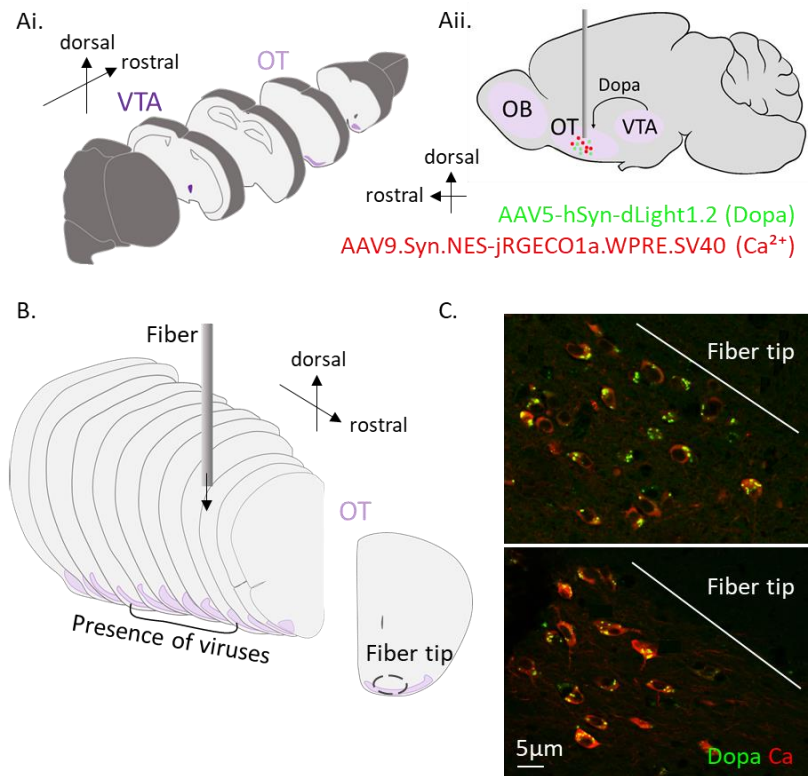


Figure 8. Injection sites, fiber placements, and fluorescence expression in the targeted brain region. **Ai.** Representation of the localizations of the OT and the VTA in the mouse brain (exploded view) (**Ai**) and with fiber position and viral expressions (**Aii**). **B.** Viruses were both expressed in a mean of 6 sections of 14 μm (5.7 mm) of the mOT. **C.** Photos of cells in the mOT expressing both viruses (x40, ZEISS® microscope).

Discussion

We presented a workflow for using ultrafast neuronal imaging to capture calcium (jRGECO1a) and dopaminergic (dLight1.2) signals during odor preference tests in freely moving adult mice. We demonstrated the effectiveness of this approach by showing increased dopaminergic release in the mOT of mice when investigating pleasant odors compared to unpleasant ones. This result highlights that a pleasant odorant can act as a natural reward and elicit dopaminergic release in the mOT. Furthermore, when examining the calcium signal, different results were observed based on the type of analysis. While the area under the curve is similar between odor hedonic conditions, a higher response was found for pleasant compared to unpleasant odors regarding the average signal. This last result aligns with previous findings showing that pleasant odors generate attraction through the recruitment of the OT and VTA (Midroit *et al.*, 2021). This difference could be explained by the nature of the analyses. The area under the curve measures the total signal amplitude over a given period, capturing the overall accumulation of activity while the mean signal represents the averaged amplitude, which appears to be more sensitive for detecting transient differences.

Our workflow can be applied to any behavior that might retrospectively become of interest. In our case, we also analyzed the “*escape*” behavior. We observed that this escape behavior occurred as frequently on the board as “*olfactory investigation of pleasant odorants*”. Interestingly, it was not associated with the same dopaminergic or calcium patterns in the OT as for the odor investigation. This supports the hypothesis that pleasant odor investigation depends on the OT activity, while escape behavior, which also drives attractiveness, does not.

Finally, the method offers additional advantages. Indeed, it allows not only for modifications to the type of event being analyzed but also for adjustments to the time window of analysis or for selection by the sequence of the event during the test trial (*e.g.*, the first event, last event, or an average of all events). This allows for a detailed analysis of the relationship between behavior and brain response.

One of the main challenges in conducting fiber photometry experiments is the data analysis. Its complexity limits the range of behavioral experiments to which it can be applied and the way to analyze them (*Bruno et al., 2021*). Our goal was to take advantage of the synchronization between behavioral and fiber photometry data to segment behavioral events into distinct time-defined events, allowing the extraction, alignment, and analysis of corresponding signals for group and condition comparisons. This approach simplifies and enables the analysis of any behavior of interest within a task. It also diminishes the need to limit fiber photometry to control experimental designs, predefined external triggers or strong paradigms such as fear conditioning. Finally, the coupling between the signal and the behavioral recordings allows spatial analysis by tracking neurotransmitter levels based on the animal's position.

However, this method alone has some limitations. It cannot precisely identify the responses of different neuronal populations within the mOT. One key limitation is the inability to record the activity of individual cells, as the probe detects fluorescence from the surrounding neuronal populations expressing the biosensor. This issue could be addressed by using transgenic animal lines that target specific neuronal subtypes (*Wang et al., 2021*) and/or cell specific promoters within the virus. Calcium release in response to pleasant odorants may occur in some sub-population of neurons, potentially in response to dopaminergic input from the VTA. Even if the overall calcium signal could reflect the combined activity of multiple neural populations, the response is likely driven by medium spiny neurons, the main population of the mOT which expresses dopamine membrane receptors (D1 and D2) (*Bertran-Gonzalez et al., 2008; Xiong & Wesson, 2016*).

In conclusion, this workflow provides valuable insight into the neural basis of olfactory hedonics, revealing that attractive odorants not only activate the mOT, but also trigger dopamine release in the structure.

More broadly, fiber photometry is one of the most sensitive and accessible techniques for recording cell-type-specific neuronal activity and modulation in brain structures of behaving animals (*Guo et al.,*

2015). Exciting advances are made in this field, giving this approach a promising future. However, much remains to be understood. For instance, it is still unclear whether the changes in calcium levels detected by fiber photometry reflect alterations in action potential firing, somatic *versus* non-somatic calcium fluctuations, or a combination of both (Legaria *et al.*, 2022). Furthermore, we do know that fiber photometry can capture events across a wide range of timescales, from sub-second to several minutes (Simpson *et al.*, 2023). However, identifying the precise timescales over which neurotransmitters are modulated by specific behavioral responses remains to be determined. Further investigation of ecological behaviors, as well as the exploration of diverse behavioral responses will enhance both technical and theoretical understanding, contributing to greater reproducibility across studies.

While there is still a growing need to ensure reproducibility and understand the neural-behavioral correlates of ecological behaviors, recent studies have provided detailed protocols and open-source software to address these challenges (Bruno *et al.*, 2021; Conlisk *et al.*, 2023; Murphy *et al.*, 2023). In this effort, we aim to contribute by presenting a comprehensive protocol for the acquisition and analysis of the relationship between exploratory behaviors such as olfactory perception and brain function.

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Annexe 1.

1. Fiber photometry signals reading and pre-processing

```
% READING DORIC FILES
flagErreurDoric1 = 0;
flagErreurDoric2 = 0;
clear StrData
StrData_nb = 0;
for n=1:size(TableProto,1)
    % Reading DORIC file and compatibility tests
    flagOK = 1;
    [TimeCtrl, SignalCtrl, TimeDopa, SignalDopa, TimeCa, SignalCa] = CartoDopa_LectureDoric(nomFicDataDoric(n));
    if size(TimeCtrl,1) == 0 || abs(sum(TimeCtrl-TimeDopa)) > 0 || abs(sum(TimeCtrl-TimeCa)) > 0
        flagErreurDoric1 = 1;
        disp(['ERREOR reading Doric file (possible timing offset) : ',nomFicDataDoric(n)]);
        % TIME BASE CORRECTION
        TimeDopa = TimeCtrl;
        TimeCa = TimeCtrl;
        flagOK = 0;
    end
    if (size(TimeCtrl,1) ~= size(TimeDopa,1)) || (size(TimeCtrl,1) ~= size(TimeCa,1))
        flagErreurDoric2 = 1;
        disp(['ERROR',nomFicDataDoric(n)]);
        flagOK = -1;
    end
end

% Reading and tests OK: DATA PRE-PROCESSING
if flagOK >= 0
    % DATA FILTERING
    [SignalCtrl_Filtre, SignalDopa_Filtre, SignalCa_Filtre] =
        CartoDopa_FiltrageData(TimeCtrl, SignalCtrl, SignalDopa, SignalCa);
    % STORAGE OF FILTERED CTRL, DOPA, CA VECTORS IN COMMON STRUCTURE
    StrData_nb = StrData_nb+1;
    StrData(StrData_nb).NumSel = n;
    StrData(StrData_nb).Time = TimeCtrl;
    StrData(StrData_nb).SignalCtrl_Filtre = SignalCtrl_Filtre;
    StrData(StrData_nb).SignalDopa_Filtre = SignalDopa_Filtre;
    StrData(StrData_nb).SignalCa_Filtre = SignalCa_Filtre;
    % CHANGE FROM BASELINE: DF/F CALCULATIONS
    SignalDopa_dFsurF = (SignalDopa_Filtre-SignalCtrl_Filtre)./SignalCtrl_Filtre;
    SignalCa_dFsurF = (SignalCa_Filtre-SignalCtrl_Filtre)./SignalCtrl_Filtre;
    StrData(StrData_nb).SignalDopa_dFsurF = SignalDopa_dFsurF;
    StrData(StrData_nb).SignalCa_dFsurF = SignalCa_dFsurF;
    % SEARCH FOR StartX/EndX FACTORS FOR ZSCORE CALUCLATIONS
    tpsStart0 = TableProtoEvt.Start0(n);
    tpsEnd0 = TableProtoEvt.End0(n);
    for i=1:size(TimeCtrl,1)-1
        if ((TimeCtrl(i)) <= tpsStart0) && ((TimeCtrl(i+1)) > tpsStart0)
            indTpsStart0 = i;
            break;
        end
    end
    for i=1:size(TimeCtrl,1)-1
        if ((TimeCtrl(i)) <= tpsEnd0) && ((TimeCtrl(i+1)) > tpsEnd0)
            indTpsEnd0 = i;
            break;
        end
    end
    % ZSCORE CALCULATIONS ON THE PERIOD OF INTEREST StartX/EndX
    periodeDopa = SignalDopa_dFsurF(indTpsStart0:indTpsEnd0);
    periodeCa = SignalCa_dFsurF(indTpsStart0:indTpsEnd0);
end
```

```

        StrData(StrData_nb).SignalDopa_ZScore = zscore(periodeDopa);
        StrData(StrData_nb).SignalCa_ZScore = zscore(periodeCa);
        StrData(StrData_nb).Time_ZScore = TimeCtrl(indTpsStart0:indTpsEnd0);
    end
end

% Detailed function CartoDopa_FiltrageData
function [cDataCtrl, cDataDopa, cDataCa] = CartoDopa_FiltrageData(Times, DataCtrl, DataDopa, DataCa)
% LOWPASS FILTER BUTTERWORTH
dt=diff(Times);
dt=dt(1);
cutoff=10 ;
Fs = (1/dt);           % Aquisition Frequency
Fn = Fs/2;            % Nyquist Frequency
Wp = cutoff/Fn;       % Passband Frequency (Normalised)
Ws = 3/Fn;            % Stopband Frequency (Normalised)
Rp = 2;
Rs = 10;
[nb,Wn] = buttord(Wp,Ws,Rp,Rs);
[b,a] = butter(nb,Wn,'low');
dnFDataCtrl = filtfilt(b,a,DataCtrl);
dnFDataDopa = filtfilt(b,a,DataDopa);
dnFDataCa = filtfilt(b,a,DataCa);
% Smoothing filter and polynomial fitting function
s=0.05 ;               % Smoothing index
dnFDataCtrl = smooth(dnFDataCtrl,s,'lowess'); % to include if the channel introduce noise
bls = polyfit(dnFDataCtrl(1:end), dnFDataDopa(1:end), 1);
Y_Fit = bls(1) .* dnFDataCtrl + bls(2);
cDataDopa = (dnFDataDopa(:)-Y_Fit(:))./Y_Fit(:);
bls = polyfit(dnFDataCtrl(1:end), dnFDataCa(1:end), 1);
Y_Fit = bls(1) .* dnFDataCtrl + bls(2);
cDataCa = (dnFDataCa(:)-Y_Fit(:))./Y_Fit(:);
cDataCtrl = dnFDataCtrl;
end % End function

```

2. Video processing (ROIs and Trajectories)

```

% READING AND ANALYZINF REGX AND TRAJ FILES
% REGX and TRAJ files are files generated with A2V program after video analysis. REGX files contain the different drawn
% regions of interests (hole and board). TRAJ files contain mouse tracking during the 2 minutes of video recordings.
flagErreurRegx = 0;
flagErreurEvtx = 0;
flagErreurTraj = 0;
clear StrTraj
StrTraj_nb = 0;
for n=1:size(TableProto,1)
    % Reading Regx file (for P1,P2,Center)
    [P1, P2, Centre, erreurRegx] = CartoDopa_LectureRegx(nomFicDataRegx);
    % Reading optional Evtx file for T1 and T2
    [DEF_Evtx, erreur] = A2V_LectureFicEvtx(nomFicDataEvtx);
    % Reading Trajectory
    [Xtraj, Ytraj, TPStraj, TpsDeb, TpsFin, erreur] =
        A2V_LectureFicTraj(nomFicDataTraj, DEF_Evtx.T1, DEF_Evtx.T2, SeuilCoordTraj);
    % TRANSLATION AND ROTATION OF THE TRAJECTORY (to control heterogeneity of video recordings (board positions)
    [Xtraj, Ytraj] = CartoDopa_TranslationTraj(Xtraj, Ytraj, Centre);
    [Xtraj, Ytraj] = CartoDopa_RotationTraj(Xtraj, Ytraj, P1, P2);
    % TRAJECTORY CONVERSION: FROM PIXELS TO MM
    % P1 and P2 stands for the two locations of the bottom edges of the board.
    distP1P2 = sqrt((P1.x-P2.x)*(P1.x-P2.x) + (P1.y-P2.y)*(P1.y-P2.y));
    coef = TailleBoard/distP1P2;
    Xtraj = Xtraj * coef;
    Ytraj = Ytraj * coef;
    % STORAGE OF TRAJECTORIES IN A COMMON STRUCTURE

```

```

StrTraj_nb = StrTraj_nb+1;
StrTraj(StrTraj_nb).NumSel = n;
StrTraj(StrTraj_nb).nbPt = size(TPStraj,1);
StrTraj(StrTraj_nb).TPStraj = TPStraj;
StrTraj(StrTraj_nb).Xtraj = Xtraj;
StrTraj(StrTraj_nb).Ytraj = Ytraj;
StrTraj(StrTraj_nb).T1 = DEF_Evtx.T1;
StrTraj(StrTraj_nb).T2 = DEF_Evtx.T2;
StrTraj(StrTraj_nb).TpsDeb = TpsDeb;
StrTraj(StrTraj_nb).TpsFin = TpsFin;
end

```

3. Merge of video and signal data

```

% CartoDopa_CalcCartographie : CALCULATIONS FOR HEAT MAPS
% Generation of matrix for presence density display
% nbCase corresponds to the size of the frame in the pixel matrix
nbCase = round(TailleCadre / TaillePasGrille);
TailleCadrePix = nbCase;

% Calculation of the Dopa and Ca density and map display
% regarding trajectories (presence in the frame of the matrix)
if StrTraj_nb > 0
    [imageZS_Dopa, imageZS_Ca] = CartoDopa_CalcDensiteDopaCa(TableProto, StrData, StrData_nb, StrTraj, StrTraj_nb, ...
        TailleCadre, TaillePasGrille, WID_toggleOptCarto.Value);
end

```

```

% Detailed function CartoDopa_CalcDensiteDopaCa
% CALCULATION OF THE MEAN SIGNAL FOR DOPA AND CA regarding mouse's position
function [imageZS_Dopa, imageZS_Ca] = CartoDopa_CalcDensiteDopaCa(TableProto, StrData, StrData_nb, StrTraj, ...
    StrTraj_nb, TailleCadre, PasGrille, modeCalcul)

% Initialize density image to zero
nbCase = round(TailleCadre / PasGrille);
imageZS_Dopa = round(zeros(nbCase,nbCase));
imageZS_Ca = round(zeros(nbCase,nbCase));
imageNB = round(ones(nbCase,nbCase));
dataOK = 0;

% Loop on all points of all selected paths
for n=1:StrTraj_nb
    if TableProto{StrTraj(n).NumSel,1} > 0
        dataOK = 1;
        % Searching for corresponding data
        indiceData = 0;
        for i=1:StrData_nb
            if StrTraj(n).NumSel == StrData(i).NumSel
                indiceData = i;
                break;
            end
        end
        % Loop on all points of all selected paths regarding the time interval between
        % 2 successive positions to average the values
        if indiceData > 0
            Periode = StrData(indiceData).Time(2)-StrData(indiceData).Time(1);
            indData0 = 1;
            for k=1:StrTraj(n).nbPt-1
                % Current point and grid index recovery
                xpos = StrTraj(n).Xtraj(k);
                ypos = StrTraj(n).Ytraj(k);
                indx = floor((ypos + TailleCadre/2)/ (PasGrille))+1;
                indy = floor((xpos + TailleCadre/2)/ (PasGrille))+1;
                % Start/end index search in ZScore data
                if indx > 0 && indx <= nbCase && indy > 0 && indy <= nbCase

```


-DISCUSSION GÉNÉRALE-

-Premier Chapitre-

Les conséquences à long terme du stress précoce

1.1) *Stress précoce et comportement maternel*

Chez l'humain, la maltraitance durant l'enfance induisant le stress précoce découle de la répétition d'événements aversifs, dont les conséquences peuvent s'accumuler et interagir. Même si le modèle LBN ne représente pas entièrement la complexité des traumatismes que peuvent subir les enfants (Burns, 2018; Walker et al., 2017), il les reproduit assez bien, puisqu'il repose sur la répétition pendant 7 jours des mauvais comportements de la mère, allant de la négligence à l'agressivité. Dans notre étude principale, le suivi du comportement maternel pendant les 7 jours de protocole de stress, a confirmé la maltraitance opérée sur les petits. En effet, nous avons observé que le modèle LBN ralentit la croissance des petits mâles et femelles et modifie leur locomotion (ÉTUDE 1 page 14, Figure 3). Dans la littérature, il est intéressant de noter que les répercussions négatives du modèle LBN concerneraient les rats dont la mère adopte le comportement le plus entropique, c'est-à-dire imprédictible. L'imprédictibilité de la mère pourrait ainsi être une mesure supplémentaire à prendre en compte dans l'étude de l'impact du stress précoce (Kangas et al., 2022).

Nous savons grâce à des études chez l'humain que la qualité du soin prodigué **après** un événement traumatique peut également impacter la santé mentale de l'individu et sa future réponse au stress (Glynn et al., 2019; Smith & Pollak, 2020). Une mauvaise prise en charge dans une famille ou un foyer qui ne prodigue pas les soins et l'attention requise peut-être à l'origine de traumatismes supplémentaires. Ceci favorise la persistance d'un environnement imprédictible délétère pour l'enfant. Dans notre étude, les petits sont restés avec la mère les jours suivants le stress précoce jusqu'à la période de sevrage (P21), dans un environnement classique. Le comportement maternel a cependant pu être impacté durablement par le modèle LBN, même une fois de retour dans des conditions d'hébergement habituelles. L'analyse du comportement maternel de P12 jusqu'à la période de sevrage (P21), ou lorsqu'une situation où le stress perdure -présence d'une autre mère ou d'un contexte de nouveau aversif- permettraient de mieux comprendre comment les interactions mère-petit évoluent après la phase aiguë de stress et si des changements comportementaux chez la mère persistent même en l'absence de stress immédiat, ce qui pourrait influencer la survenue d'un phénotype vulnérable ou résilient chez les petits (Santarelli et al., 2017).

De plus, l'adolescence (autour de P20 chez la souris) est une deuxième fenêtre de neurodéveloppement sensible à l'environnement avec des phénomènes importants de remodelages morphologiques, fonctionnels et comportementaux (Andersen, 2016; Casey et al., 2008; Leussis & Andersen, 2008; McCormick & Mathews, 2010; Spear, 2000; Tsoory et al., 2007). Un comportement maternel altéré jusqu'à cette période pourrait avoir des conséquences néfastes supplémentaires influençant l'individu adulte.

En conclusion, il reste une part à investiguer pour comprendre l'influence du comportement maternel sur le devenir des petits. Les études sont généralement faites chez le jeune ou bien chez l'adulte et la recherche pourrait bénéficier d'une approche longitudinale.

1.2) Stress précoce : stress physiologique et perçu

Le stress précoce provoque l'hyperactivité de l'axe HPA (Bath et al., 2016; Breton et al., 2024; Danese & McEwen, 2012; Gunnar & Quevedo, 2007; Tan et al., 2021), et des études bloquant cette hyperactivité ont montré des effets partiels de sauvetage (Arp et al., 2016; Lesuis et al., 2018; Sanguino-Gómez & Krugers, 2024). Le stress précoce agit donc *via* d'autres prismes -neurodéveloppemental, génétique ou encore épigénétique- et ses effets ne sont pas dépendants uniquement de l'axe HPA. Enfin, vis-à-vis de la perception olfactive, nous rappelons que les impacts du stress dépendent de son poids sur l'humeur (induction d'émotions négatives, perturbation des régulations émotionnelles) plus que sur les niveaux de glucocorticoïdes (Hoenen et al., 2017). Ainsi, notre étude ne s'est pas centrée sur le stress physiologique et nous n'avons pas réalisé de mesures des niveaux de corticostérone. Des études ont d'ores et déjà confirmé que le modèle LBN est souvent associé à des hausses de corticostérone chez l'animal (Breton et al., 2024). Nous nous sommes donc plutôt focalisés sur le stress perçu, observable à travers un comportement inadapté pour valider le caractère traumatisant du modèle LBN. Enfin, le développement du système olfactif, et plus spécifiquement celui du BO se déroule pendant la période critique (Tufo et al., 2022), moment où les odeurs jouent un rôle crucial pour l'individu (Moriceau et al., 2009, 2010; Moriceau & Sullivan, 2006; Opendak et al., 2019; Opendak & Sullivan, 2019). C'est donc l'impact à long terme du stress précoce sur le développement du système et du sens olfactif que nous avons jugé pertinent de tester. Comme nous allons le détailler plus tard, notre étude a montré que le développement postnatal du BO est perturbé par le stress précoce (ÉTUDE 1, page 18, Figure 6), ce qui est susceptible de chambouler la vie future de l'individu, son approche aux odeurs et donc, d'autres comportements qui dépendent du sens olfactif.

1.3) Stress précoce et phénotype émotionnel adulte

Les études **chez l'humain** évaluent l'impact psychologique du stress précoce avec des échelles standardisées (Smith & Pollak, 2020) reposant sur le souvenir de l'individu et sa fiabilité, ce qui peut induire un biais (Birn et al., 2017; Daskalakis et al., 2013).

Chez le modèle animal, l'impact du stress précoce sur le comportement émotionnel est évalué via des mesures comportementales variées (Andersen, 2022), ce qui peut également compliquer l'analyse et l'interprétation des résultats. En effet, l'expérimentateur choisit d'évaluer un ou des comportement/s parmi un spectre complexe des effets du stress précoce (Bolton et al., 2017). Dans notre étude, nous avons utilisé la préférence au sucre, l'*openfield*, la *light/dark box*, et le *novelty induced hypophagia*. Il s'est avéré que les animaux stressés étaient perturbés sur chacun de ces tests par rapport aux contrôles (ÉTUDE 1, page 15, Figure 4). Le modèle LBN altère bel et bien les comportements émotionnels chez les souris adultes mâles en induisant des comportements de type anxieux et dépressif. En combinant ces résultats dans un index comportemental, nous concluons que les souris adultes mâles ont un phénotype émotionnel altéré par rapport aux souris contrôles.

Au sein des groupes et entre les études, des différences de résultats vis-à-vis du comportement émotionnel peuvent en partie être expliquées par la variabilité individuelle. Les différences individuelles face au stress précoce, bien que peu étudiées (Smith & Pollak, 2020) sont en effet majeures. Chez l'humain comme le rongeur, le stress précoce n'affecte pas les individus de manière uniforme, générant des effets variés qui peuvent dépendre de l'âge, du sexe, du comportement maternel, de la personnalité et du comportement basal (Birn et al., 2017). Dans ce contexte, l'index de stress, qui a premièrement confirmé le caractère traumatisant du modèle LBN sur le comportement émotionnel global, a un intérêt qui dépasse cette simple validation. Cet index pourrait permettre d'aller plus loin dans la distinction de profils comportementaux : des animaux contrôles qui se comportent comme la moyenne ou à tendance

plus anxieuse, des animaux stressés fortement impactés par le modèle LBN ou au contraire plutôt résilients. Une telle approche pourrait affiner les résultats comportementaux et neuronaux observés après le stress précoce et permettre d'explorer l'existence de patterns.

Dans notre étude, le stress précoce a donc eu des impacts profonds sur le comportement et le phénotype émotionnel des souris mâles. De façon intéressante, nous n'avons pas observé un tel impact sur le comportement des souris femelles, ce qui nous invite à nous interroger sur la notion de résilience, ou résistance face au stress, qui fait l'objet du deuxième chapitre de cette discussion.

-Deuxième Chapitre- **Stress précoce et résilience**

Même si la plupart des études se concentrent sur les aspects négatifs du stress précoce -dont son impact sur les troubles affectifs et psychiatriques à l'âge adulte- un certain nombre relèvent que le stress précoce peut ne pas avoir d'effet, voire qu'il peut être bénéfique sur le comportement et/ou la neurobiologie de l'humain et de l'animal (*Daskalakis et al., 2013; Krugers et al., 2017; Southwick & Charney, 2012*).

Le mot « **résilience** » veut dire étymologiquement « rebondir ». La définition de ce terme a également fait un passage du côté de la physique où il fait référence à la capacité d'un matériau de résister à un choc (*Centre National de Ressources Textuelles et Lexicales*). La résilience désigne donc la capacité d'un individu à surmonter des épreuves difficiles et à s'adapter aux situations adverses. Dans des définitions plus humanistes, la résilience « c'est la résistance à la destruction et la construction d'une existence valant d'être vécue » (*Manciaux, 2001*). Emilie Werner, une psychologue américaine influente sur ce sujet, parle des enfants traumatisés qu'elle a suivis pendant de longues années comme des individus « vulnérables, mais invincibles » (*Manciaux, 2001*). D'autres personnalités telles que Boris Cyrulnik ou John Bolwby, font partie de ceux qui ont beaucoup travaillé autour de cette notion et participé à sa vulgarisation dans la psychologie clinique dès les années 50. Depuis, la recherche autour de la résilience s'est enrichie, prenant en compte des facteurs biologiques, psychologiques et sociaux autour de l'idée que certains ont des capacités d'adaptation au stress, parfois innées, parfois de l'ordre de la transformation personnelle.

Différentes théories tentent ainsi d'expliquer la vulnérabilité ou la résilience au stress précoce **chez l'humain**.

Le modèle du stress cumulatif postule que si l'accumulation de stress dépasse un certain seuil, l'apparition de troubles affectifs est favorisée (*Danese & McEwen, 2012; McEwen, 2000; McEwen & Gianaros, 2011*). L'adversité cumulée augmenterait ainsi progressivement le risque de maladie, et la résilience dépendrait alors directement du seuil de chaque individu.

La théorie de **concordance/non-concordance développementale** dit que le stress précoce pourrait préparer à un environnement adulte difficile et représenter une source d'adaptation active (*Schmidt, 2011; Schmidt et al., 2011; Van Bodegom et al., 2017*). Si l'environnement adulte est conforme aux attentes programmées par les expériences précoces (concordance), cela favorise la résilience. À l'inverse, un décalage entre l'expérience précoce et adulte (non concordance) peut exacerber la vulnérabilité aux troubles de la santé mentale. Dans tous les cas, la vulnérabilité ou la résilience

dépendent également des prédispositions génétiques des individus, c'est **la susceptibilité différentielle à l'influence de l'environnement** (Daskalakis et al., 2013).

La théorie des trois coups, comme les trois points essentiels de la vulnérabilité ou de la résilience est la combinaison de ces théories (Daskalakis et al., 2013). Le coup-1 correspond à la prédisposition génétique, le coup-2 dépend de l'environnement de la petite enfance, et le coup-3, de celui de la vie ultérieure. Ce concept est fondamental pour comprendre comment les individus peuvent être aptes à faire face à un environnement précoce difficile et aux défis futurs en étant résilients (« eustress »), ou bien incapables de le faire et succombent à une variété de troubles et leurs comorbidités (« distress »), dans des circonstances à l'origine identiques.

La recherche animale tente également d'apporter des éléments de réponse depuis quelques années, si bien que les études autour de la résilience se multiplient. Chez le primate et le rongeur, des expériences stressantes modérées (séparation maternelle brève) peuvent renforcer la résilience, avec une meilleure régulation émotionnelle et une réponse HPA atténuée. À l'inverse, les privations sociales prolongées et intenses entraînent plus de vulnérabilité avec des comportements anormaux et des altérations neurobiologiques persistantes (Bolton et al., 2017; Köhler et al., 2019; Maestripieri et al., 2006). Les adversités précoces peuvent même conférer une résilience contre les effets neuroendocriniens, comportementaux et moléculaires du stress chronique à l'âge adulte (Santarelli et al., 2017), avec par exemple une amélioration des performances mnésiques (Oomen et al., 2010). De plus, il est utile de rappeler que le modèle LBN induit des comportements maternels allant de la maltraitance à l'hypervigilance (Gallo et al., 2019), ce qui pourrait conditionner la prévalence d'un phénotype vulnérable ou résilient.

La résilience, dont il a été montré qu'elle dépend en partie de **la génétique** chez l'humain (Daskalakis et al., 2013), pourrait dépendre chez l'animal de la souche utilisée (Burns, 2018; Savignac et al., 2011). Cette observation supporte la nécessité d'interpréter l'historique des altérations émotionnelles dues au stress précoce à la lumière de la prédisposition génétique.

Concernant **les mécanismes neurobiologiques** impliqués, préserver l'activité de certaines structures telles que celles du striatum pourrait favoriser la résilience face aux troubles dépressifs (Ironsides et al., 2018). Comme chez le primate non humain, la manière dont est modulé l'axe HPA par le stress chez le rongeur peut influencer la vulnérabilité ou la résilience au stress précoce (Daskalakis et al., 2013; Dudek et al., 2021). En effet, l'activité du système noradrénergique initié dans le LC est très liée à celle de cet axe et donc aux réponses physiologiques du système cardio-vasculaire et immunitaire. La suractivation du LC en parallèle de l'hyperactivité de l'axe HPA est ainsi devenue un marqueur des effets du stress (C. S. Wood et al., 2017). À noter que d'autres paramètres sont à prendre en compte dans les processus de vulnérabilité et/ou la résilience face au stress tels que la réponse du système immunitaire, les processus inflammatoires (Dudek et al., 2021), les facteurs génétiques et épigénétiques (Feder et al., 2019), ou encore le fonctionnement de différentes populations de cellules (astrocytes, microglies ou encore interneurons à parvalbumine) et l'activité de diverses structures cérébrales (amygdale, striatum, CPF, hippocampe) (Favoretto et al., 2023).

Pour conclure, la résilience est soutenue par des facteurs psychosociaux et des mécanismes biologiques actifs. Certains de ces éléments dépendent directement du comportement de l'individu (Dudek et al., 2021). Un support social suffisant ou un enrichissement environnemental peut par exemple prévenir la survenue des symptômes dits dépressifs (Planchez et al., 2019). La résilience pourrait ainsi découler d'une réserve cognitive propre à l'individu, dépendant de son expérience, son environnement, son comportement et sa génétique.

Dans notre étude, la question de la résilience se pose vis-à-vis des **femelles** qui n'expriment pas, contrairement aux mâles, d'effet délétère du stress précoce à l'âge adulte. L'absence d'observation d'un impact comportemental peut-elle dire que le stress précoce n'a véritablement pas eu d'effet ? Les femelles ont-elles un phénotype altéré qui s'exprime différemment des mâles ? Sont-elles résilientes, résistantes au stress ? Si oui, cette résilience est-elle innée ou bien acquise et développée plus tard ? À quel moment ? Basé sur quels mécanismes neurobiologiques ? Dans quel(s) contexte(s) cette résilience se met-elle en place et de quoi dépend-elle ? Nos résultats et ces questions en suspens soulignent la nécessité de creuser l'étude de l'impact du stress précoce sur les femelles adultes. Ces études permettraient d'aller plus loin dans l'identification des mécanismes impliqués dans ce phénomène de résilience.

Maintenant que nous avons parlé de la vulnérabilité et/ou la résilience des individus face au stress précoce, nous allons discuter autour des nouveaux éléments recueillis vis-à-vis du lien entre le stress précoce et la perception olfactive, au niveau comportemental et cellulaire.

-Troisième Chapitre-

Impacts du Stress précoce sur la Perception Olfactive

3.1) Stress précoce et perturbations du comportement olfactif

3.1.1) L'Anhédonie olfactive

Dans notre **ÉTUDE 1**, nous avons révélé que le stress précoce altère la perception hédonique des odeurs. Il diminue l'intérêt que les animaux ont pour des odorants naturellement plaisants/attractifs sans signification biologique particulière (*Kermen et al., 2016*). Ceci se traduit par une diminution du temps passé au niveau de la source odorante.

Dans la littérature, la sévérité des troubles olfactifs prédit celle de la dépression (*Kohli et al., 2016*). De manière intéressante, dans des analyses complémentaires de l'**ÉTUDE 1**, l'anhédonie olfactive corrèle avec l'anhédonie au sucrose chez les souris mâles stressées (**Figure 13**), mais pas avec les symptômes mesurés dans nos autres tests tels que l'*openfield* ou le *novelty induced hypophagia*. Cela suggère que les troubles olfactifs peuvent être liés à des indicateurs de certains symptômes dépressifs, sans pour autant affecter l'ensemble des manifestations de la dépression.

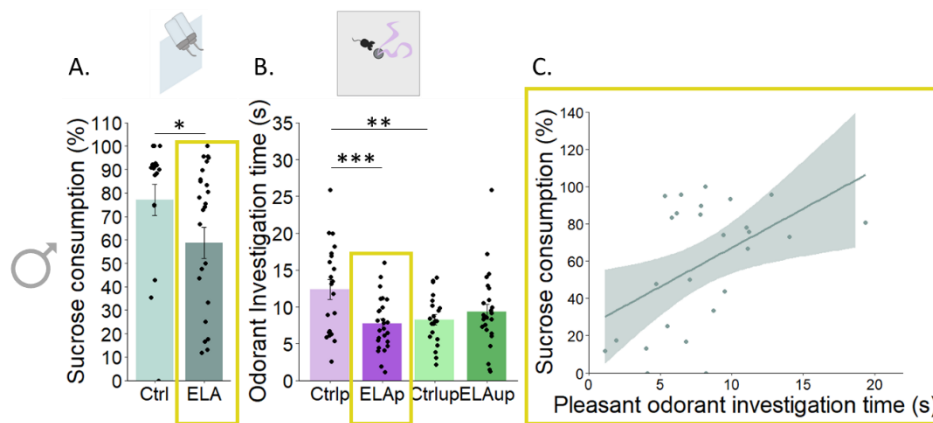


Figure 13. Analyse complémentaire : l'anhédonie au sucre corrèle positivement avec l'anhédonie olfactive chez les souris adultes mâles ELA. A-B. Rappel des résultats de l'ÉTUDE 1 avec une diminution de la consommation de sucre chez les souris ELA (A.), ainsi qu'une diminution de l'intérêt porté aux odorants plaisants (B.) par rapport aux souris contrôles. Ces deux altérations comportementales sont corrélées positivement (C. Corrélation de Spearman, $p=0.01$, $r=0.47$).

-Ctrl : contrôle ; ELA : Early Life Adversity ; p : pleasant/plaisant ; up : unpleasant/déplaisant

L'anhédonie olfactive corrélant uniquement avec l'anhédonie au sucre, les futures études se focalisant sur l'impact du stress précoce sur le phénotype émotionnel et la perception olfactive auraient intérêt à orienter l'analyse vers les comportements anhédoniques : le comportement social voire sexuel, le plaisir gustatif et la prise alimentaire (Planchez *et al.*, 2019) ou encore l'ajustement des choix et du comportement selon la probabilité d'obtenir une récompense (Kangas *et al.*, 2022) par exemple. Cela pourrait permettre de mieux caractériser la spécificité de l'anhédonie olfactive dans les altérations émotionnelles liées au stress précoce et de rechercher les bases neurales, communes ou pas, de ces types d'anhédonies.

Par ailleurs, notre étude n'a pas mis en évidence d'**alliesthésie olfactive**, soit la perception encore plus négative d'un stimulus olfactif à l'origine déplaisant (Atanasova *et al.*, 2010; Naudin *et al.*, 2012). Cela soutient l'idée selon laquelle les valeurs hédoniques positives et négatives des odeurs sont encodées par des mécanismes neuronaux au moins en partie distincts (Kermen *et al.*, 2016; Midroit *et al.*, 2021), qui réagissent de surcroît différemment au stress précoce. En effet, nous avons observé chez les souris contrôles une activité cellulaire du TO et de l'ATV plus importante en réponse aux odorants plaisants par rapport aux déplaisants, et que cette activité est altérée chez les souris stressées en réponse aux odorants plaisants uniquement (ÉTUDE 1, page 23, Figure 8).

Le stress précoce pourrait avoir un impact sélectif sur le circuit de la récompense, affectant principalement la capacité à ressentir du plaisir -anhédonie- sans modifier la sensibilité aux stimuli peu plaisants. L'absence d'alliesthésie olfactive pourrait également être un mécanisme visant à protéger l'organisme contre une perception excessivement négative des odeurs. En d'autres termes, une forme de résilience pourrait atténuer l'aggravation de la perception négative, même si le plaisir olfactif est altéré. À l'inverse, l'alliesthésie lorsqu'elle s'exprime, pourrait tendre à recréer une distinction entre le plaisant et le déplaisant en amplifiant la perception négative des stimuli aversifs.

En conclusion, ces hypothèses ouvrent la voie à de futures recherches sur les circuits sous-tendant la perception d'odorants plaisants et déplaisants ainsi que leurs implications dans les réponses comportementales au stress précoce.

Enfin, il est connu que la perception de la valeur hédonique des odeurs dépend chez l'humain de facteurs tels que la familiarité (*Delplanque et al., 2008*), l'attention (*Prescott et al., 2008*). On peut ainsi imaginer que prendre en compte ces autres facteurs permettrait de mieux comprendre les effets du stress précoce sur la perception hédonique des odeurs.

3.1.2) Stress précoce et autres fonctions olfactives

La perturbation de la perception hédonique des odeurs apparaît parfois dans la liste des symptômes de la dépression, mais cette observation ne fait pas l'objet d'un consensus (*Atanasova et al., 2010; Clepce et al., 2010, 2012; Croy & Hummel, 2017; Marin et al., 2023; Naudin et al., 2012; Pabel et al., 2018; Rochet et al., 2018*). Nous nous sommes donc focalisés sur les impacts du stress précoce sur cette fonction olfactive qui est au cœur du traitement émotionnel du stimulus olfactif. Il faut noter que nous n'avons pas mis en évidence dans notre étude d'altération de la détection d'odorants à 1 Pa après un stress précoce. Nous avons ici vérifié que les odorants étaient bien détectés par tous les animaux, à la concentration utilisée dans la tâche de préférence olfactive. Nous n'avons pas testé l'impact du stress précoce sur le seuil de détection olfactive et ne pouvons pas conclure à ce stade, même s'il semblerait qu'il n'y ait pas d'effet (*Pardasani et al., 2023*). Enfin, étant donné que nous avons identifié des atteintes anatomo-fonctionnelles du stress précoce au niveau du BO -la première structure du traitement des informations olfactives- il est raisonnable de supposer que d'autres fonctions olfactives, non évaluées dans notre étude, pourraient être affectées, telles que les performances d'apprentissages olfactifs perceptif et associatif.

Ainsi, le stress précoce, traumatisant émotionnellement, détériore la capacité des souris mâles adultes à être attirées par les odorants normalement plaisants. Ces impacts comportementaux du stress précoce retrouvés à l'âge adulte nous amènent à discuter dans la prochaine partie des bases neurobiologiques associées.

3.2) Les bases neurobiologiques du stress précoce sur le système olfactif

3.2.1) Stress précoce et BO

Stress précoce et morphologie des interneurones. Dans notre **ÉTUDE 1**, des répercussions neurobiologiques du stress précoce sont retrouvées dès le point de départ cortical du système olfactif : le BO. Le stress précoce diminue la complexité de l'arborisation dendritique basale des cellules granulaires répondant aux odeurs plaisantes et la densité synaptique de leurs dendrites (**ÉTUDE 1**, page 19/20, **Figure 6**). Si la tendance était observée chez les épines de types fines et champignons, la différence était portée par les épines de types filopodes. Les cellules immatures -comme nos cellules granulaires au moment du stress- ont un niveau élevé de formation et de rétraction des filopodes, une dynamique qui diminue à mesure que les cellules mûrissent (*Breton-Provencher et al., 2014*). Des niveaux de densité différents touchant particulièrement les épines de type filopodes dans notre étude pourraient ainsi refléter un défaut de maturation.

Une étude soutient que la densité synaptique des cellules granulaires peut être modifiée par l'inflammation, lorsque celle-ci a lieu durant une fenêtre temporelle courte, juste après la naissance de nouvelles cellules (*Lazarini et al., 2014*). L'inflammation générée par le stress précoce a donc pu impacter la maturation et la différenciation des cellules granulaires et modifier leur morphologie à long terme.

De plus, dans des analyses complémentaires préliminaires, nous nous sommes penchées sur la morphologie fine des épines dendritiques -la taille du cou ou encore le diamètre de la tête- entre les

groupes contrôle et ELA (Figure 14). Nous avons observé des modifications morphologiques entre les groupes, qui pourraient être associées à des changements de plasticité synaptique influençant la communication entre les neurones et, par extension, les processus cognitifs et émotionnels. Le stress précoce altère donc profondément la structure des cellules granulaires, de leur morphologie générale à la structure fine de leurs épines et donc potentiellement leur capacité à former et maintenir des connexions synaptiques efficaces.

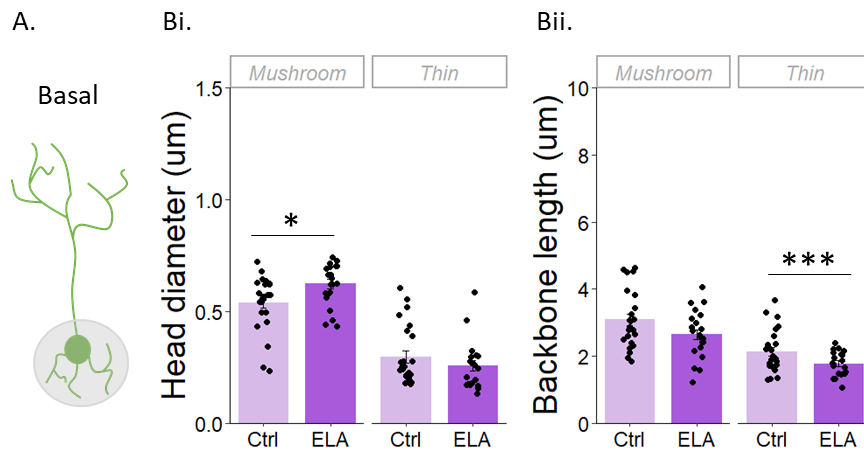


Figure 14. Analyse complémentaire : le stress précoce modifie la morphologie fine des épines situées sur les branches basales des interneurons répondant aux odorants plaisants. A. Schéma de la localisation analysée. Bi. Le stress précoce augmente le diamètre de la tête (« Head diameter ») des épines de type « Mushroom » ou champignon. Bii. Le stress précoce diminue la longueur du cou (« Backbone length ») des épines fines. $n=18$ neurones de souris Ctrl et $n=16$ neurones de souris ELA. (ANOVA 2, $*p < 0.05$, $***p < 0.0001$). -Ctrl : contrôle ; ELA : Early Life Adversity-

Au vu de ces résultats, une piste intéressante pour de futures études serait d'analyser la connectivité synaptique des interneurons granulaires pour mieux comprendre la fonctionnalité de ce circuit (Druckmann et al., 2014). Le LC que nous avons décrit précédemment, innerve massivement le BO (Brunert & Rothermel, 2021), est impliqué dans le traitement spontané des odeurs, ou lié à une motivation (tâche associative ou non) (Linster, 2019; Linster & Escanilla, 2019), et joue un rôle dans la vie précoce et sa sensibilité au stress (Sheppard et al., 2024). De par ces caractéristiques, l'hypothèse principale serait que la connectivité entre les neurones noradrénergiques (provenant du LC) et les dendrites basales des cellules granulaires pourrait être touchée par le stress précoce. Pour vérifier cette hypothèse, nous pourrions utiliser une méthode d'ingénierie moléculaire contrôlée génétiquement pour détecter sélectivement les synapses formées entre les neurones d'une source définie et une population cible : mGRASP (Druckmann et al., 2014; Feng et al., 2014; Kim et al., 2012; Song et al., 2018). Ceci nous permettrait en effet d'aller plus loin en identifiant la distribution des contacts synaptiques fonctionnels spécifiques ainsi que des changements de connectivité synaptique dans le BO après une exposition au stress précoce, susceptibles d'être impliqués dans les modulations de la perception hédonique des souris adultes.

Stress précoce et neurogenèse. Le BO est très plastique, il recrute tout au long de la vie des nouveaux neurones qui mûrissent, se différencient et s'intègrent fonctionnellement pour faire partie du réseau olfactif (Alonso et al., 2024; Imayoshi et al., 2008; Lazarini & Lledo, 2011; Lledo et al., 2006, 2008).

Nous avons vu dans notre étude que le modèle LBN impacte la neurogenèse adulte dans le BO (**ÉTUDE 1**, page 22/23, **Figure 7**), qui pourtant arrive dans une fenêtre temporelle éloignée de celle du stress précoce.

Dans la littérature, il existe un lien direct entre la diminution de la survie des neurones nés à l'âge adulte et l'humeur dépressive, et à l'inverse des antidépresseurs augmentent la survie des nouveaux neurones, ce qui améliore l'humeur (*Dioli et al., 2021; Lepousez et al., 2015; Siopi et al., 2016*). Il est donc plutôt surprenant d'observer chez nos souris ELA une hausse de la survie des nouvelles cellules formées à l'âge adulte. Ce phénomène pourrait être un exemple de plasticité du BO, qui essaye de remplacer une population neuronale défaillante. Ceci a déjà été observé dans l'étude de (*Czarnabay et al., 2019*), où un retard de maturation des cellules neuronales de l'hippocampe et du BO ainsi qu'une augmentation de la prolifération cellulaire et du nombre de cellules immatures étaient observés après un stress précoce. De plus, dans notre étude nous avons remarqué qu'un plus grand pourcentage de ces cellules nées à l'âge adulte sont recrutées en réponse aux odeurs déplaisantes (**ÉTUDE 1**, page 22/23, **Figure 7**). Si l'hyperexcitabilité des neurones nés à l'âge adulte est bien décrite (*Lepousez et al., 2015*), ici cette hypersensibilité semble dirigée plutôt vers les odeurs déplaisantes, ce qui pourrait remodeler à long terme l'ensemble du réseau.

Enfin, les interneurones granulaires nés à l'âge adulte sont connus pour être importants dans les processus d'adaptation olfactive, d'apprentissage perceptif simple et perceptif complexe (*Forest et al., 2019*). En lien avec le paragraphe « 3.1.2 », tester les effets d'un stress précoce vis-à-vis d'autres tâches comportementales pourrait être utile pour comprendre l'implication des répercussions observées sur ces interneurones.

- APARTE : stress précoce et stress chronique -

Pendant ma thèse, j'ai étudié les effets du stress chronique imprédictible adulte (CUMS) sur la perception hédonique des odeurs et la neurogenèse adulte bulbaire (**Annexe 1**). Il est intéressant d'interpréter ces résultats au regard de ceux de **l'ÉTUDE 1**. Premièrement, dans les deux cas, l'intérêt pour les stimuli hédoniques positifs est diminué. Deuxièmement, le point commun des deux études est que le stress, précoce ou chronique, diminue le pourcentage de cellules granulaires nouvellement formées qui sont activées face aux odeurs plaisantes. La perception des odorants plaisants est donc globalement très sensible au stress, quel qu'il soit.

Néanmoins, le stress chronique diminue la survie des nouveaux interneurones du BO tandis que le stress précoce l'augmente. Ainsi, le stress précoce et le stress chronique affectent différemment le réseau bulbaire, probablement à travers des variations des mécanismes biologiques impliqués (niveaux de cortisol, de facteurs neurotrophiques etc). Le stress chronique impacte directement leur survie, alors que dans le cas du stress précoce, les interneurones naissent bien après. Le stress précoce pourrait augmenter la survie des interneurones nés à l'âge adulte pour compenser les altérations des interneurones nés pendant la période de stress. Le stress chronique à l'âge adulte pourrait lui, conduire à une réduction de la survie des neurones certainement en partie à cause des effets prolongés du cortisol et de la réduction de la plasticité cérébrale.

Le stress précoce augmente également le pourcentage de cellules granulaires nées à l'âge adulte qui répondent aux odeurs déplaisantes par rapport aux contrôles. En revanche, le stress chronique ne change pas la réponse des cellules nées à l'âge adulte face aux odeurs déplaisantes. Ces résultats suggèrent que le stress durant la période critique impacte la morphologie des cellules granulaires préexistantes -donc probablement leur fonctionnement- ce qui pourrait perturber l'intégration des nouveaux neurones. Ainsi, un réseau plus large est affecté, ce qui peut expliquer l'impact à la fois sur la réponse aux stimuli

plaisants et déplaisants. Il serait intéressant dans ce contexte, d'étudier si la survie, l'intégration et le fonctionnement des cellules granulaires préexistantes -nées pendant la période critique- sont altérés, en plus de leur morphologie, afin d'avoir une vision plus détaillée du fonctionnement du réseau bulbaire après un stress précoce. Pour terminer, ces résultats dans leur ensemble confirment que le type de stress est important à considérer dans l'étude des effets du Stress avec un grand S sur la perception olfactive.

Stress précoce et activité des structures impliquées dans la perception hédonique. L'équipe a précédemment montré que les odorants déplaisants provoquent une plus grande activité dans la partie postéro-ventrale du BO, tandis que les odorants plaisants induisent une hausse d'activité dans la zone antéro-ventrale des couches de cellules granulaires et glomérulaires (Kermen et al., 2016). Dans notre étude nous avons observé une augmentation de l'activité des cellules granulaires dans le BO antérieur chez des souris stressées investiguant des odorants plaisants pour lesquelles elles ont perdu de l'intérêt (**ÉTUDE 1**, page 22/23, **Figure 7**). Sur la base des résultats de Kermen et al, (2016) nous nous attendions à une diminution de l'activité des interneurons du BO antérieur pouvant expliquer la perte d'intérêt pour les odeurs plaisantes. Cette contradiction pourrait s'expliquer par une méthodologie de cartographie différente entre les deux études, prenant en compte la partie ventrale du BO (dans l'article de Kermen et al., 2016) ou l'ensemble du BO (**ÉTUDE 1**). Nos résultats sont cohérents avec le fait que nous avons identifié une diminution dans l'activité des cellules mitrales du BO antérieur chez les souris stressées en réponse aux odorants plaisants. Ainsi le stress précoce altère le message de sortie du BO relatif aux odorants plaisants.

Enfin, nous avons produit les mêmes résultats que (Midroit et al., 2021), avec une activité supérieure du TO et de l'ATV en réponse aux odorants plaisants par rapport aux odorants déplaisants chez les souris contrôles. Cette réponse est altérée chez les souris ayant subi un stress précoce et étant exposées aux odorants plaisants. Ces résultats suggèrent un défaut d'activité du système DAergique de la récompense en réponse aux odorants plaisants après un stress précoce (**ÉTUDE 1**, page 24, **Figure 8**).

Chez les animaux ELA, le BO pourrait donc échouer à recruter le système de récompense en réponse aux odorants plaisants. Les structures appartenant au système de la récompense pourraient également être touchées directement par le stress précoce (Farrell et al., 2016; He et al., 2021; Ironside et al., 2018; Novick et al., 2018; Opendak et al., 2021; Teicher & Samson, 2016), et ne répondraient plus correctement aux odorants plaisants.

C'est pour aller plus loin dans la part que joue le système DAergique dans la perception des odorants plaisants et son altération chez les souris stressées que nous avons développé dans l'équipe la méthodologie de photométrie par fibre comme présentée dans **l'ÉTUDE 2**.

3.2.2) Stress précoce, émotion et olfaction, qui est l'œuf et qui est la poule ?

Nous avons vu qu'il existe un lien direct entre le système olfactif et le système émotionnel. Si l'on est sûr que le stress précoce affecte ces deux systèmes, on ne sait en revanche pas si les deux sont des cibles directes ou si les dégâts du stress précoce subis par l'un entraînent la chute de l'autre. Pour répondre à la question « est-ce l'altération de la perception des odeurs qui altère le comportement émotionnel ou l'inverse ? », il existe plusieurs pistes de réponses.

L'olfaction est le système sensoriel primordial pendant la période critique pour la recherche de proximité, l'attachement du mamelon, l'allaitement, le comportement social et donc la formation de liens solides entre les petits et les figures parentales. Ces comportements à la fois dépendent et modulent la perception hédonique des odeurs (Opendak et al., 2019; Raineki et al., 2010; Sullivan & Leon, 1986; Sullivan & Wilson, 1991a, 1991b). Comme nous l'avons vu dans **l'ÉTUDE 1**, le développement du

système olfactif est perturbé profondément et durablement par le stress précoce. Cela pourrait impliquer que le stress précoce impacte le premier apprentissage de la vie de l'individu : celui de la mise en mémoire de l'odeur de la figure parentale. Ceci est applicable à l'humain même si les nouveau-nés n'utilisent pas que leur système olfactif (Ehret, 2012). En effet l'olfaction joue aussi chez l'humain un rôle essentiel dans les relations avec le/les parent(s). Ainsi, l'altération précoce du système olfactif pourrait être le point de départ de la cascade d'effets néfastes sur le comportement émotionnel du stress précoce.

D'un autre côté, l'appréciation des odeurs est un phénomène complexe et dépendant de multiples facteurs cognitifs plus généraux. Des structures essentielles aux comportements émotionnels et participant à différentes fonctions olfactives -comme le LC ou l'amygdale- mûrissent pendant la période critique même si la majorité des neurones sont déjà nés durant l'ontogenèse (Czarnabay et al., 2019; Manella et al., 2013; Moreno et al., 2012; Moriceau et al., 2009, 2009; Opendak et al., 2019; Opendak & Sullivan, 2019). Le stress précoce pourrait donc altérer directement ces structures, ce qui se répercuterait ensuite sur le système olfactif (Wilson et al., 2014). Endommager la connectivité entre le LC et le BO ou le système de la récompense et les structures olfactives secondaires pourrait non seulement affecter la mémoire olfactive, cruciale pour l'attachement (Landers & Sullivan, 2012), mais aussi le réseau du traitement hédonique des odeurs (Barr et al., 2009; Moriceau et al., 2009). Nous rappelons que l'anhédonie est un symptôme de la dépression (Nestler & Carlezon, 2006), elle correspond à une perte de motivation et est donc au moins en partie dépendante du système mésolimbique DAergique. La diminution d'activité observée dans l'ATV dans notre étude pourrait être le résultat d'un impact direct du stress précoce, susceptible d'expliquer nos résultats comportementaux à la fois sur la perception olfactive et sur le comportement émotionnel.

Répondre à la question « est-ce l'altération de la perception des odeurs qui altère le comportement émotionnel ou l'inverse ? », pourrait donc bénéficier d'une autre approche dans de futures études. Nous avons identifié des défauts de structure et/ou de fonctionnement après un stress précoce dans le BO, le TO et l'ATV, des régions clés dans l'encodage de la valeur hédonique des odeurs (Kermen et al., 2016, 2021; Midroit et al., 2021). Dans ce contexte, l'activation optogénétique de certaines structures chez les souris ELA serait une approche adéquate et une piste à suivre dans la suite de ce travail pour étudier le lien causal existant entre l'activité du TO et de l'ATV, et l'anhédonie olfactive.

Pour aller plus loin, en introduction nous avons évoqué d'autres structures, faisant partie des systèmes olfactif, limbique et de la récompense (Figure 12), participant à la perception hédonique des odeurs -comme le COF, l'amygdale, le noyau accumbens- et pouvant être altérées dans les troubles affectifs et par le stress. De plus, nous avons fait l'hypothèse dans ce manuscrit que le codage des valeurs hédoniques positives et négatives dépendent de circuits différents. Tout ceci renforce la pertinence de pousser l'analyse des effets du stress précoce au niveau de la connectivité fonctionnelle des réseaux sous-tendant l'encodage de la valeur hédonique des odeurs. Cette approche développée dans l'équipe (Terrier et al., 2024), serait utile sur différents points : déchiffrer les bases neurales de la perception des odorants plaisants ou déplaisants, constater les effets larges du stress précoce sur un réseau entier, ainsi que comprendre les atteintes comportementales de ce stress précoce en fonction des différences dans la connectivité fonctionnelle observée lors d'une tâche comportementale (anhédonie olfactive ou générale) ou des phénotypes d'individu (stressés ou résilients).

-Quatrième Chapitre-

Effets de sexe

La susceptibilité au stress précoce dépend de facteurs individuels tels que la génétique ou l'âge. Parmi ces facteurs, le sexe joue également un rôle majeur. Les différences sexuelles sont particulièrement difficiles à caractériser car elles impliquent beaucoup de sous-facteurs que nous allons aborder ici. Si la recherche s'intéresse progressivement aux caractéristiques du dimorphisme sexuel dans les troubles affectifs, il a pendant longtemps été mis de côté (OMS, 2017). C'est dans ce contexte que nous avons décidé d'inclure les mâles et les femelles dans notre **ÉTUDE 1**. Pour l'instant et à la lumière de nos résultats, le mystère autour du dimorphisme sexuel en réponse à un stress précoce persiste. Vis-à-vis des tests comportementaux que nous avons utilisés, nos femelles adultes ayant subi le même stress précoce que les mâles ne présentent pas d'altération du phénotype émotionnel, en termes de symptômes dépressifs ou d'anxiété (**ÉTUDE 1**, page 16, **Figure 4**). Sur le plan physiologique, le retard de croissance qui a persisté uniquement chez les mâles, était observé au départ chez les deux sexes. Ceci exclut la première hypothèse à laquelle nous avons pensé, comme quoi les femelles n'auraient tout simplement pas été impactées par le stress précoce. La deuxième hypothèse postule que le stress perçu dépend des soins parentaux qui peuvent différer selon le sexe (Bath, 2020). Des études montrent que chez les rats, les mâles nécessitent plus de soins et de contacts alors que les femelles sont plus susceptibles de recevoir des soins négligents ou abusifs dans certains contextes (Kosten et al., 2014; Moore & Morelli, 1979). Cependant, dans notre cohorte nous ne mettons en évidence aucune différence de traitement maternel entre les mâles et les femelles stressés pendant la période critique ce qui écarte cette hypothèse. L'hypothèse principale reste donc que les femelles ont développé une résilience comme abordé dans le deuxième chapitre de cette discussion et que ce phénomène pourrait être en partie expliquée par le dimorphisme sexuel, sur lequel nous allons maintenant nous concentrer.

4.1) Le dimorphisme sexuel dans la réponse au stress

Des différences sexuelles existent dans le contexte du stress précoce, que ce soit chez l'humain ou chez l'animal (Touchant & Labonté, 2022).

Chez l'humain, une distinction majeure réside dans la nature des traumatismes précoces : les hommes sont davantage victimes d'abus physiques, tandis que les femmes subissent plus souvent des abus sexuels, ce qui perturbe différemment l'individu (Jaworska-Andryszewska & Rybakowski, 2019). Sur le plan comportemental et physiologique, les conclusions vis-à-vis du sexe ne font pas l'objet d'un consensus (Kinner et al., 2016). Les hommes semblent parfois plus sensibles au stress précoce (Alastalo et al., 2013), et parfois plus résistants que les femmes (Syal et al., 2014). Sur le plan clinique, les troubles affectifs liés au stress sont plus fréquents chez les femmes (J. B. Becker et al., 2007), avec un risque accru de dépression et de toxicomanie (MacMillan et al., 2001), des symptômes dépressifs plus variables, violents, sévères et précoces, un nombre d'épisodes dépressifs plus élevé, des symptômes atypiques et imprédictibles, et des niveaux d'anxiété supérieur (Eid et al., 2019; Nolen-Hoeksema et al., 1999; Salk et al., 2017; Touchant & Labonté, 2022; Weissman & Klerman, 1985). Les hommes quant à eux, auraient une plus grande tendance à l'agressivité et aux comportements addictifs et à risque (Martin et al., 2013). Ceci étant dit, il est suggéré que les femmes ont une connaissance et une conscience émotionnelle supérieures à celles des hommes (J. B. Becker et al., 2007). Elles exprimeraient ainsi plus et plus facilement leurs émotions malgré une expérience similaire (Dimberg & Lundquist, 1990; Kret & De Gelder, 2012; Kring & Gordon, 1998; Poláčková Šolcová & Lačev, 2017). Cette plus grande propension à ressentir et exprimer les émotions pourrait contribuer à l'expression du dimorphisme sexuel des troubles affectifs après un stress précoce.

Chez le rongeur, l'utilisation des deux sexes pour modéliser les troubles de l'humeur n'était pas à l'origine systématique, si bien que les mâles ont été majoritairement étudiés alors que deux-tiers des patients dépressifs sont des femmes (*Touchant & Labonté, 2022*). Néanmoins, depuis quelques années, le dimorphisme sexuel du rongeur dans la réponse au stress précoce reçoit plus d'attention (*Alonso et al., 2024; Walker et al., 2017*). Les altérations comportementales semblent plus fréquentes chez les mâles (*Arp et al., 2016; Loi et al., 2014*). Toutefois, ces résultats dépendent fortement du domaine cognitif étudié. La mémoire et les capacités d'apprentissage semblent plus sensibles chez les mâles (*Arp et al., 2016; Bath et al., 2017; Naninck et al., 2015; Walker et al., 2017*), qui sont également plutôt sujets à l'anhédonie, l'hypophagie, la prise de risque et des fluctuations de poids après un stress (*Burke et al., 2016; Llorente-Berzal et al., 2011*). Les femelles stressées précocement présentent parfois des niveaux supérieurs d'anxiété (*Kanatsou et al., 2016*) et des phénotypes dépressifs (*Bath et al., 2017*). Dans d'autres cas, elles ne sont pas plus anxieuses que les mâles (*Guadagno et al., 2020; Guadagno, Wong, et al., 2018*). Vis-à-vis des comportements sociaux, les femelles stressées précocement ont une plus forte contagion émotionnelle entre congénères, ainsi que des comportements prosociaux réduits par rapport aux femelles contrôles. Chez les mâles, le stress précoce diminue les comportements sociaux dits affiliatifs (blotissement) (*Breton et al., 2024*). Cette dernière étude renforce l'idée selon laquelle observer l'impact du stress précoce chez un sexe ou l'autre nécessite parfois de s'attarder sur des réponses très fines d'un comportement particulier. Enfin, dans un paradigme de conditionnement pavlovien, une équipe a découvert un dimorphisme sexuel basal -les femelles sont plus actives face à l'adversité tandis que les mâles adoptent des comportements plus passifs- qui était complètement effacé par le modèle LBN (*Manzano Nieves et al., 2023*). Ainsi, le stress précoce peut aussi effacer le dimorphisme sexuel existant à l'origine. Ceci met en lumière l'importance d'une baseline pour étudier les conséquences comportementales de l'adversité précoce selon le sexe. Enfin, les impacts du stress en fonction du sexe dépendent également de l'âge de l'animal et donc du timing développemental (*Bath, 2020*). Le stress précoce pourrait conférer une résilience chez le mâle adolescent, qui développerait ensuite des dysfonctionnements à l'âge adulte par exemple (*Burns, 2018; Loi et al., 2014; Suri et al., 2013*).

Ces arguments sont étayés par des **observations neurobiologiques**, avec des niveaux d'activation distincts dans les structures du système limbique entre l'homme et la femme (*Filkowski et al., 2017*). Le dimorphisme sexuel est notamment visible dans le cortex frontal qui se développe et mature plus précocement chez les femmes (*Cullity et al., 2019*). Ainsi, un stress pendant la période critique, survenant au même moment chez les hommes et les femmes n'aura pas les mêmes impacts sachant que plusieurs structures seront à des étapes de développement différentes. Ceci est vrai aussi pour l'hippocampe, qui a un volume réduit après un stress précoce chez l'homme mais pas chez la femme (*Samplin et al., 2013*). Le stress précoce impacte également différemment la connectivité des réseaux cérébraux associés au traitement de la récompense et des émotions (*Kogler et al., 2016*).

Chez les primates non humains et les rongeurs, le cortex frontal -dont le CPF- le noyau accumbens, le striatum, l'hippocampe ou encore l'amygdale ont des timings développementaux dépendant du sexe (*Perry et al., 2021*). Des différences cérébrales dans la distribution des récepteurs aux glucocorticoïdes, des niveaux de BDNF, de GABA, de glutamate et d'autres neurotransmetteurs ou encore dans l'innervation de système DAergique existent entre mâles et femelles, suggérant des impacts différents du stress précoce à une même période entre les sexes (*Daskalakis et al., 2015; González-Pardo et al., 2020; Perry et al., 2021; Touchant & Labonté, 2022*). À terme, cela induit des niveaux d'activation différents selon le sexe dans certaines structures après un stress précoce comme c'est le cas pour l'amygdale (*Demaestri et al., 2024*). Ces résultats soulignent la complexité des interactions entre le sexe et les régions cérébrales dans les effets du stress précoce. De plus, la neurogenèse hippocampique

est modulée par le stress précoce selon l'âge et le sexe, avec une augmentation chez les rats mâles jusqu'à la puberté, puis une diminution à l'âge adulte, tandis que chez les femelles, le schéma semble inversé (Bath et al., 2017; Loi et al., 2014; Oomen et al., 2010).

La génétique et l'épigénétique jouent également un rôle certain dans le dimorphisme sexuel face au stress précoce (Jaworska-Andryszewska & Rybakowski, 2019; Perry et al., 2021; Shepard & Nugent, 2020). On observe aussi que 25 % des différences entre mâles et femelles dans les symptômes dépressifs sont liées à l'expression des gènes présents sur les chromosomes sexuels, tandis que 23 % à 31 % sont associées aux hormones gonadiques (Paden et al., 2020). Enfin, le fonctionnement de l'axe HPA est aussi dépendant du sexe, en baseline et en réponse à un stress, expliquant une part des différences observées (Dudek et al., 2021).

En conclusion le dimorphisme sexuel face au stress précoce se manifeste à différents niveaux (neurobiologique, hormonal, génétique et épigénétique, physiologique) et s'explique en partie par un développement du cerveau spécifique au sexe chez l'humain et l'animal (An et al., 2022; Goodwill et al., 2019; Oomen et al., 2010). Néanmoins, les observations quant au stress précoce ne sont pas systématiquement faites dans les deux sexes et les données neurobiologiques font souvent défaut. Une grande hétérogénéité est présente dans les résultats tant sur le plan comportemental que cellulaire. Si certaines études soutiennent que les femmes sont plus vulnérables aux troubles affectifs après un stress précoce, dans de nombreux cas, les données physiques, physiologiques et neurobiologiques ne vont pas toutes dans la même direction. Ainsi, aucun consensus n'a été établi à ce jour pour expliquer les bases neurales de cette vulnérabilité prétendument accrue chez les femmes. Elle pourrait être en partie expliquée par la conscience émotionnelle supérieure des femmes qui tendraient plus facilement à parler de leurs troubles affectifs. Le dimorphisme sexuel et ses mécanismes restent donc mal compris alors que comprendre la vulnérabilité différenciée entre les sexes dans les modèles animaux et les populations cliniques humaines est crucial pour adapter les traitements des troubles affectifs en fonction du sexe (Eliot & Richardson, 2016).

Maintenant que nous avons discuté du dimorphisme sexuel basal et face au stress il est important de noter que celui-ci existe aussi au regard de la perception olfactive.

4.2) Le dimorphisme sexuel dans la perception olfactive

Dimorphisme sexuel et perception olfactive chez les individus sains. Il existe des différences sexuelles dans différentes fonctions du sens olfactif **chez l'humain** (Bontempi et al., 2021; Sorokowski et al., 2019; Triscoli et al., 2014). Concernant la perception hédonique des odeurs, les femmes ont tendance à évaluer les odeurs agréables et désagréables de manière plus extrême que les hommes. Cependant, ce phénomène dépend du type d'odeur (corporelles ou non) (Bontempi et al., 2021), de l'odeur elle-même et des conditions expérimentales (intensité et répétition de l'exposition). En résumé, le dimorphisme sexuel relatif à la perception hédonique des odeurs est complexe et dépendant de multiples sous-facteurs chez l'humain, ce qui n'est que très peu testé chez le rongeur.

En effet, il a été montré **chez le rongeur** que les femelles ont des réponses plus rapides et étendues aux odorants, une meilleure capacité de discrimination et d'identification et un système olfactif plus efficace (Kass et al., 2017). Cependant ces conclusions sont issues d'études majoritairement sur les odeurs biologiques (phéromones ou composés présents dans l'urine par exemple), les comportements sexuels et sociaux et les circuits associés. Ces études mettent en évidence des différences sexuelles dès l'épithélium olfactif et les neurones olfactifs sensoriels (Vihani et al., 2020), dans la représentation de la population DAergique dans le BO (Gómez et al., 2007), ou encore dans les réponses du BO et de

l'amygdale (*Cherry & Baum, 2020*). Ainsi, lorsque le dimorphisme sexuel est étudié chez le rongeur, ce n'est pas au regard d'odorants sans signification biologique particulière.

Dans notre **ÉTUDE 1**, nous avons effectué le test de préférence olfactive -de la même façon que pour les mâles- chez nos femelles. Les souris femelles adultes du groupe contrôle ont exprimé le même niveau d'attraction pour toutes les odeurs présentées, qui témoigne par rapport aux mâles d'une appréciation plutôt neutre des odorants : ni plaisants ni déplaisants. De surcroît ce niveau d'attraction est similaire aux souris femelles stressées. Ces résultats suggèrent que les souris femelles adultes contrôles ont des préférences olfactives différentes de celles des souris mâles. En réalité la majorité des études faites sur les fonctions olfactives du rongeur se concentre sur les mâles (*Kermen et al., 2016, 2021; Kobayakawa et al., 2007; Levinson et al., 2020; Linster, 2019; Linster et al., 2011; Midroit et al., 2021*) et les femelles ne sont que très rarement voire pas étudiées. Nous n'avons ainsi à ce jour aucune idée des préférences olfactives des souris femelles. Ceci pourrait expliquer que le stress précoce n'a pas modifié l'intérêt des souris femelles pour les différents odorants présentés par rapport aux contrôles. À la lumière de ces résultats et de la littérature existante, il y a un véritable besoin d'étudier les fonctions olfactives des souris femelles, leurs préférences et leurs capacités, afin d'élucider les mystères de la perception hédonique chez elles aussi, et comment elle peut être altérée par le stress précoce.

Enfin, comme évoqué précédemment les femmes semblent avoir une conscience émotionnelle supérieure aux hommes, ce qui pourrait s'étendre au sens olfactif (*Athanassi et al., 2021; Nováková et al., 2014; Ohla & Lundström, 2013*). La perception olfactive pourrait être plus émotionnelle chez les femmes dont la conscience est davantage orientée vers la dimension plaisante ou déplaisante des odeurs et l'imagination olfactive qui comporte des composantes émotionnelles (*Bensafi & Rouby, 2007*). Cependant, d'autres études ne montrent pas d'effet du genre sur la perception émotionnelle relative aux odeurs chez l'humain (*Chen et al., 2022*). Il paraît donc intéressant dans ce contexte d'explorer s'il existe un schéma spécifique au sexe dans l'association entre le stress perçu et l'hédonisme olfactif. Une étude de (*Hu et al., 2024*) montre que chez les femmes, un niveau plus élevé de stress chronique est associé à une diminution du plaisir olfactif qui dépend de la conscience qu'elles ont des odeurs. En effet, sous l'effet du stress, les femmes qui sont attentives aux odeurs de manière positive voient leur plaisir olfactif diminuer davantage que celles qui en ont une conscience négative. Ainsi, les effets du stress sont modulés par la manière dont la valeur hédonique des odeurs est perçue à l'origine. Ceci souligne l'importance de prendre en compte les préférences aux odeurs, et si possible de manière individuelle. Enfin, il faut noter que dans le dimorphisme sexuel de la perception hédonique des odeurs, le statut hormonal des individus joue évidemment un rôle, mais surtout dans la perception d'odeurs biologiques (*Brémond-Bostoen et al., 2024; Hummel et al., 1991*).

Dimorphisme sexuel et perception olfactive chez les individus atteints de troubles affectifs. Pour terminer, les données sont moins nombreuses sur la manière dont les troubles affectifs affectent la perception olfactive en fonction du sexe. Certaines études ne trouvent aucune différence de genre dans le jugement hédonique des odeurs chez les patients schizophrènes (*Hudry et al., 2002; Kamath et al., 2013; Lui et al., 2020*). D'autres révèlent une anhédonie olfactive uniquement ou préférentiellement chez les hommes schizophrènes (*Moberg et al., 2003; Robabeh et al., 2015; Walsh-Messinger et al., 2018*). Dans d'autres contextes pathologiques, tels que la dépression (*Clepce et al., 2010*), il ne semble pas y avoir d'effet de genre sur le jugement hédonique des odeurs. S'il existe un manque d'investigation des effets des troubles affectifs et notamment du stress précoce sur la perception hédonique des odeurs, ce manque est encore plus prononcé en ce qui concerne le dimorphisme sexuel qui y est associé.

En conclusion, s'il est admis à l'issue de ce travail de thèse que le stress précoce altère la perception hédonique des individus mâles, celle des femelles reste à étudier. J'aimerais à présent terminer la discussion de ce manuscrit en partageant les réflexions qui animaient principalement ma troisième et dernière année. En effet, ma thèse s'inscrit dans un projet ANR (COBRA – ANR-23-CE17-0065) plus grand dont un des objectifs est d'étudier les mécanismes de remédiation aux troubles affectifs après un stress précoce et leurs bases neurobiologiques.

-Cinquième Chapitre- **Remédiation par les odeurs**

La dernière partie de cette discussion concerne une technique de remédiation face aux troubles affectifs et dont les acteurs principaux sont les odeurs. Il a été remarqué que les patients dépressifs prêtent moins attention à leur environnement quotidien et donc aux odeurs. Ils ont une capacité réduite à traiter les stimuli olfactifs notamment agréables, favorisant l'anhédonie olfactive (*Dal Bo', 2023*). Ceci renforce l'hypothèse selon laquelle **un entraînement ou enrichissement olfactif**, à travers une conscientisation et une attention nouvelle portée aux odeurs dans l'environnement, pourrait être bénéfique pour prévenir et/ou traiter certains aspects des troubles émotionnels (*Oleszkiewicz et al., 2022*).

Il convient tout d'abord de rappeler brièvement les stratégies thérapeutiques actuelles pour traiter les troubles affectifs, dont la dépression. Il existe diverses approches aux résultats variables selon les patients et les modèles animaux (*Leon & Woo, 2022*). Les thérapies pharmacologiques ne sont par exemple efficaces que dans 40 à 60% des cas (*Doty et al., 2021; Dudek et al., 2021, 2021; Malhi et al., 2020*). D'autres options non médicamenteuses existent telles que la stimulation magnétique transcrânienne (*Bolwig, 2002; Fregni et al., 2021*) ou la stimulation cérébrale profonde (*Aibar-Durán et al., 2022*). Parallèlement, d'autres stratégies ont vu le jour pour améliorer et potentialiser les bénéfices des traitements classiques. Ces approches incluent l'acupuncture, le rééquilibrage du microbiote intestinal, le yoga ou encore la méditation (*Bajaj et al., 2016; J. D. Bremner et al., 2020; Joss & Teicher, 2021; Kong et al., 2019; Rosenkranz et al., 2016; Slyepchenko et al., 2014; Tan et al., 2021*). L'une des approches dans ce contexte est l'enrichissement environnemental, protecteur contre le vieillissement cognitif chez l'humain et l'animal et bénéfique pour les capacités émotionnelles et olfactives (*Joushi et al., 2022; Kuzumaki et al., 2011; Leon & Woo, 2022*). Parmi les enrichissements environnementaux possibles, **l'enrichissement olfactif** est une piste intéressante pour soutenir la perception olfactive et améliorer la santé émotionnelle. En effet, les odeurs ont un pouvoir unique d'induction des émotions positives et peuvent également agir comme modérateur et suppresseur de stress (*Masuo et al., 2021*).

L'enrichissement olfactif consiste en une exposition répétée à un ou plusieurs odorants ou encore des mélanges odorants plus ou moins complexes sur une période prolongée.

Chez l'humain, cette procédure a été largement étudiée, notamment par T.Hummel et son équipe, qui ont défini un protocole d'exposition systématique à quatre odorants, deux fois par jour pendant douze semaines (*Hummel et al., 2009*). L'efficacité de l'enrichissement olfactif dépend de sa durée, de l'étiologie de la perte olfactive (s'il y'en a une), de l'âge, des molécules utilisées, ou encore de la répétabilité de l'exposition (*Pieniak et al., 2022*). Il peut améliorer la détection, la discrimination et l'identification olfactive chez les patients souffrant des pertes olfactives diverses.

Au-delà des bénéfices sensoriels, l'enrichissement olfactif peut diminuer le sentiment d'anxiété, améliorer la réponse au stress, la cognition, l'humeur, et le fonctionnement émotionnel de sujets sains et dépressifs, ainsi que diminuer la perte cognitive associée au vieillissement (*Ballanger et al., 2019; Leon & Woo, 2022; McDermott et al., 2022; Pieniak et al., 2022; Vance et al., 2024*). Les bénéfices de l'enrichissement olfactif sont observables également chez les personnes souffrant de troubles tels que l'autisme (*Woo et al., 2015*), la maladie de Parkinson (*Haehner et al., 2013*), ou bien la démence (*Buetow & MacLeod, 2024*). Différentes huiles essentielles ont ainsi des bénéfices cliniques variés (*Fung et al., 2021*). La lavande, la bergamote la camomille, l'orange, le yuzu ou encore la rose sont très utilisés en prévention (relaxation) ou même en traitement de la dépression et de l'anxiété (*Conrad & Adams, 2012; Ebrahimi et al., 2022; Goes et al., 2012; Lehrner et al., 2000; Matsumoto et al., 2014*).

Les mécanismes sous-tendant ces effets pourraient être de différents ordres : régulation des niveaux de monoamines, induction de la production de facteur neurotrophique, augmentation de la neurogenèse, régulation du système endocrine, activation de différentes régions cérébrales. Les bénéfices de l'enrichissement olfactif pourraient reposer sur le fait qu'il confère une « réserve cognitive », favorisant la mise en place des mécanismes de plasticité compensant les symptômes de divers troubles psychiatriques (*Leon & Woo, 2022; Stern et al., 2019*). Néanmoins, certaines études n'ont pas mis en évidence d'effets émotionnels significatifs liés à l'enrichissement olfactif (*Oleszkiewicz et al., 2021, 2022*) ce qui soulève la question des conditions spécifiques dans lesquelles il serait bénéfique. Il est important de rappeler que le système olfactif est étroitement connecté aux systèmes cognitifs et émotionnels du cerveau incluant l'amygdale, l'hippocampe, le noyau accumbens ainsi que le CPF, impliqués dans la cognition et l'humeur (*Leon & Woo, 2022*). Certaines études ont d'ailleurs montré que l'enrichissement olfactif modifie la connectivité fonctionnelle dans les régions cérébrales qui ne se limitent pas au traitement de l'information olfactive (*Jiramongkolchai et al., 2021; Kollndorfer et al., 2015; Vance et al., 2024*). Par ailleurs, l'enrichissement olfactif est capable d'augmenter le volume de matière grise dans des structures telles que l'hippocampe, le thalamus, ou le COF (*Gellrich et al., 2018; Han et al., 2021*).

Chez l'animal, les odeurs modulent l'anxiété, la réponse au stress, la gestion du conflit, et l'expression de symptômes dits dépressifs (*de Sousa et al., 2015; Faturi et al., 2010; Fung et al., 2021; Harada et al., 2018; Linck et al., 2010; Nakatomi et al., 2008; Tsang & Ho, 2010*). Une exposition quotidienne de trente minutes chez des souris à des huiles essentielles sur une période de trois mois améliore les performances mnésiques et stimule la neurogenèse dans l'hippocampe et le BO, à des niveaux comparables à ceux des animaux vieillissants dans un environnement enrichi classique (*Rusznák et al., 2018*). Une exposition quotidienne à un nouvel odorant pendant trente à quarante jours favorise la survie des nouveaux neurones du BO, et améliore les performances de la mémoire olfactive à court terme (*Rey et al., 2012; Rochefort et al., 2002*). Une étude menée dans l'équipe a également révélé que les souris adultes soumises à un enrichissement olfactif avaient une meilleure capacité de discrimination, même à un âge avancé, ainsi qu'une meilleure mémoire spatiale et une plus grande flexibilité cognitive. Ces bénéfices étaient supportés par une augmentation de l'innervation noradrénergique dans le BO et l'hippocampe ainsi qu'un remodelage des réseaux associés à un meilleur vieillissement cognitif (*Terrier et al., 2024*). D'autres mécanismes sous-tendant les bénéfices de l'entraînement olfactif pourraient reposer sur l'activation plus ou moins directe du système olfactif et du réseau émotionnel, par les molécules odorantes via leur passage dans le cerveau par voie nasale ou transdermique (transférabilité cérébrale) (*Masuo et al., 2021*), mais la compréhension de ces mécanismes nécessite encore des investigations (*Pieniak et al., 2022*). Mieux comprendre les bénéfices et les bases neurales de l'enrichissement olfactif pourrait enrichir la recherche sur de nouvelles stratégies

thérapeutiques innovantes, plus naturelles, moins invasives et coûteuses, pour combattre les conséquences néfastes des troubles affectifs liés à un stress précoce.

Le dernier point abordé dans ma thèse s'inscrit dans ce souhait, puisqu'il s'agit de tester les bénéfices d'un enrichissement olfactif sur les conséquences comportementales et neurobiologiques d'un stress précoce. Cet objectif a initié la mise en place, le développement et l'entretien d'une lignée de souris transgéniques (TRAP2) permettant de marquer l'activité cellulaire à deux temps différents (DeNardo et al., 2019; Guenthner et al., 2013; Lee et al., 2020). Ainsi, sur ces souris TRAP2 ayant subi un stress précoce, il sera possible d'étudier la connectivité fonctionnelle des réseaux neuronaux impliqués dans le traitement des odeurs, avant et après un enrichissement olfactif ainsi que leurs comportements émotionnels et olfactifs. Les hypothèses testées sont que l'altération de la perception des odeurs après le stress précoce est due à une modification du/des réseau/x sous-tendant la valeur hédonique des odeurs et que l'enrichissement olfactif seul ou combiné à un traitement pharmacologique peut restaurer cette perception ainsi que le fonctionnement de ce/s réseau/x. Ce projet constitue la suite de ce travail et est également au cœur d'une approche appliquée et clinique, en collaboration avec l'équipe PSYR2 testant les effets de la combinaison d'un enrichissement olfactif et d'un traitement rTMS sur des patients atteints de trouble dépressif majeur (« Conséquences d'un traumatisme précoce sur la perception des odeurs et sur les circuits neuronaux qui la sous-tendent, stratégie de remédiation » COBRA, ANR-23-CE17-0065). Cette collaboration a abouti notamment à la publication d'un article méthodologique présenté en **Annexe 3** : *Combining a Breath-Synchronized Olfactometer with Brain Simulation to Study the Impact of Odors on Corticospinal Excitability and Effective Connectivity*.

En conclusion, des études comportementales suivies d'analyses approfondies des bases neurales de l'enrichissement olfactif permettraient d'identifier et décrire les mécanismes responsables de ces bénéfices, dans le cadre de troubles affectifs survenant notamment après un stress précoce.

-ÉPILOGUE-

Au terme de ce travail une petite conclusion s'impose.

J'ai apporté différents éléments de réponses quant aux bases neurales de l'appréciation innée d'odorants -sans signification biologique particulière et non familiers pour la souris- et comment celle-ci peut être transformée par une perturbation précoce : le stress.

Nous avons confirmé que les souris expriment en condition basale des préférences olfactives distinctes. Elles sont attirées par des odorants considérés comme plaisants, ce qui se traduit par un recrutement du système de la récompense : le TO et l'ATV (**ÉTUDE 1**), et une augmentation des niveaux de libération DAergique dans le TO (**ÉTUDE 2**). Le stress précoce -issu du modèle LBN- induit une anhédonie olfactive soit une diminution de l'intérêt pour les odorants plaisants, qui est en corrélation avec certaines altérations du phénotype émotionnel. Cette anhédonie olfactive est en partie sous-tendue par une hyperactivité de la couche granulaire et à l'inverse une réponse plus faible des cellules de projections du BO antérieur, du TO et de l'ATV. Elle pourrait également être partiellement expliquée par une atteinte du développement et un défaut de la maturation des interneurons postnatals du BO.

J'ai eu le plaisir de faire des mesures et des observations comportementales et cellulaires autour de ce phénomène pour en améliorer la compréhension. Je me suis attaquée à une montagne méthodologique : la photométrie par fibre, dont les applications relatives à ce sujet et à d'autres questionnements de l'équipe sont multiples et prometteurs.

Enfin j'ai eu la chance d'observer et de participer à une approche clinique et thérapeutique chez l'humain qui suscite mon plus grand intérêt, si bien que nous avons établi un projet parallèle à cette approche chez l'animal, pour en appréhender les mécanismes.

Dans un projet, il est toujours frustrant de voir les questions se multiplier au fur et à mesure que l'on répond aux précédentes. Si je devais donner mes envies pour la suite, il s'agirait de poursuivre ce travail de thèse en l'approchant de manière longitudinale, de multiplier et d'individualiser les mesures comportementales chez les mères et les petits, de la période de stress à un âge avancé, en passant par l'adolescence et la vie d'adulte, et ce tout en observant les répercussions sur les réseaux de connectivité anatomique et fonctionnelle cérébrales des mâles et des femelles.

Enfin, je tiens une fois de plus à remercier tous les acteurs de ce travail, cités en début de manuscrit. Merci également au lecteur qui parcourra ces quelques pages résumant trois années de travail et de cheminement scientifique, professionnel et personnel.

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-ANNEXE I-

*Chronic unpredictable mild stress alters odor hedonics and adult
olfactory neurogenesis in mice*

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Chronic unpredictable mild stress alters odor hedonics and adult olfactory neurogenesis in mice

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Experiencing chronic stress significantly increases the risk for depression. Depression is a complex disorder with varied symptoms across patients. However, feeling of sadness and decreased motivation, and diminished feeling of pleasure (anhedonia) appear to be core to most depressive pathology. Odorants are potent signals that serve a critical role in social interactions, avoiding danger, and consummatory behaviors. Diminished quality of olfactory function is associated with negative effects on quality of life leading to and aggravating the symptoms of depression. Odor hedonic value (I like or I dislike this smell) is a dominant feature of olfaction and guides approach or avoidance behavior of the odor source. The neural representation of the hedonic value of odorants is carried by the granule cells in the olfactory bulb, which functions to modulate the cortical relay of olfactory information. The granule cells of the olfactory bulb and those of the dentate gyrus are the two major populations of cells in the adult brain with continued neurogenesis into adulthood. In hippocampus, decreased neurogenesis has been linked to development or maintenance of depression symptoms. Here, we hypothesize that chronic mild stress can alter olfactory hedonics through effects on the olfactory bulb neurogenesis, contributing to the broader anhedonia phenotype in stress-associated depression. To test this, mice were subjected to chronic unpredictable mild stress and then tested on measures of depressive-like behaviors, odor hedonics, and measures of olfactory neurogenesis. Chronic unpredictable mild stress led to a selective effect on odor hedonics, diminishing attraction to pleasant but not unpleasant odorants, an effect that was accompanied by a specific decrease in adult neurogenesis and of the percentage of adult-born cells responding to pleasant odorants in the olfactory bulb.

KEYWORDS

olfactory bulb, odor hedonics, adult neurogenesis, chronic mild stress, emotional alteration

Introduction

Depressive symptoms can be complex and vary widely between individuals as well as within individuals with recurrent episodes. In major depressive disorder (MDD), core symptoms include anhedonia and diminished motivation. In humans, depression is often also associated with disturbance in olfactory sensory function (Negoiias et al., 2016; Croy and Hummel, 2017; Pabel et al., 2018; Athanassi et al., 2021). Like depression, olfactory sensory function is complex (including features such as familiarity, intensity, and identity) with the hedonic perception of odor dominating. Hedonic value is generally the first criterion used by humans to describe and categorized odorants (Schiffman, 1974; Richardson and Zucco, 1989; Yeshurun and Sobel, 2010) and represents a valuable source of information for decision-making and guiding goal-oriented motivated behaviors. Across species (including humans), the hedonic value of odorants is a critical driving force for food intake or social interactions supporting survival and regulation of the emotional state. Interestingly, odor hedonic value (pleasantness) is the dimension of olfactory perception most strongly affected in depression (Naudin et al., 2012). Consequently, altered odor hedonics can cause disturbances in food intake or mood which worsen depressive symptoms and contribute significantly to negative outcomes across a range of diseases with comorbid depression. The critical neural substrate influencing odor hedonics appears to be the granule cell layer of the olfactory bulb (OB), a modulator of the first cortical relay of olfactory information (Kermen et al., 2016). Optogenetic inhibition of granule cell activity modulates approach behavior to odorants in rodents, which is used to evaluate whether an odorant is pleasant or unpleasant in an animal model (Kermen et al., 2016; Midroit et al., 2021). The granule cells, significantly influence processing of hedonic information and interestingly are the largest population of cells with ongoing adult neurogenesis (Lledo and Valley, 2016). Stem cells located in the subventricular zone of the lateral ventricles give rise to neuroblasts that migrate along the rostral migratory stream to reach the OB and functionally integrate the pre-existing network (Alvarez-Buylla and García-Verdugo, 2002; Carlén et al., 2002; Belluzzi et al., 2003; Carleton et al., 2003; Magavi et al., 2005). These new neurons are key elements of odor processing by shaping the activity of the relay cells (Schoppa and Urban, 2003; Lledo et al., 2005; Lledo and Saghatelyan, 2005). The other major brain structure with high rates of adult neurogenesis, is the hippocampus (Lledo et al., 2006; Kempermann, 2015). Prior work has implicated alterations in hippocampal neurogenesis on both the development and response to treatment for depression (Santarelli et al., 2003; Planchez et al., 2019). Like the hippocampus, the OB serves a significant role in cognitive processes, memory formation and retrieval, and emotional regulation (Bagur et al., 2021; Mofleh and Kocsis, 2021; Salimi et al., 2022).

Despite prior work implicating disturbance in neurogenesis in hippocampus with depression few studies have investigated the relationship between depressive-like behaviors and OB neurogenesis and olfactory processing (Lledo and Valley, 2016; Siopi et al., 2016). Chronic stress is known to increase the risk for depression, alter morphology and functioning of the hippocampus including alteration of adult neurogenesis (McEwen and Magarinos, 1997). Prior work has established that chronic

unpredictable mild stress (CUMS) can reliably induce behavioral profiles in mice that resemble depressive symptoms in humans (Pothion et al., 2004; Nollet et al., 2012; van Boxelaere et al., 2017).

Here, the aim of this work was to test the impact of CUMS on olfactory hedonics in mice, its effects on adult OB neurogenesis, and the activity of adult-born neurons during hedonic valuation. In CUMS exposed and control mice, behavioral measures of both depressive-like and anxiety-like behaviors (Willner et al., 1987; Papp and Moryl, 1994; Willner, 2017) were assessed. In conjunction, the hedonic processing of olfactory signals was tested in an olfactory preference test (Kermen et al., 2016; Midroit et al., 2021). Finally, measures of adult-born neuron number and activity were measured in the OB of mice following hedonic preference testing. The CUMS protocol led to higher levels of anhedonia and increased anxiety-like behaviors compared with control mice. Importantly, the CUMS paradigm induced olfactory hedonic alteration, significantly diminishing interest in pleasant but not unpleasant odorants. At the cellular level, CUMS decreased the density of adult-born neurons in the OB as well as the percentage of adult-born neurons responding to pleasant but not unpleasant odorants.

Materials and methods

Animals

Twenty adult male C57Bl6/J mice (8 weeks, Charles River Laboratories, L'Arbresle, France) were used in this study. Mice were randomly assigned to one of two groups, one control group ($n = 10$; housed in groups of five in standard laboratory cages) and one stressed group ($n = 10$; placed in individual standard laboratory cages). All experiments were done in accordance with the European Community Council Directive of 22nd September 2010 (2010/63/UE) and the National Ethics Committee (Agreement APAFIS#20702_2019072614086203_v2). Mice were kept on a 12 h light/dark cycle at a constant temperature of 22°C with food and water *ad libitum* except during the stress protocol (Table 1). All efforts were made to minimize animal suffering.

TABLE 1 CUMS protocol stressors (see Supplementary Table 1 for examples of daily sequences of events).

Stimuli	Duration
Sawdust bedding removed	About 6 h
Mouse cage change	Permanent
Cage tilting at 45°	About 1 h
Wet sawdust (200 mL of water for 100 g sawdust)	3 to 12 h
Nycthemeral rhythm perturbation	About 3 h through the night
Food and water deprivation	About 12 h
Mice pair housed (with or without dry or wet sawdust)	About 3 h or through the night
Mice hung by their tail	About 1 min
Change sawdust	Twice (each hour)

Chronic unpredictable mild stress

The CUMS protocol was performed every day over 7 weeks in which mice were subjected to mild stressors several times per day (Table 1). Some stressors were combined such as: cage tilting and wet sawdust, mice were housed 2 per cage for food and water deprivation, mice were placed 2 per cage for cage tilting, etc. Details of the procedure are described in Supplementary Table 1. All stress manipulations were performed in a room different from the housing room. When no overnight stress was planned, mice were returned to and remained in the housing room.

Behavioral testing

Sucrose Preference Test. Animals were placed in a cage with free access to two pipettes connected to two bottles of water: one contained only water and the other one contained 1% sucrose dissolved in water. The test was conducted during 3 days (d1 to d3). The sucrose solution was freshly prepared each day. To avoid neophobia, sucrose at 1% was presented to all animals for a period of 24 h, a week before the test. During the test, the pipettes and bottles containing sucrose were reversed every 12 h to avoid potential side biases. Sucrose preference was calculated as follow: Sucrose Consumption (mg)/Total Consumption (mg). Preference of CUMS mice was expressed as a percentage of that of control mice.

Light/Dark Box test. This apparatus is composed of two compartments: one (2/3 of the box) is directly highly illuminated (820 lm) and the other one (1/3 of the box) is dark. The animal can move freely from one compartment to another. For testing, a single mouse was placed in the left corner of the highly illuminated compartment of the box and the trajectory of the mouse was video recorded and analyzed using A2V Volcano®. The time spent in the illuminated side was used as a measure of anxiety-like behavior with avoidance of the brightly lit area being considered a measure of heightened anxiety-like behavior. The test lasted 5 min and the box was cleaned after each trial.

OpenField test. Animals were placed in an OpenField maze composed of a wall-enclosed area to prevent the mouse from escaping. For testing, a single mouse was placed at the top left corner, several motor and behavioral activities were video recorded for 5 min, including: time spent in the inner zone, number of entries into the inner zone, duration of grooming, number of fecal boli deposited, latency and number of rearing events (mouse standing on their hind limbs). An increased number of fecal boli, increased rearing, decreased latency of rearing and decreased grooming as well as decreased time spent in the inner zone were used as metrics of increased anxiety-like behavior.

Odor hedonic value assessment. Based on work done in mice (Kermen et al., 2016; Midroit et al., 2021), we selected six odorants for this test: three well documented to have positive hedonic values (Limonene + Lim; 5,989-27-5; 0.2% dilution for 1 Pa vapor), Citronellol (Citro; 106-22-9; 17.8% dilution for 1 Pa vapor) and Camphor (Cam; 76-22-2; 0.46% dilution for 1 Pa vapor) and three with well-documented negative hedonic values (Guaiacol (Gua; 90-05-1; 2.08% dilution for 1 Pa vapor), P-cresol (Cre; 6,032-29-7; 1.8% dilution for 1 Pa vapor), and Pyridine (Pyr; 110-86-1; 2% dilution for 1 Pa vapor)). Hedonic preference was defined as approach and avoidance behavior and was validated in reference to investigation times for biologically relevant odors with known aversive or appetitive properties (Kermen

et al., 2016). All odorants were diluted on the day of the test in order to have similar vapor pressures (1 Pa; similar perceived intensity) (Mandairon et al., 2006; Moreno et al., 2009).

The test was performed on the one hole-board apparatus as follows: 60 μ L of pure odorant was put on cotton batting and placed at the bottom of a pot covered with bedding. The pot was put in the central hole of the board. Mice were placed at the bottom right corner of the board and allowed to freely explore for 2 min. After the 2 min test, the mouse and the pot were removed, and the board was cleaned. The order of odor presentation was randomized. The total time spent exploring the hole (as measured by a photobeam break at the hole entrance) was recorded for each mouse.

Neurogenesis assessment

Sacrifice. To identify populations of cells that were activated in response to a given odorant, mice were odor stimulated for 1 hour with either an unpleasant odorant (Guaiacol; 100 μ L of odorant at 1 Pa) or a pleasant odorant (+Limonene; 100 μ L of odorant at 1 Pa). Odorants were presented in a tea ball. One hour after the end of odor stimulation, mice were sacrificed by Urethane injection (2 g/kg) followed by intracardiac perfusion of 50 mL of cold fixative (paraformaldehyde 4% in PBS, pH 7.4). Brains were dissected, sunk in sucrose and sectioned (14 μ m thick).

BrdU injections. Adult-born cells of the OB require several days to migrate from the subventricular zone to the OB, where they differentiate into mature granule and periglomerular cells. To determine the impact of CUMS on integration and differentiation of adult-born cells in the OB, mice were injected with bromodeoxyuridine (BrdU; Sigma; 50 mg/kg in saline three times daily at 2 h intervals, i.p.) 20 d after the beginning of stress procedure. The CUMS procedure continued for several weeks following BrdU injection, with animals being sacrificed at the completion of behavioral testing (38 d after the BrdU injections). This time course of BrdU administration allowed for assessment of CUMS effects on the birth, differentiation, and survival of adult-born cells in the OB during the CUMS procedure.

BrdU immunocytochemistry. The protocol has been described in detail previously (Forest et al., 2019). Sections were incubated overnight in a mouse anti-BrdU antibody (1:100, Millipore Bioscience Research Reagents) at 4°C followed by a biotinylated anti-mouse secondary antibody (1:200, Vector Laboratories) for 2 h. The sections were then processed through an avidin-biotin-peroxidase complex (ABC Elite Kit, Vector Laboratories). Following dehydration in graded ethanols, the sections were defatted in xylene and coverslipped in DPX (Fluka, Sigma).

Double labelling BrdU/cFos immunocytochemistry. To investigate the density of BrdU-positive cells responding to pleasant and unpleasant odorants, double labelling was performed using mouse anti BrdU (1/100, Merck) and monoclonal rat anti c-Fos (1/2000, Synaptic System) respectively. Appropriate secondary antibodies were used (goat anti-mouse Alexa 546 (1/200, Molecular probes); goat anti-rat Alexa 488 (1/200, Molecular probes) to label cBrdU+/-Fos+ cells.

Data analysis (BrdU and c-Fos levels)

Every fifth coronal section of the olfactory bulb was processed for immunostaining (sampling interval = 70 μ m; 3–5 sections per animal

were analyzed). Within each analyzed section, every BrdU-positive cell was counted in the granule layer of the right OB using mapping software (Mercator, Explora Nova, La Rochelle, France) coupled to a Zeiss microscope. The mean positive cell density was calculated and averaged within each experimental group.

Fluorescent counting was done using AxioVision (Zeiss) software coupled to a pseudo-confocal Zeiss microscope. We calculated the percentage of BrdU+ cells expressing c-Fos per mouse and averaged the values per group (23 ± 5 cells per animal were assessed). As an additional control, we also counted the density of c-Fos+/BrdU- in the OB (3 sections were analyzed and averaged per animal). All counting was done blind with regards to the identity and treatment group of the animal.

Statistics

All analyses were performed using Rstudio. For behavioral experiments were used, preliminary tests including Kolmogorov–Smirnov and Levene tests for normality for all data. This resulted in the use of parametric tests: repeated measures analysis of variance (ANOVA) (day as factor) followed by Bonferroni-corrected *post-hoc* *t*-tests for the sucrose consumption test and two-ways ANOVA (group and hedonic as factors) followed by Bonferroni-corrected *post hoc* *t*-tests for the olfactory preference test and cellular analysis. For other behavioral tests and analysis of cellular densities, the 2 experimental groups were compared using two sample *t*-tests. Unilateral *t*-tests were used when prior evidence of effects of the CUMS model on depressive-like behavior and anxiety in rodents were reported (Antoniuk et al., 2019; Markov, 2022). The validity of the *t*-test positive results was confirmed using permutation tests. No statistical methods were used to predetermine sample sizes, but our sample sizes were similar to those reported in previous publications (Forest et al., 2019). Data collection and animal assignment to the various experimental groups were randomized and data collected blind to condition.

Results

Chronic unpredictable mild stress induced emotional changes in adult mice

To confirm the effectiveness of the CUMS protocol to alter expression of anxiety and depressive-like behaviors, sucrose preference was assessed in mice over 3 days (Goodwill et al., 2019). The repeated measures ANOVA revealed a day effect on sucrose consumption in CUMS compared to control group ($F(2,18) = 3.82, p = 0.04$). More precisely, CUMS mice showed a significant reduction of sucrose consumption at D2 and D3 compared to controls (unilateral one sample *t*-test D1 $p = 0.6$; D2 $p = 0.006$; D3 $p = 0.03$; Figure 1A).

To assess the impact of CUMS on the level of anxiety-like behaviors, mice were tested on two standard behavioral assays, the Light/Dark Box and the OpenField. The Light/Dark Box test relies on the relative time spent exploring each of the two compartments (one illuminated and the other dark). A decrease in time spent in the illuminated compartment is used as a measure of increased of anxiety-like behavior. Results showed that the CUMS group spent less time in the illuminated compartment compared to the control group

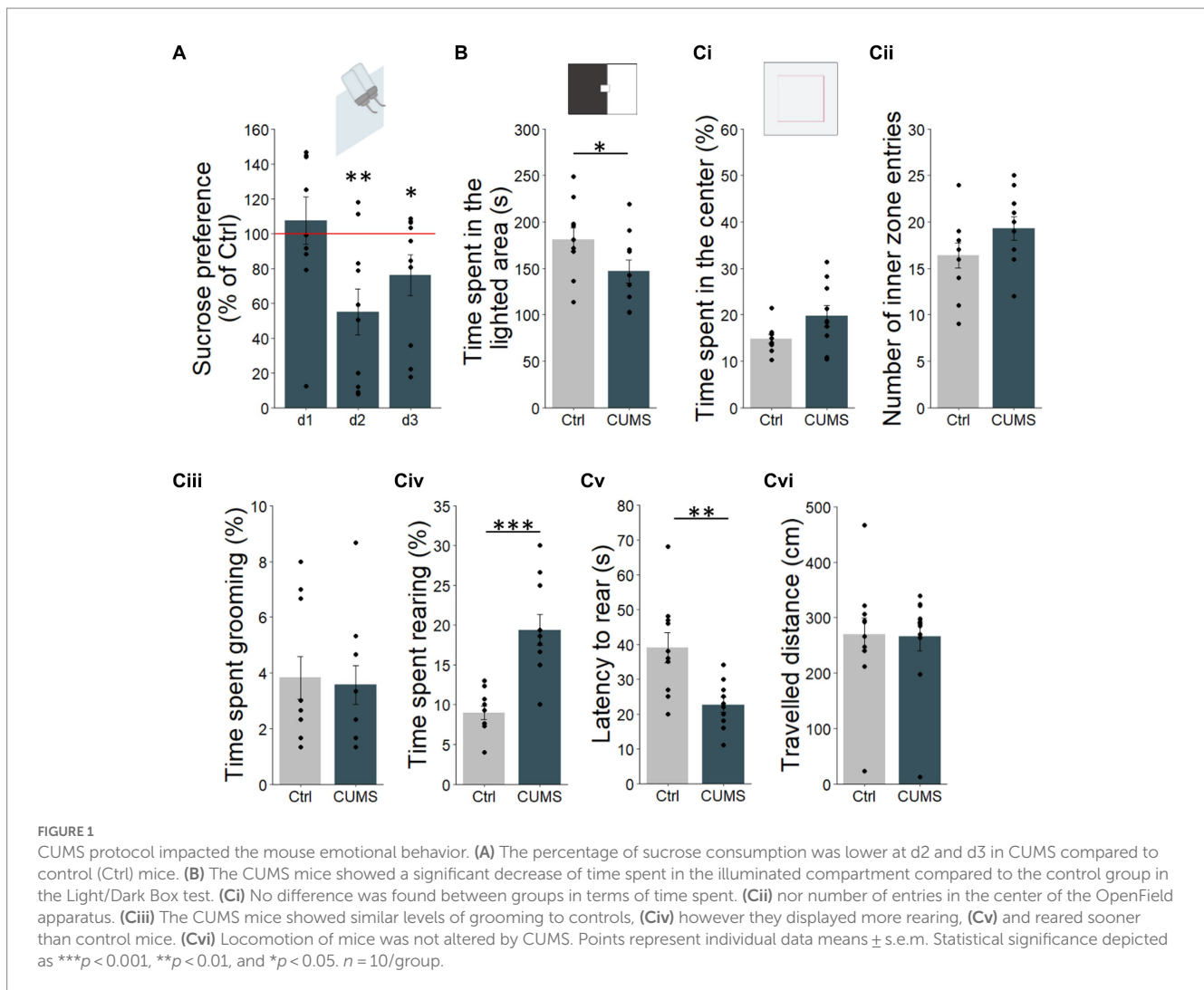
(unpaired unilateral *t*-test, $p = 0.02$; permutation test, $p = 0.03$, 100,000 permutations; Figure 1B), suggesting a higher level of anxiety in CUMS mice. Similarly, the middle of the OpenField, is generally considered more anxiogenic. Thus, the amount of time that the animal spent in the inner zone (center) of the setup, and the number of entries into center was used as an index of anxiety-like behavior. Here, no significant differences were found between CUMS and control mice (unpaired *t*-test, $p = 0.96$ and $p = 0.93$ respectively; Figures 1Ci,ii). As additional measures, CUMS mice spent a similar amount of time to control animals grooming (unpaired *t*-test, $p = 0.25$; Figure 1Ciii). We observed an increase in time spent rearing (unpaired *t*-test, $p = 0.005$; permutation test, $p < 0.0001$, 100,000 permutations; Figure 1Civ) and a decrease of latency to rear (unpaired *t*-test, $p = 0.0003$; permutation test, $p = 0.001$, 100,000 permutations; Figure 1Cv) in CUMS compared to control mice. To ensure that these effects were not due to changes in locomotor activity, we assessed the total distance travelled by animals in the OpenField for the two groups and found no difference between control and CUMS groups (unpaired *t*-test, $p = 0.44$; Figure 1Cvi). Taken together, these results suggest that CUMS influences multiple aspects of anxiety and depressive-like behavior.

Chronic unpredictable mild stress induced alterations in odor hedonics

Odor hedonics were automatically assessed using an odor preference test (Mandairon and Linster, 2009), and the time the mouse investigated the odorants was used as an index of odor hedonics (Kermen et al., 2016; Midroit et al., 2021; Figure 2 and Supplementary Figure 1). As expected, investigation time of pleasant odorants was higher compared to unpleasant ones in control mice (2-ways-ANOVA, odor effect: $F(1,36) = 5.039, p = 0.03$; Bonferroni *post-hoc* test, $p = 0.02$; Figure 2). Interestingly, we observed a significant effect of experimental group (CUMS versus control: 2-ways-ANOVA; $F(1,36) = 8.035, p = 0.007$) and an interaction ($F(1,36) = 4.2, p = 0.04$) indicating that the effect of the experimental group depends on the odor hedonic value. In particular, the investigation time of the pleasant odorants was lower in the CUMS group compared to the control one (Bonferroni *post hoc* test, $p = 0.007$; Figure 2) while the investigation time of the unpleasant odorants remained unchanged (Bonferroni *post hoc* test $p = 0.9$; Figure 2).

Chronic unpredictable mild stress altered adult neurogenesis and reduced the density of adult-born cells responding to pleasant odorants in the OB

We then tested whether the altered odor hedonic perception after CUMS protocol was associated with changes in olfactory neurogenesis. To do this, we labelled a cohort of adult-born cells integrating in the OB during CUMS protocol by injecting BrdU 20 days after the beginning of the CUMS procedure. This delay allows neuroblasts to migrate from the subventricular zone to the OB and integrate into the pre-existing neural network. We assessed adult-born cell density in the granule cell layer. We found a lower density of BrdU-positive cell in the OB in CUMS compared to control group (unpaired bilateral *t*-test, $p = 0.006$; permutation test, $p = 0.0043$, 924 permutations; Figure 3A).



We then assessed the effect of CUMS on the activity of adult-born neurons responding to pleasant versus unpleasant odors by analyzing *c-Fos* expression in BrdU labeled cells 1 h following stimulation with either a hedonically pleasant or unpleasant odorant. Using 2-way-ANOVA, we found an effect of experimental group ($F(1.36) = 19.837$, $p = 0.0007$), of odor hedonics ($F(1.36) = 5.039$, $p = 0.03$) and an interaction ($F(1.36) = 6.634$, $p = 0.02$) indicating that the effect of the experimental group depended on the odor hedonics. Specifically, we found that while the percentage of double labeled BrdU/*c-Fos* cells is not different between the two experimental groups in response to unpleasant odorant (Bonferroni post-hoc test, $p = 0.3$; Figure 3B), a lower percentage of double labeled cells was found in response to pleasant odorant in CUMS compared to control group (Bonferroni post-hoc test, $p = 0.0002$; Figure 3B). This was not due to an overall decrease in neuronal activity in the OB of CUMS animals. Indeed, when assessing the density of *c-Fos*+/*BrdU*-labeled cells, a 2-way-ANOVA revealed no effect of experimental group ($F(1.13) = 0.45$, $p = 0.51$), nor of the hedonic value of odorants ($F(1.13) = 0.972$, $p = 0.7$) (Figure 3C). Thus, the decrease in BrdU/*c-Fos* double labelling reflects less involvement of adult-born neurons in processing pleasant odorants in CUMS group, and not an overall decrease in sensitivity of CUMS animals to pleasant odorants.

Discussion

In the current experiments, the CUMS paradigm evoked patterns of behavior consistent with increased anxiety and depressive-like behavior and also altered olfactory hedonics. Specifically, our findings confirmed that CUMS induced depressive-like state, with evidence of anhedonia in the sucrose preference test. We also observed an increased anxiety-like behavior in the Light/Dark Box test and the assessment of rearing behavior in the open field. Importantly, we made the novel observation that CUMS altered odorant hedonics by specifically lowering interest for pleasant odors. To date, the focus of studies assessing the effect of stress and depression on olfactory perception focus on threshold or intensity (Schablitzky and Pause, 2014). Here, in order to focus on odor hedonics, we chose the strategy of using largely supraliminal concentration of odors so that the mice, even if they had an altered threshold for odor detection, could still detect the odorant. This is confirmed by the fact that the level of investigation of unpleasant odors is the same for control and CUMS mice. In addition, simple discrimination is known to be unaffected by chronic stress (Athanassi et al., 2021). While olfactory hedonics is almost never studied in rodent models of stress, several studies in humans have found that pleasant odors were perceived

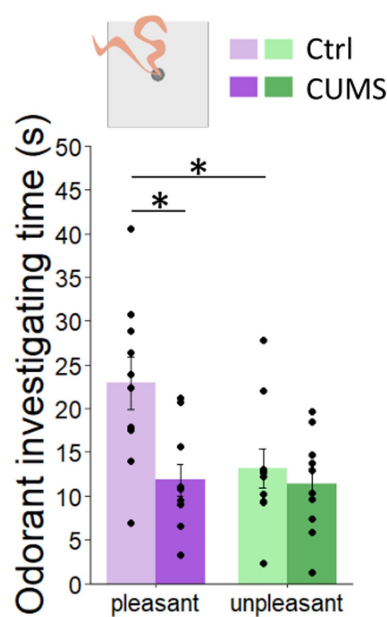


FIGURE 2
CUMS protocol altered the perception of pleasant odors. (Top) Experimental set up. (Bottom) (Ctrl) mice spent more time investigating pleasant odors compared to unpleasant ones. Odor investigation time of the unpleasant odors was similar between control and CUMS mice while it was reduced for the pleasant odors in CUMS compared to control group. Points represent individual data means \pm s.e.m. Statistical significance depicted as $*p < 0.05$. $n = 10$ /group.

as less pleasant in patients with depression (anhedonia) and unpleasant ones perceived as more unpleasant (negative alliesthesia) (Atanasova et al., 2010; Naudin et al., 2012; Kazour et al., 2020). In the present study, no difference between control and CUMS groups were found in response to unpleasant odors suggesting a specific effect of CUMS on the perception of pleasant odorant but a preserved avoidance behavior for unpleasant odors.

A neural signature of odor hedonics is believed to reside in the granule cell layer of the OB (Kermen et al., 2016). As OB granule cells are born throughout life, we investigated the impact of CUMS on adult neurogenesis. More specifically, we studied the impact of CUMS on the density of adult-born in the OB and their response to odors to test a possible neural bases for observed effects on olfactory hedonics. First, our results showed that CUMS decreased the density of adult-born neurons in the OB. The lower cell density in the OB could be the result of a reduction of adult-born cell survival in the OB and/or of cell proliferation in the subventricular zone. Here, the time course of labeling was designed to assess aggregate effects on proliferation, integration, and survival of newly born cells, but did not test for selective effects on proliferation. Thus, the contribution of CUMS effects on proliferation versus survival remains open since other studies using various stressors showed different effects on olfactory proliferation (Yang et al., 2011; Belnoue et al., 2013; Siopi et al., 2016; Czarnabay et al., 2019; Wang et al., 2022). The diversity of findings may indicate that the impact of stress on neurogenesis may rely on either discrete (proliferation vs. survival) or synergistic (proliferation and survival) mechanisms in the OB as well as the hippocampus since in the hippocampus, neurogenesis levels and more

specifically cell proliferation is altered after stress (corticosterone injections), or CUMS (Guo et al., 2009; Roni and Rahman, 2015; Huang et al., 2019; Li et al., 2021, 2022; Parul et al., 2021). Here, we did not test for changes in hippocampal neurogenesis in our model. While some olfactory associative tasks may involve the hippocampus (Kesner et al., 2011), many of them do not and do not modulate hippocampal neurogenesis (Kaut and Bunsey, 2001; Kaut et al., 2003; Sultan et al., 2010, 2011; Mandairon et al., 2011). Moreover, the olfactory preference task that we use here is a spontaneous olfactory task that do not rely on learning or recall. Thus, even though many studies have found alterations in hippocampal neurogenesis by CUMS, there is no evidence that hippocampal recruitment is involved in olfactory preference testing. Thus it is unlikely that CUMS effects on hippocampal neurogenesis explain olfactory anhedonia.

Interestingly, hippocampal (Huang et al., 2019) and bulbar (Lledo et al., 2006; Hitoshi et al., 2007; Siopi et al., 2016) neurogenesis are restored after anti-depressive treatment. It would be interesting in this context to assess the effect of anti-depressive treatment on olfactory hedonics.

Here, we provided evidence of impaired activity of adult-born granule cells in response to pleasant but not unpleasant odors after CUMS. Indeed, stressed animals not only displayed lower neurogenesis but also a weaker integration of these cells to the mature network of adult-born granule cells processing pleasant odors specifically. This suggests that adult born neurons involved in processing pleasant but not unpleasant odors are not correctly integrated. This suggests a specific sensitivity of OB sub circuits that underlie response to pleasant odors and could be due to a vulnerability of olfactory sensory neurons recognizing specific molecular features of pleasant odors and/or a vulnerability of feedback projections to the OB that may modulate survival of newly born cells. This alteration of odor hedonics and bulbar neurogenesis is consistent with recent studies revealing a link between adult-born neuron activity in the hippocampus and hedonic behavior with alleviated depression-like behaviors and an increased resilience against chronic stress when the activity of adult-born neurons is increased (Anacker et al., 2018; Rawat et al., 2022). Finally, granule cells coding for olfactory hedonics regulate mitral cell activity projecting directly to the olfactory tubercle, another key structure of odor hedonics (Midroit et al., 2021). Therefore, any functional alterations of adult-born granule cells in the OB would modulate olfactory processing in the OB and possibly also one of its direct targets, the olfactory tubercle, contributing to odor hedonic impairment.

Olfactory perception and emotional behavior have a bidirectional relationship with olfactory disturbances leading to behavioral profiles indicative of depression-like state in rodents and humans and *vice-versa* (Atanasova et al., 2008; Croy and Hummel, 2017; Taalman et al., 2017; Rochet et al., 2018). Since the OB is tightly linked to the limbic system whose structures are involved in emotional regulation, changes in OB outputs can also modify emotional circuit functioning and behavior (Eiland et al., 2012; Ma et al., 2021). Conversely, alteration of structures of the emotional circuit after stress, like the amygdala, which projects to the OB granule cells (Wen et al., 2019), can lead to change in olfactory processing and perception. Altogether, this may explain why the olfactory perception can be a marker of depression (Croy and Hummel, 2017). Finally, strategies increasing adult hippocampal neurogenesis can alleviate anhedonia (Eliwa et al., 2021), leading to questions about whether a similar mechanism could be employed in the olfactory bulb.

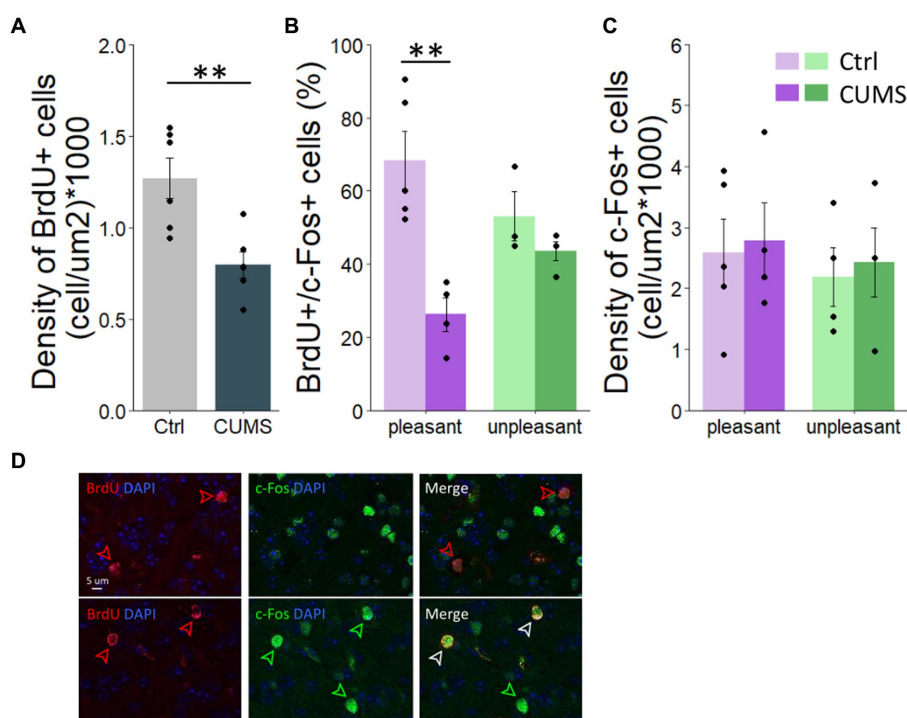


FIGURE 3

CUMS protocol altered adult-born survival and the activity of adult-neurons involved in pleasant odorant processing. (A) The density of BrdU-positive cells was lower in CUMS compared to controls. (B) The percentage of BrdU-positive cells responding to pleasant odors was lower in CUMS mice compared to controls while no difference was found in response to unpleasant odors. (C) The density of c-Fos + cell is similar between groups and hedonic value of odors. (D) Photo of BrdU⁺ cells (red arrows), c-Fos + cell (green arrows) and BrdU+/c-Fos + double-labelled cells (white arrows). Points represent individual data means \pm s.e.m. Statistical significance depicted as $**p < 0.001$. $n = 5$ control and 4 CUMS mice stimulated with pleasant odors and $n = 3$ control and 4 stressed mice stimulated with unpleasant odors.

In summary, these represent the first study linking CUMS with altered hedonic processing of odors, a phenotype that is associated with increased anxiety and depressive-like behavior. We provide novel data indicating that CUMS effects on either integration and activity of adult-born cells may underlie alterations in olfactory hedonics. However, more specific characterization of the activity and interaction of adult-born and pre-existing neurons would be an asset to better understand the mechanistic underpinnings of the altered odor hedonics observed here. Such change in OB activity could contribute significantly to our understanding of the mechanisms of hedonic disturbance of olfactory signals in human population and their contribution to either symptom presentation or worsening quality of life in depressed populations. In addition to enhancing our understanding of the mechanisms underlying impairments in pleasant odor perception in depression, our findings could provide useful information to set up more naturalistic, non-pharmacological approaches to improve olfactory perception, and thereby overall well-being of individuals who have suffered from chronic stress. Indeed, by demonstrating a specific effect of CUMS on positive odor hedonics, future studies could focus on the effects of olfactory training on the hedonic response to odors in order to assess if its effects are mediated by an increase in olfactory hedonic perception. Furthermore, fostering training with consensual pleasant odors could be more efficient in improving mood disorders in humans, than previous studies using odors without considering their hedonic value (Umezū, 1999; Ceccarelli et al., 2004; de Almeida

et al., 2004; Tokumo et al., 2006; Faturi et al., 2010; Linck et al., 2010; Cryan and Sweeney, 2011; Birte-Antina et al., 2018; Pieniak et al., 2022).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by European Community Council Directive of 22nd September 2010 (2010/63/UE) and the National Ethics Committee (Agreement APAFIS#20702_2019072614086203_v2).

Author contributions

MB, AD, and NM contributed to conception and design of the study. MB performed the behavioral tests. AA performed the cellular analysis. AA and LC performed the statistical analysis. LC, AA, JB, KB, and NM wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

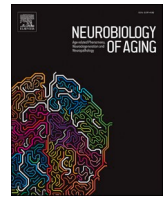
The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnins.2023.1224941/full#supplementary-material>

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-ANNEXE 2-

*Age-related differences in Perception and Coding of Attractive
Odorants in Mice*

(publié)



Age-related differences in perception and coding of attractive odorants in mice

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ABSTRACT

Hedonic perception deeply changes with aging, significantly impacting health and quality of life in elderly. In young adult mice, an odor hedonic signature is represented along the antero-posterior axis of olfactory bulb, and transferred to the olfactory tubercle and ventral tegmental area, promoting approach behavior. Here, we show that while the perception of unattractive odorants was unchanged in older mice (22 months), the appreciation of some but not all attractive odorants declined. Neural activity in the olfactory bulb and tubercle of older mice was consistently altered when attraction to pleasant odorants was impaired while maintained when the odorants kept their attractiveness. Finally, in a self-stimulation paradigm, optogenetic stimulation of the olfactory bulb remained rewarding in older mice even without ventral tegmental area's response to the stimulation. Aging degrades behavioral and neural responses to some pleasant odorants but rewarding properties of olfactory bulb stimulation persisted, providing new insights into developing novel olfactory training strategies to elicit motivation even when the dopaminergic system is altered as observed in normal and/or neurodegenerative aging.

1. Introduction

Sensory information robustly elicits emotional responses which guide approach or avoidance to stimuli. Our unlearned emotional responses to stimuli, often referred to as their hedonics, possess major implications for aging and countless age-related neurological conditions wherein impairments in stimulus hedonics may impact quality of life as well as metabolic health. For example, deficit in hedonic evaluation of food odorants decreases food intake (Joussain et al. 2017) and may perpetuate bodily wasting disorders (Ali and Garcia, 2014; Frontera et al. 2000; Madsen et al. 1997) – which are common in aging and age-related dementias (Joussain et al. 2013, 2016) and represent a major public health problem. The lack of mechanistic understanding of age-related impairments in odor hedonics, especially at the neural circuit level, prevents us from developing remediation strategies.

Our team revealed the existence of a pathway in young adult mice that transmits the hedonic information of the odor from the olfactory system to the reward system to drive approach behavior. Specifically, we uncovered evidence that odor hedonics is represented along the antero-posterior axis of the olfactory bulb (OB) (Kermen, Midroit et al. 2016; Kermen, Mandairon, Chalençon, 2021) – the first cortical level for processing odor information. This neural signature of hedonic value present in the OB is transferred into down-stream brain structures. Among the main structures targeted by OB axons, we revealed recently that only the olfactory tubercle (OT, which is part of the ventral striatum) preferentially responds to attractive odorants in young adult mice, as does the ventral tegmental area (VTA) (Midroit, Chalençon et al. 2021). To further emphasize the functional connection between the OB and the reward system, we tested whether direct stimulation of OB neurons could be rewarding. Intracranial self-stimulation has been

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extensively used to determine brain areas belonging to the reward system and unravel the neural bases of motivation (Olds and Milner, 1954; Olds and Fobes, 1981; Carlezon and Chartoff, 2007). This technic consists in an operant conditioning in which the stimulation of a specific brain area serves as a reinforcer. We previously showed that self-stimulation of the OB is able to induce operant conditioning and is accompanied by an activation of the VTA in young adult mice (Midroit, Chalénçon et al. 2021). This accounted for how the olfactory system solicits the reward system to drive motivated behavior. Altogether these data showed that spontaneous attraction induced by pleasant odorants is dependent upon the solicitation of the reward circuit. However, whether this brain mechanism may be affected by aging remains unknown.

The biological substrate of the reward circuit includes dopamine (DA) release from VTA neurons in the ventral striatum. With aging and/or age-related neurological conditions, numbers of dopaminergic neurons and their receptors in the midbrain decline (Collier, Kanaan, and Kordower, 2017) as do forebrain DA levels (Araki et al. 1997; Irwin et al. 1994; Schuligoi et al. 1993; Barili et al. 1998; Watanabe, 1987; Norrara et al. 2018). Degeneration of this neuromodulatory pathway is involved in abnormal reward valuation in aging (Marschner et al. 2005; Sojitra et al. 2018) and thus may underlie odor hedonic dysfunction that is observed in aging (Wang et al. 2005; Markovic et al. 2007; Jousain et al. 2013; Vieillard et al. 2020). In this context, the first goal of this project was to assess how odor hedonics and its neural signature is modulated by aging. Since neural representation of odor hedonics has been found along the antero-posterior axis of the OB, we hypothesized changes in this neural representation linked to the change in behavioral performances. The second goal was to determine to what extent attractive odorants could still drive motivation and activation of the olfactory and reward circuits with aging as it was observed in young adult mice (Midroit, Chalénçon et al. 2021).

2. Materials and methods

2.1. Animals

133 adult male C57Bl6/J mice (Charles River Laboratories, L'Arbresle, France) were used for all experiments (86 mice in experiment 1, 47 mice in experiment 2). “younger” mice were aged of 2 months and “older” mice were aged 22 months at the beginning of the experiments. Experiments were done following procedures in accordance with the European Community Council Directive of 22nd September 2010 (2010/63/UE) and the National Ethics Committee (Agreement DR2013–48 (vM)). Mice were kept on a 12-hr light/dark cycle (22°C) with food and water *ad libitum*. Mice were housed by groups of 5, then individually after surgery (when applicable). All experiments started after at least 1 week of acclimatization to the animal facility.

2.2. Statistical analysis

All statistical analyses were conducted using Statistica. We verified normal distribution of data and homogeneity of variances for each group using Kolmogorov-Smirnov and Levene tests. If either normality or variance homogeneity was not met, non-parametric tests were applied (Friedman, Wilcoxon or Mann-Whitney tests). For data that met these two conditions, statistical analyses were performed using parametric tests (ANOVA or t-test). One-Tailed tests were performed when we had specific predictions about the direction of the difference based on the data of young adult mice. Bonferroni corrections for multiple comparisons were applied when needed. Heatmaps and permutation tests were performed using custom-made algorithms in MATLAB. No statistical methods were used to predetermine sample sizes, but sample sizes were similar to those reported in previous publications (Midroit, Chalénçon et al. 2021; Kermen, Midroit et al. 2016; Moreno et al. 2009; Mandairon et al. 2011a, 2011b; Sultan et al. 2011; Moreno et al. 2012).

2.3. Experiment 1. Olfactory performances and neuronal activity in response to odorants with different hedonic values

2.3.1. Odorants

Six monomolecular odorants were used: three attractive in young adult mice (camphor: CAM; citronellol: CITRO; +limonene: LIM) and three unattractive (pyridine: PYR; guaiacol: GUA; p-cresol: CRE). The behavioral attraction of the odorants was described previously (Kermen, Midroit et al. 2016) and replicated in two independent experiments (Midroit, Chalénçon et al. 2021; Athanassi, Breton et al. 2023). To ensure similar air concentration, the odorants were all diluted in mineral oil proportionally to their vapor pressure to reach a pressure of 1 Pascal (Pa) (Table S1) (Midroit, Chalénçon et al. 2021; Kermen, Midroit et al. 2016; Kermen et al. 2011).

2.3.2. Odorant detection

At 1 Pa, the odor concentration was shown to be supraliminal (above the detection threshold) and thus easily detected by older mice (Rey et al. 2012; Moreno et al. 2014). Here, we confirmed that older mice were able to detect odorants at 1 Pa by submitting three groups of mice to a preliminary detection test session. 28 mice aged of 22 months conducted the odorant detection test (n=10 CITRO, n=10 GUA, n=8 CAM). The session consisted of 4 successive trials (50-sec duration, 5 min inter-trial interval). During the first three trials, only mineral oil (no odor condition; NO) was presented in a tea ball hanging from the lid of the cage (habituation phase: NO1 to NO3). For the fourth trial, the tea ball was odorized with CITRO, CAM or GUA at 1 Pa (cross habituation). Time spent to investigate the tea ball was recorded (active sniffing 1 cm around the tea ball, using Volcan software (Hegoburu et al., 2009; Richard et al., 2010)). Detection was recognized as a significant change in the exploration time of the odorized tea ball when compared to the mean exploration time of the last habituation trial (NO3 versus odor trial; Two-Tailed Wilcoxon).

2.3.3. Assessment of odor attraction (or preference test)

10 younger and 25 older mice performed an olfactory preference test. Odor attraction was measured in mice using odorant investigation time (Kobayakawa et al. 2007; Mandairon, Poncelet, et al. 2009; Kermen, Midroit et al. 2016). All odorants were novel for the mice and tested once so that differences in investigation time could be ascribed to different levels of attraction and not novelty. As previously described (Kermen, Midroit et al. 2016), we used a computer-assisted one-hole-board apparatus fitted with sensors to automatically monitor the duration of nose poking into the central hole (Mandairon, Poncelet, et al. 2009; Mandairon, Sultan, et al. 2009). A polypropylene swab impregnated with 60 µL of odorant at 1 Pa was placed at the bottom of the hole, under a grid covered with clean bedding. The bedding was replaced after each trial. All mice were allowed to explore each of the six odorants and one trial was performed in a no odor control condition (mineral oil) (one odorant/day; 2 min/trial; Fig. 1A). Odorants were presented in random order and animals performed no more than two consecutive days of testing. Behavioral data from young adult mice (Figure 1B) replicated previous findings in young adult mice using an additional group (n=10 Fig. S2) (Athanassi, Breton et al. 2023; Midroit, Chalénçon et al. 2021; Kermen, Midroit et al. 2016). For each animal, investigation time of each odorant was expressed as the percentage of the no odor condition (used as a reference value, 100%) and differences from 100% was assessed by one sample t-tests.

2.3.4. Odorant stimulation and sacrifice

Over the 25 older mice that performed the olfactory preference test, 20 mice have been randomly selected to examine their odor-evoked neuronal activity. To do so, we mapped the expression of the immediate early gene c-Fos in several olfactory and reward areas. The protein c-Fos is a transcription factor whose expression reflects neural activity. Before sacrifice, mice were submitted to odorant stimulation for one

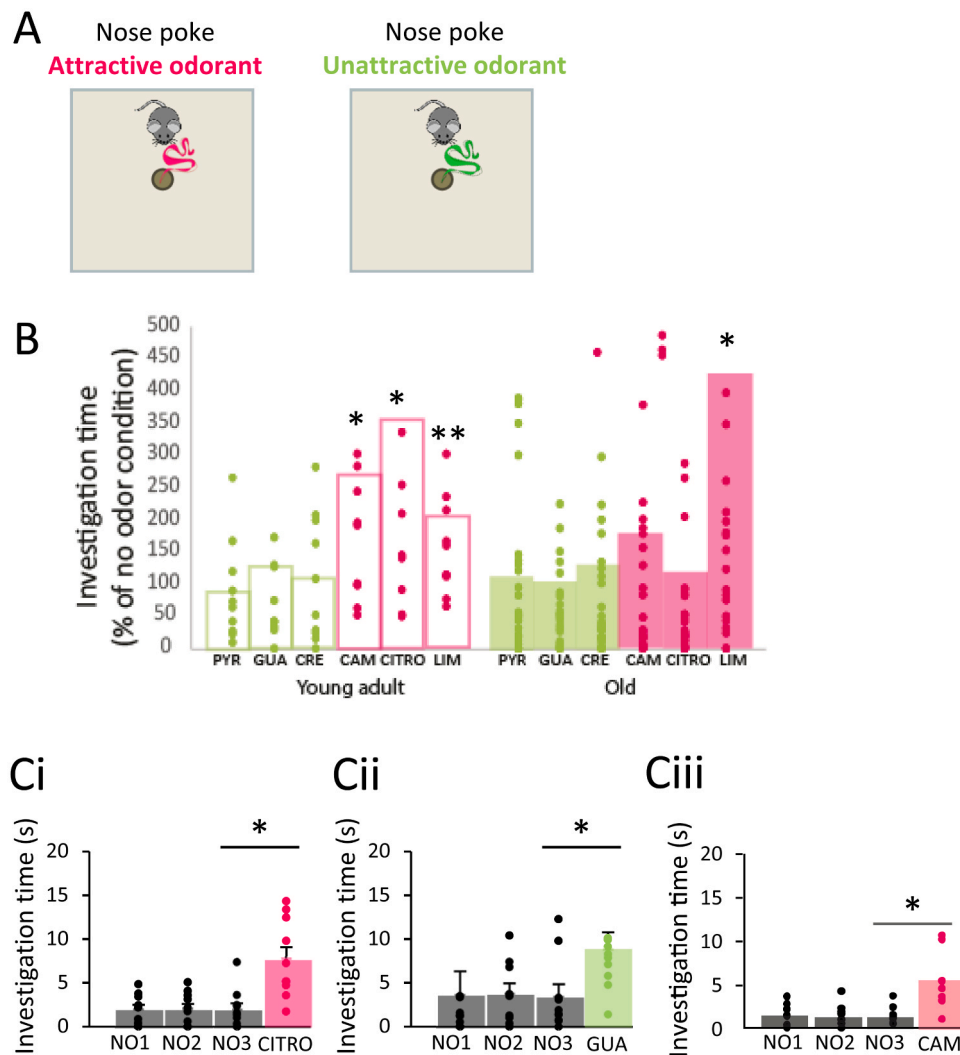


Fig. 1. Odor-driven attraction is altered during aging. **A.** Experimental setup. **B.** Investigation time measured in young adult ($n=10$) and older mice ($n=25$) in response to 3 odorants known to be attractive (pink: CAM, CITRO, LIM) and 3 unattractive in young adult mice (green: PYR, GUA, CRE). For each age condition, the mouse investigation time of each odorant was expressed as a percentage of the no odor condition (young mice, mean = 3.63 se.m. = 0.92 and aged mice, mean = 9.34 se.m. = 2.33) and analyzed by one sample t-test for difference from 100% (* $p<0.05$; ** $p<0.01$; *** $p<0.001$). CAM, CITRO and LIM were explored more than no odor in young adult mice while in older mice CAM and CITRO were no longer explored more than no odor. LIM remained attractive and explored above no odor level. For clearer representation, 10 data points (out of 210) above 600% are not shown and are distributed as follows: in older animals, Lim: 1845.23, 1454.89, 2440.95, 1126.47; Citro: 1454.14; Cam: 1588.29, 733.33; Cre: 1522.66; Gua 681.8 and in young adult animals, Citro: 6442.94. **C.** To assess detection of odorants at 1 Pa, an habituation/cross habituation test was used. The significant difference of investigation time between NO3 and CITRO (Ci; ($n=10$)), NO3 and GUA (Cii, ($n=10$)) or NO3 and CAM (Ciii, ($n=8$)) indicated efficient odor detection by older mice. For all graphs, bars represent means of individual data points \pm sem. *: $p<0.05$; ***: $p<0.001$. PYR: pyridine, GUA: guaiacol, CRE: p-cresol, CAM: camphor, CITRO: citronellol, LIM: +limonene, NO: no odor. [Midroit et al., 2021](#).

hour and killed one hour after the end of the stimulation. This 1-hour delay has previously been shown to enable the expression of c-Fos in response to odorant stimulation ([Kermen, Midroit et al. 2016](#); [Dielenberg et al., 2001](#); [Kovács, 2008](#)). Briefly, mice were first placed in individual clean cages for 1 h, with an empty tea ball hanging from the top of the cage. A polypropylene swab impregnated with one odorant (60 μ L, 1 Pa) was then placed in the tea ball for an additional hour. Mice were sacrificed using dolethal (2 g/Kg) and intracardiac perfusion of 50 ml of fixative (paraformaldehyde 4%, pH = 7.4). The brains were removed, post-fixed overnight, cryoprotected in sucrose (20%), frozen rapidly, and then stored at -20°C before sectioning with a cryostat (14 μm thick; 210 μm intervals).

2.3.5. c-Fos immunohistochemistry and mapping in the ventral granule cell layer of the OB

For each animal, c-Fos immunohistochemistry was performed on OB sections ($n=5$ mice randomly chosen for each hedonic condition; 210

μm interval; coordinates from the bregma [antero-posterior (AP) +5.56 to +4 mm]). A rabbit anti-c-Fos primary antibody was used (1: 5000; Santa Cruz Biotechnology, Santa Cruz, CA, USA; ref: Sc-189) and combined to biotinylated goat anti-rabbit secondary antibody (1: 200; Vector Laboratories, Burlingame, CA, USA; ref: 24541). Brain sections were then processed through an avidin-biotin-peroxidase complex (ABC Elite Kit, Vector Laboratories). Following dehydration in graded ethanols, sections were defatted in xylene and coverslipped in DPX (Fluka, Sigma).

For each OB section analysed, the external contour of the right hemisphere was outlined and c-Fos⁺ cells were automatically counted in the ventral granule cell layer in line with our previous finding showing that the hedonic representation lies in this part of the OB. 14 sections were analysed per mouse using a mapping software (Mercator, Explora Nova, La Rochelle, France) coupled to a Zeiss microscope. All cell counts were conducted blind with regard to the experimental group. Then, the coordinates of each labelled cell (c-Fos⁺) and the contour point were

exported into a homemade software allowing high-accuracy matching of experimental brain sections with a reference brain atlas (Midroit et al. 2018). This method allows precise, automatic assignment of the labelled cells to a brain structure and permits between-group comparisons. In the atlas, we delineated the ventral granule cell layer (corresponding to the half-inferior part of the granule cell layer). The layer was represented along its antero-posterior axis with each bin corresponding to 120 μm of the layer along the antero-posterior axis (missing sections were interpolated; for more method explanations see Midroit et al. 2018). For each mouse, we first calculated the density of c-Fos-positive cells for each of the 14 bins. As the mean of the bins' density per animal could vary as a result of technical factors (i.e. difference in labeling intensity etc.). We computed z-scores on the c-Fos density of the 14 bins in order to standardize the data within each animal (mean density of the 14 bins of each animal = 0 and SD = ± 1). To compare spatial patterns of labelled cells between groups, we then averaged the standardized density maps across animals within each group and used Mann-Whitney tests to compare the c-Fos density of each bin between groups (mean density of a bin of "A" group compared to the respective bin of "UnA" or "PA" group). Non-parametric tests were used as the distribution of the density maps across animals was not normal. Importantly, z-scores and Mann-Whitney tests have been performed on different dataset (14 bins of one animal for the z-scores (1 z-score per animal), 5 animals of one group vs 5 animal of another group for the Mann-Whitney tests (1 Mann-Whitney per bin)). We compared maps of three groups of mice based on the behavioral results: unattractive (n=5), attractive in young but no more in older mice called previously attractive (n=5) and attractive (n=5) odorants. The statistical differences between groups were confirmed by odorant permutation (252 permutations) on each bin of the z-scored arrays followed by One-Tailed Mann-Whitney tests.

2.3.6. Double labelling analysis

To investigate the activity of medium spiny neurons of the OT (coordinates from the bregma [AP +2.09 to -0.11 mm]), we performed double immunostaining against c-Fos and DARPP-32 (a marker of medium spiny neuron of the striatum and the OT (Murata et al. 2015)). Briefly, rabbit anti-DARPP-32 (1: 500; AbCam, ref: 40801) and mouse anti-c-Fos (1: 500; ProteinTech, ref: 66590) primary antibodies were combined respectively with Alexa 488 goat anti-rabbit (1:250; Molecular Probes, ref: 11034) and Alexa 546 goat anti-mouse (1: 250; Molecular Probes, ref: 11030) antibodies. The percentage of double-labelled cells (c-Fos⁺ among DARPP-32⁺=DARPP-32⁺/c-Fos⁺) was assessed (5 sections/animal; ~ 220 cells/animal, 5 animals/group). One-Way ANOVA was used to test group differences followed by Bonferroni corrected *post hoc* t-tests for paired comparisons.

To investigate the activity of the VTA (coordinate from the bregma [AP -3.3 mm]), we performed double immunostaining against c-Fos and Tyrosine Hydroxylase (one section/animal; 5 animals/group). Briefly, chicken anti-TH (1: 2000; AbCam, ref: 76442) and rabbit anti-c-Fos (1: 2000; ProteinTech, ref: 26192) primary antibodies were combined respectively with Alexa 546 goat anti-chicken (1: 250; Molecular Probes, ref: 11040) and Alexa 488 goat anti-rabbit antibodies (1: 250; Vector Laboratories, Burlingame, CA, USA, ref: 32731). The percentage of double-labelled cells (c-Fos⁺ among TH⁺=TH⁺/c-Fos⁺) was calculated (1 section/animal; ~16 cells/animal, 5 animals/group) and Kruskal-Wallis ANOVA was used to test the group effect. The percentage of TH⁺/c-Fos⁺ cells was also calculated and One-Way ANOVA was used to assess the group effect.

After both immunohistochemistries, sections were cover-slipped in Vectashield medium combined with Dapi (Vector laboratories). All fluorescent counting was done blind with regards to the identity of the animal and using AxioVision (Zeiss) software coupled to a fluorescent pseudo-confocal Zeiss microscope.

2.4. Experiment 2. Optogenetic intracranial self-stimulation

The aim of the optogenetic experiment was to inhibit the inhibitory granule cells of the ventro-posterior OB using a NpHR expressing lentivirus that was shown to effectively inhibit granule cells and consequently disinhibit mitral cells using slice patch clamp recording (Kermen, Midroit et al. 2016).

2.4.1. Lentivirus injection and optical fiber implantation in the ventro-posterior OB

Prior to surgery, 47 mice aged of 22 months were anaesthetized with an intraperitoneal cocktail injection of 50 mg/kg ketamine and 7.5 mg/kg xylazine and secured in a stereotaxic instrument (Narishige Scientific Instruments, Tokyo, Japan). 150 nl of Lenti-hSyn-eNpHR3.0-EYFP lentivirus ($9,22 \times 10^6$ IU/ml, expressing halorhodopsin and the yellow fluorescent protein; NpHR mice, n=27) or 300 nl of control Lenti-hSyn-EYFP lentivirus ($1,1 \times 10^6$ IU/ml, expressing only EYFP; Ctrl mice, n=20) were injected bilaterally in the ventral granule cell layer of the ventro-posterior part of the OB (with respect to the bregma: AP, +4.3 mm; ml, ± 0.75 ; DV, -2 mm) at the rate of 150 nl/min. Following virus infusions, a dual optical fiber (200-nm core diameter, 0.22 N.A.; Doric Lenses) was implanted into the OB at the same coordinates as the virus infusion. Behavioral experiments started 8 weeks after surgery. 5 mice died immediately after surgery and 15 during the 8-week time window after surgery, resulting in 16 NpHR mice and 11 Ctrl mice available for the behavioral experiment. The rate of post-surgery death is higher than in our previous studies and could be attributed to the age of the mice (22 months) (Midroit, Chalçon et al. 2021; Forest et al. 2020; Forest, Moreno et al. 2019; Kermen, Midroit et al. 2016).

2.4.2. Optical self-stimulation in freely moving animals

8 weeks after surgery, NpHR and Ctrl mice were submitted to the conditioning phase during which they were allowed to move freely on the one-hole-board apparatus for a 2-min trial per day, during 5 days (Midroit, Chalçon et al. 2021). The bilateral continuous light stimulation (Crystal Laser, 561 nm, 10–15 mW) was automatically triggered by the entry of the mouse's nose within 1 cm (light-triggering zone) of the hole (VideoTrack, Viewpoint) and stopped automatically when the mouse's nose exited the zone (Light phase: Light1, Light2, Light3, Light4 and Light5). This phase was followed by an extinction phase (Extinc1, Extinc2, Extinc3, Extinc4 and Extinc5) in which the nose presence in the zone did not trigger light stimulation any longer and one last trial (Light1') in which light stimulation was available again (2 trials per day). Total duration of nose time spent in the light-triggering zone was recorded and Friedman tests were used to test trial effect and was followed by Two-Tailed Wilcoxon tests for specific trial comparisons.

2.4.3. Cellular analysis

All mice were sacrificed as described in Experiment 1 one hour after a behavioral session in which light stimulation was available. Behavioral performances of this session were not different from those of the last behavioral session (Light1') (Two-Tailed Wilcoxon for difference between session the day of sacrifice and Light1', NpHR post group: $p=0.782$; EYFP post group: $p=0.700$).

OB sections were double labelled after immunohistochemistries of EYFP and c-Fos proteins to control the effect of light stimulation on transfected granule cells. Briefly, rabbit anti-c-Fos (1: 5000; Santa Cruz Biotechnology, Santa Cruz, CA, USA, ref: Sc-189) and chicken anti-EYFP (1: 1000; Anaspec TEBU, ref: 55423) primary antibodies were combined respectively with Alexa 546 goat anti-rabbit (1: 250; Vector Laboratories, Burlingame, CA, USA; ref: 11035) and Alexa 488 goat anti-chicken (1: 250; Molecular Probes; ref: A32931TR) antibodies. The total density of EYFP⁺ and the percentage of double-labelled cells (EYFP⁺/c-Fos⁺) were assessed in the ventral granule cell layer of the OB at the level of the injection site (ventro-posterior OB, 2 sections/animal; ~50 cells/animal; 27 animals) and at 0.96 mm anterior to the injection

(2 sections/animal; ~8 cells/animal) (ventro-anterior OB; coordinates from the bregma [AP +5.33 to +4.85 mm]; ventro-posterior OB; coordinates from the bregma [AP +4.49 to +3.89 mm]). Bonferroni corrected Two-Tailed Wilcoxon were used to compare EYFP⁺ density between the injection site (ventro-posterior OB) and ventro-anterior OB. Two-Tailed Mann-Whitney was used to compare the percentage of EYFP⁺/c-Fos⁺ between groups. Neural activity in the VTA and OT was assessed by calculating the percentage of c-Fos-TH and c-Fos-DARPP-32 double-labelled cells as described in Experiment 1 (Two-Tailed Mann-Whitney for between group comparisons).

3. Results

3.1. Perception of some but not all pleasant odorants becomes aberrant with aging

To test whether odor hedonics is altered during aging, we analyzed the duration of odor investigation as a reflection of attraction to odorant (Kobayakawa et al. 2007; Mandairon, Poncelet, et al. 2009; Kermen, Midroit et al. 2016; Midroit, Chalçon et al. 2021) (Figure 1A) in 22-month-old mice and in younger mice. The 6 odorants used (Supplementary Table 1) were novel for the mice and tested once. Replicating previous finding (Kermen, Midroit et al. 2016; Midroit, Chalçon et al. 2021), PYR, GUA, CRE, 3 odorants documented as unattractive in young adult mice, induced investigation times in young adults similar those of the no odor condition (one Sample t-test, PYR $p=0.1$; GUA $p=0.79$; CRE, $p=0.76$), thus showing low level of attractivity. This was also true for older mice (one Sample t-test, PYR $p=0.54$; GUA $p=0.86$; CRE, $p=0.62$; Fig. 1B). Odorants documented as attractive in young adult mice (CAM, CITRO and LIM) showed here also increased attractivity compared to the no odor control condition in young mice (one Sample t-test, CAM $p=0.01$; CITRO $p=0.03$; LIM, $p=0.005$). In older mice, CAM and CITRO were no longer attractive (one Sample t-test, CAM $p=0.22$; CITRO $p=0.77$; Fig. 1B) and only LIM remained as attractive as in young adult mice (One Sample t-test, LIM $p=0.01$; Fig. 1B).

To ensure that the changes in hedonic perception observed in aged mice was not due to a lack of odor detection in older mice, we performed a cross habituation test. In this task, a tea ball containing odorless mineral oil was presented 3 times before one presentation of a pleasant (CITRO or CAM, Figure Ci and Ciii) or unpleasant odorant (GUA, Figure Cii), at the same concentration than during the preference test (1 Pa). An increase of investigation time was observed in response to odorants compared to the last trial of mineral oil indicating that the three odorants were detected at 1 Pa (Friedman trial effect, CITRO $F_{(3,6)}=13.163$ $p=0.001$, GUA $F_{(3,6)}=9.000$ $p=0.029$, CAM $F_{(3,8)}=12.26$ $p=0.006$; Two-Tailed Wilcoxon for difference between NO3 and odorant, CITRO $p=0.013$, GUA $p=0.037$, CAM $p=0.012$; Fig. 1C).

Altogether, our data revealed an age-dependent decrease of odor-driven attraction specific to some attractive odorants that we will call now previously attractive odorants (PA).

3.2. Neural activity in the OB reflects odor attractiveness

To investigate the neural bases of the alteration of odorant attraction with aging, we compared neural activity in response to odorants that are unattractive (UnA; PYR GUA CRE), that were attractive in young adult mice but no longer in older mice (PA; CAM CITRO) and that were still attractive in aged mice (A; LIM). The expression of the immediate early gene c-Fos was used to evaluate neural activity in the OB, OT and VTA in response to the three types of odorants (Midroit et al. 2018; Midroit, Chalçon et al. 2021).

Regarding the OB, we first found no difference in global c-Fos density between the three experimental groups (One-Way ANOVA group effect, $F_{(2, 12)}=0.513$, $p=0.611$). We then focused our analysis on the antero-posterior axis of the ventral granule cell layer (Figure 2Ai-Aii) as the neural signature of odor hedonics was previously identified in this

region (Kermen, Midroit et al. 2016). Indeed, in this previous study performed in young adult mice, a higher activity was found in the ventro-anterior granule cell layer in response to attractive odorants and in the ventro-posterior granule cell layer in response to unattractive odorants (Kermen, Midroit et al. 2016). The pattern of activity in the ventral granule cell layer in older mice evoked by odorants is depicted in Figure 2Aiii and statistical comparisons between these patterns in Fig. 2B. C-Fos positive cell density was higher in the ventro-anterior OB in response to still attractive compared to unattractive odorants (A>UnA, Figure 2Aiii and 2B first line) and higher in the ventro-posterior OB in response to unattractive compared to attractive odorants (UnA>A, Figure 2Aiii and 2B first line). These results are in line with our previous study (Kermen, Midroit et al. 2016). Odorants that lost their attractive power with aging showed a pattern different from that of attractive odorants in the ventro-anterior OB (A>PA, Figure 2Aiii and 2B second line) and did not differ neither from the attractive nor from the unattractive patterns in the ventro-posterior OB (Fig. 2B, PA>A second line; UnA>PA and PA>UnA third line). The statistical significance of these results was confirmed by permutation tests (Fig. S1). Our current data revealed the persistence of a neural signature of odor hedonics within the granule cell layer similar to that of young mice, characterized by a representation of attractive odorants in the ventro-anterior OB and of unattractive odorants in the ventro-posterior OB in older mice. In addition, attractive odorant becoming unattractive with aging showed a pattern similar to that of initially unattractive odorant. The concomitant changes of odorant driven-attraction and OB odorant-evoked activity observed with aging support the functionality of this neural trace to represent hedonics, even in older mice.

3.3. Activity in the OT is enhanced only in response the odorant remaining attractive

Since the OB projects to the OT which is involved in olfactory perception and hedonic processing, we analyzed neural activity in the OT and more particularly in the relay cells (medium spiny neurons) of the OT using the medium spiny neurons-specific DARPP-32 marker. We found different levels of neural activity based on the odorant groups (One-Way ANOVA group effect, $F_{(2,3)}=13.146$ $p<0.001$; Fig. 2C). Indeed, we observed an increase of double labelled DARPP-32⁺/c-Fos⁺ cells in A group compared to UnA group (Bonferroni corrected *post hoc* t-test $p<0.001$) or compared to PA group (Bonferroni corrected *post hoc* t-test $p=0.019$). We found no difference between PA and UnA (Bonferroni corrected *post hoc* t-test, $p=0.325$). Together these data indicated an increase of medium spiny neuron activity, specifically in response to odorants that remain attractive in older mice.

To better understand the role of the reward system in hedonic processing in aged mice, we further investigated the neural activity of dopaminergic neurons (TH⁺ cells) of the VTA in the three odorant groups. Surprisingly, we found no difference in TH⁺/c-Fos⁺ cells between the three groups (Kruskal-Wallis ANOVA group effect, $H_{(2,3)}=1.078$ $p=0.583$; Fig. 2D) suggesting that attraction induced by odorants in older mice is not underlined by an increased activity in the VTA. No difference in the activity of non-dopaminergic (TH⁻) VTA neurons was neither observed between the groups (One-Way ANOVA attractiveness effect, $F_{(2,3)}=0.205$ $p=0.818$; Fig. 2E).

Altogether, these results revealed that when attraction to odorants was altered in older mice, the neural activity in the OT and VTA was impaired. Moreover, when the odorant retained its attractive power, an increased activity in the OT was observed compared to unattractive odorants, but not in VTA. This suggests that the increased in OT activity is sufficient to lead to LIM attraction. Whether behavioral attraction and its associated activity in the OT will vanish as aging progresses remains unclear.

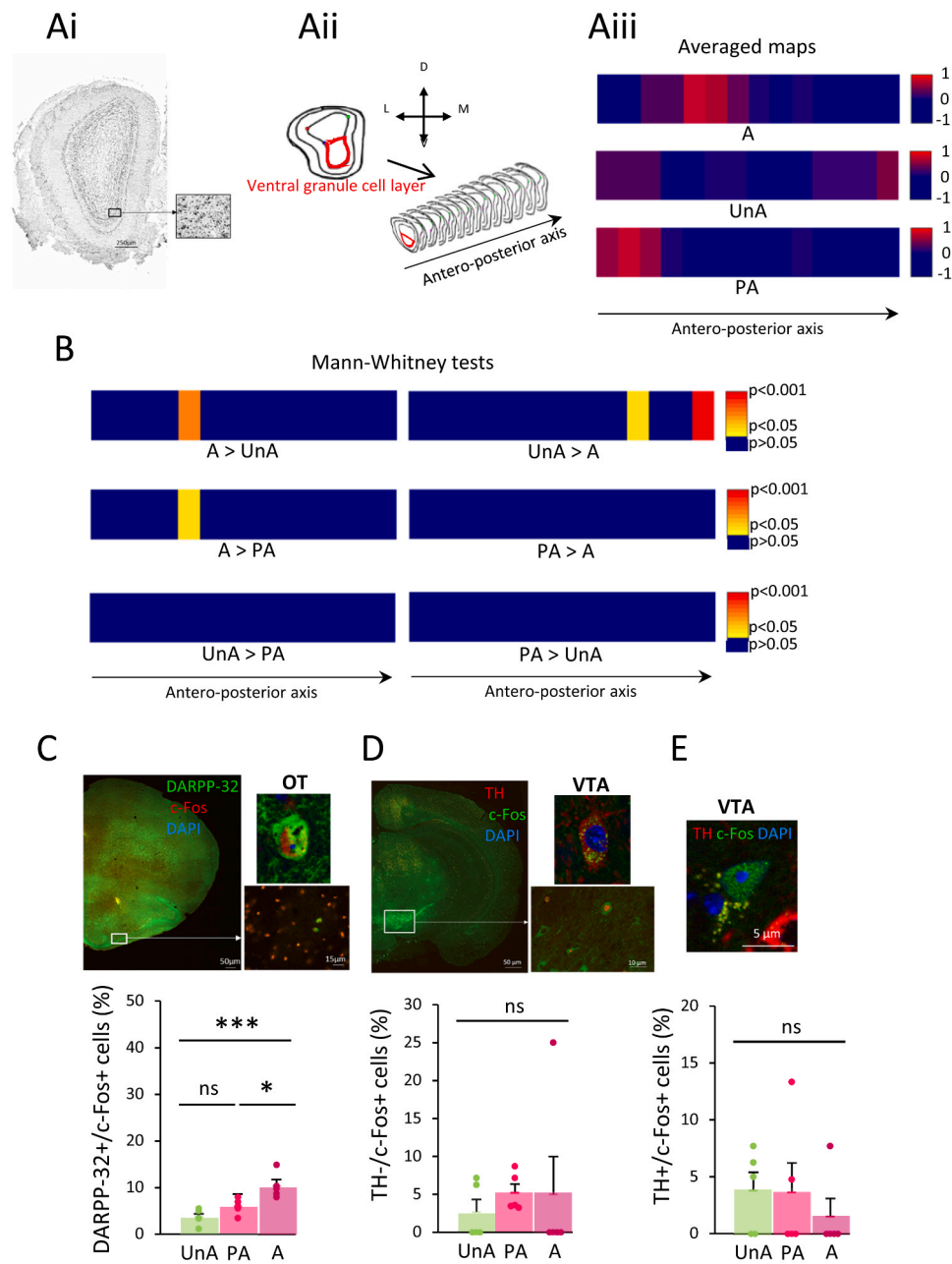


Fig. 2. Neural activity in the pOB, OT and VTA depends on the attractive power of odors. **A.** C-Fos positive-cell mapping. **(Ai).** Photo of c-Fos labelling in the OB. **(Aii).** Delineation of the ventral granule cell layer of in the OB. **(Aiii).** Averaged maps of z-scored c-Fos density (cells/mm²) in the OB ventral granule cell layer represented along the antero-posterior axis in response to attractive odors (A, n=5), unattractive (UnA, n=5) and previously attractive (PA, n=5). **B.** Mann-Whitney tests for between-group comparisons of the maps. Significance cutoff was set at p<0.05. **C.** Up. Representative image of the OT on a section (left) and double labeled DARPP-32+/c-Fos+ cell in the OT (right). Down. The percentage of DARPP-32+/c-Fos+ cells was higher in response to A compared to PA and UnA odors. There was no difference between PA and UnA groups (n=5/group). **D.** Up. Representative image of the VTA on a section (left) and double labeled TH+/c-Fos+ cell in the VTA (right). Down. There was no difference in the percentage of TH+ cells expressing c-Fos in the VTA among experimental groups. **E.** Up. Representative image of TH- cell expressing c-Fos in the VTA (n=5/group). Down. There was no difference in the percentage of TH-/c-Fos+ cells in the VTA among experimental groups (n=5/group). For all graphs, bars represent means of individual data points \pm sem. *p<0.05 ***p<0.001.

3.4. The olfactory bulb remains a rewarding site for intracranial self-stimulation in older mice

We previously showed that the granule cell layer of the ventro-posterior OB is a site for intracranial self-stimulation in young adult mice (Midroit, Chalénçon et al. 2021), revealing the capacity of this sensory area to recruit the reward circuit to drive motivated behaviors. To evaluate the functional integrity of the connection between the OB and the reward system, we investigated here whether optogenetic stimulation in the ventro-posterior OB would retain the ability to

support operant conditioning in older mice. To this aim, a bilateral injection of Lenti-hSyn-eNpHR3.0-EYFP virus was performed in the ventro-posterior granule cell layer in 22-month old mice (NpHR group) in order to reduce granule interneuron activity and leading to a disinhibition of OB relay cells (mitral/tufted cells) (Kermen, Midroit et al. 2016). In other words, self-stimulation is applied to the ventro-posterior granule cell layer in order to inhibit the inhibitory granule cells that are preferentially activated in this area by unattractive odors (Kermen, Midroit et al. 2016). A control group was injected with a control Lenti-hSyn-EYFP virus at the same coordinates (Ctrl group). During

behavioral testing, bilateral light stimulation was automatically triggered by mice nose poking within 1 cm around the hole of a one-hole-board apparatus (light-triggering zone), and lasted as long as the nose poke (Fig. 3A). Total nose pokes duration of NpHR mice increased across days of training (Friedman trial effect $F_{(4,10)}=28.45$ $p<0.001$, Wilcoxon Light1 vs Light5 $p<0.001$; Fig. 3B), revealing that older mice learned to nose poke to receive optogenetic stimulation in the ventro-posterior OB. During the extinction phase, when the light was no longer available, nose pokes duration quickly decreased (Friedman trial effect $F_{(4,10)}=27.97$ $p<0.001$, Wilcoxon Light5 vs Extinc5 $p<0.001$). On the last trial, when the light was available again, nose pokes duration increased again (Wilcoxon Extinc5 vs Light1' $p<0.001$), revealing a reinstatement of self-stimulation. This learning/extinction/reinstatement sequence confirmed that self-stimulation behavior is timely dependent on optogenetic inhibition of granule cells and that optogenetic stimulation serves as reinforcer in the operant learning in older mice. In Ctrl mice, a modulation of nose pokes duration was observed during the initial sessions of light stimulation (Friedman trial effect $F_{(4,$

$10)=10.109$ $p=0.039$; Fig. 3B), but no significant change was observed between Light 1 and Light 5 (Wilcoxon Light1 vs Light5 $p=0.929$). The investigation time remained stable during extinction (Friedman trial effect $F_{(4,10)}=0.218$ $p=0.994$, Wilcoxon Light5 vs Extinc5 $p=0.155$) and reinstatement (Wilcoxon Extinc5 vs Light1' $p=0.131$) phases. Interestingly, during the first trial, nose pokes duration in NpHR mice was lower than in Ctrl mice suggesting a neophobic response induced by the optogenetic manipulation (Bonferroni corrected One-Tailed Mann-Whitney, Light1 $p=0.039$). It is worth noting that nose pokes duration differed between NpHR and Ctrl mice from the 4th trial of training (Bonferroni corrected One-Tailed Mann-Whitney, Light2 $p=0.812$, Light3 $p=0.081$, Light4 $p=0.010$, Light5 $p=0.008$, Fig. 3B). This time lag was no longer observed during the extinction phase (Bonferroni corrected One-Tailed Mann-Whitney; Extinc1 $p=1$, Extinc2 $p=1$, Extinc3 $p=1$, Extinc4 $p=1$, Extinc5 $p=0.315$) but reappears during re-instatement (Light1' $p=0.030$).

To verify the posterior location of viral injection and of the effect of light on neuronal activity, we quantified the density of EYFP⁺ cells and

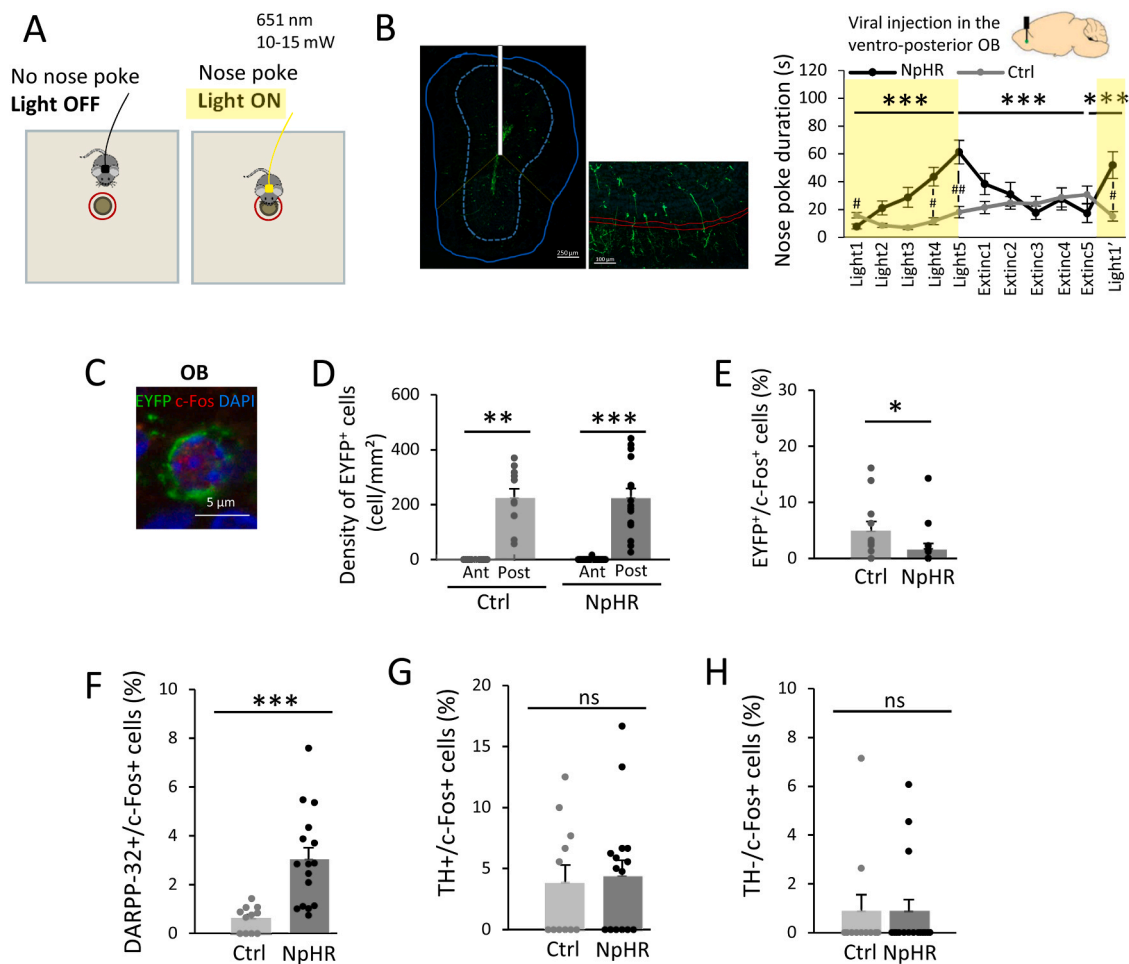


Fig. 3. The pOB remains a site for optogenetic self-stimulation in old mice. **A.** Experimental setup. **B. Left.** Image of EYFP expression in the OB. Blue line: OB boundary; dotted blue line: OB granule cell layer boundary, red line: mitral cell layer. **Right. Up.** Injection of NpHR-EYFP (NpHR, $n=16$) or control virus (Ctrl, $n=11$) in the ventro-posterior granule cell layer of the OB. **Down.** NpHR mice learned to nose poke to trigger light stimulation. This learning was subjected to extinction when light was no more available. On the last trial, light was available again and nose pokes started again. In Ctrl, nose pokes significantly decreased during the light stimulation phase, but no change was observed between Light1 and Light5. No difference was observed during extinction phase or when the light was available again. The difference between NpHR and Ctrl mice was observed from the 4th trial and was no longer observed during the extinction phase. Values represent means \pm sem. * stands for within-group differences and # for between-group differences. **C.** Representative image of double labelled EYFP⁺/c-Fos⁺ cell in the OB. **D.** Density of EYFP⁺ neurons is higher in ventro-posterior OB (the injection site) compared to ventro-anterior OB for both groups (NpHR, $n=16$; Ctrl, $n=11$). **E.** A lower percentage of EYFP⁺ cells expressing c-Fos was observed in ventro-posterior OB of NpHR compared to Ctrl mice NpHR, $n=16$; Ctrl, $n=11$. **F.** The percentage of DARPP-32⁺/c-Fos⁺ cells was higher in NpHR than in Ctrl mice NpHR, $n=16$; Ctrl, $n=11$. **G.** No difference in TH⁺/c-Fos⁺ cells was observed between NpHR and Ctrl groups NpHR, $n=16$; Ctrl, $n=11$. **H.** No difference in TH⁻/c-Fos⁺ cells was observed between NpHR and Ctrl mice. For D-H, bars represent means of individual data points \pm sem. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; #: $p<0.05$; ##: $p<0.01$.

analyzed the percentage of EYFP⁺ cells expressing c-Fos in the ventral granule cell layer of the OB. We found a higher density of EYFP⁺ cells in the ventro-posterior granule cell layer compared to the ventro-anterior part in both NpHR and Ctrl groups (Bonferroni corrected Two-Tailed Wilcoxon, NpHR $p < 0.001$ Ctrl $p = 0.007$; Fig. 3C-D) suggesting similar injection success. The percentage of EYFP⁺/c-Fos⁺ cells was lower in NpHR than in Ctrl mice (Two-Tailed Mann-Whitney, $p = 0.032$; Fig. 3E), indicating that the optogenetic manipulation efficiently induced an inhibition of ventro-posterior OB granule cells.

We then investigated the effect of self-stimulation behavior on neural activity in the OT and VTA. We found a higher percentage of activated relay cells double labelled by DARPP-32 and c-Fos in NpHR compared to Ctrl mice in the OT (Two-Tailed Mann-Whitney, $p < 0.001$; Fig. 3F). Remarkably, no difference between NpHR and Ctrl groups was observed for activated dopaminergic TH⁺/c-Fos⁺ cells (Two-Tailed Mann-Whitney, $p = 0.904$; Fig. 3G) and for the non-dopaminergic activated TH⁺/c-Fos⁺ cells (Two-Tailed Mann-Whitney, $p = 1$; Fig. 3H) in the VTA. These data indicated that while ventro-posterior OB self-stimulation is behaviorally rewarding, it led to no enhancement of the activity of the VTA in contrast to the OT suggesting less efficient recruitment of the reward system in older mice.

These results indicate that the optogenetic self-stimulation of the OB was reinforcing even without modulation of VTA activity.

4. Discussion

Age-related alteration of odor hedonics (Wang et al. 2005; Markovic et al. 2007; Joussain et al. 2013; Vieillard et al. 2020) is a major issue for the quality of life in human elderly (Joussain et al. 2017). While modifications of olfactory perception such as changes in odor detection (Bodyak and Slotnick, 1999; Kraemer and Apfelbach, 2004; Patel and Larson, 2009), fine but not gross discrimination (Moreno et al. 2014; Yoder et al. 2017; Rey et al. 2012) or learning and memory (Moreno et al. 2014; Dardou, Datiche, and Cattarelli, 2008; Kraemer and Apfelbach, 2004; LaSarge et al. 2007) have been already shown during aging in rodents, the present study revealed that as in humans (Joussain et al. 2013), an hedonic dysfunction specific to pleasant odorants is observed in older mice leading to a depreciation of these odorants. Indeed, we found that some odorants lost their attractive power while other remained attractive in aged mice. The behavioral changes we observed were ascribed to altered hedonic perception rather than to threshold or discrimination deficits. Indeed, we provided evidence that with the supraliminal odorant concentration we used, mice well detected the odorants. In addition, it appears that this age-dependent hedonic quality does not preclude discrimination between dissimilar odorants (Rey et al. 2012; Moreno et al. 2014).

Our results offer a good model to look for neural mechanisms underlying odor hedonic quality during aging and more specifically investigate the distinct pathways processing positive and negative odor hedonics.

The impact of aging at different levels of the olfactory circuit could explain changes in odor hedonics. A study in humans has shown that the spatial distribution of neural activity in the olfactory epithelium at least partly reflects olfactory pleasantness (Lapid et al. 2011). Alteration of olfactory epithelium integrity (Doty and Kamath, 2014; Naessen, 1971; Nakashima, Kimmelman, and Snow, 1984; Morrison and Costanzo, 1990; Paik et al. 1992), number of olfactory sensory neurons (Conley et al. 2003; Weiler and Farbman, 1997) and change in expression of some olfactory receptor genes have been observed with aging in humans (Cavallin et al. 2010; Khan, Vaes, and Mombaerts, 2013). In addition, some age-related modifications were observed also in the OB and secondary olfactory structures in term of volume in rodent model (Hinds and McNelly, 1981; Rey et al. 2012; Moreno et al. 2014) and human (Bhatnagar et al. 1987; Yousem et al. 1998; Sama-ul-Haq, Tahir, and Lone, 2008; Segura et al. 2013), synaptic and cellular organization (Richard, Taylor, and Greer, 2010; Gocel and Larson, 2013; Adjei et al.

2013) and odor-evoked activity (Wang et al. 2005; Suzuki et al. 2001). Interestingly, the age-related changes in hedonic appreciation has been correlated to EEG modification in beta-band oscillations in humans (Joussain et al. 2013). However, it remains unclear from these previous studies which neural alterations could be relevant to hedonic coding versus other dimension of olfactory perception such as thresholds of discrimination and importantly why the aged-related alteration in hedonics would be specific to some pleasant odorants. To shed light on this question, we analyzed odor-evoked activity in the OB, OT and VTA, a relevant pathway involved in odor-guided behavior (Midroit, Chalénçon et al. 2021).

Our previous study revealed that the ventral granule cell layer of the OB carries a neural signature of odor hedonics, with a higher activity in the ventro-anterior OB in response to pleasant odorants and in the ventro-posterior OB to unpleasant odorants (Kermen, Midroit et al. 2016). Moreover, optogenetic manipulation of these hot spots changed hedonic perception (Kermen, Midroit et al. 2016). Here, we confirmed the significance of these bulbar hot spots using aging as an ecological model of modifications of perception and of OB activity maps. Indeed, we confirmed in older mice an increase in neural activity in the ventro-anterior OB in response to an attractive odorant compared to unattractive odorants and in the ventro-posterior OB in response to unattractive compared to attractive odorant. Interestingly, when some odorants lose their attractive power, the increased neural activity in the ventro-anterior OB is no longer observed, further supporting the functional value of this pattern. The mitral cells of the OB, project onto the OT (Scott, McBride, and Schneider, 1980; Schwob and Price, 1984). Accordingly, in aged mice, we found an activation of the OT medium spiny neurons in response to attractive odorants, but not in response to odorants that have lost their attractive power or to unattractive ones. As a part of the ventral striatum receiving direct connections from the OB, the OT is considered as an open gate for olfactory information processing by the reward system (Wesson and Wilson, 2011). In addition, the OT is closely connected to the VTA (Ikemoto, 2007) and in young adult mice, pleasant odorants induced a higher activity of DAergic VTA neurons compared to unpleasant ones (Midroit, Chalénçon et al. 2021). Notably, this difference was not retrieved in older mice. In addition, since VTA glutamatergic and GABA neurons were involved in motivated behaviors (Wang et al., 2005; Morales and Margolis 2017), we also investigated the activity of non DAergic VTA neurons. No difference in activity depending on the hedonic value of the odorants was observed for non DAergic VTA neurons. These data suggest that the combined OB-OT activity is sufficient to determine the appreciation of pleasant odorants in older mice and that the lack of modulation of odor-evoked activity in the VTA may not reflect the lack of odorant positive appreciation.

Our data further raise the question of why certain odors maintain their attractiveness with aging while others do not. Our observation that LIM remained attractive in 22-month-old mice suggests that some odorants maintain their attractiveness and that neural circuits underlying these odorants are preserved. This could be due to higher ability of LIM to stimulate olfactory receptors or/and more remaining receptors able to bind LIM since individual patterns of aging related changes have been described for different receptors (no change, increase or decrease of expression; Khan, Vaes, and Mombaerts, 2013). We did not record overall higher activity in the OB in response to LIM compared to the previously attractive odorants to substantiate this hypothesis but more specific recording of individual receptor's activity would be necessary to conclude. In addition, increased activity in the OT is observed in response to attractive odorants but not unattractive or previously attractive ones. This suggests that some level of activation of the OT is required for perceived attraction to odorants. While the detailed central projection of individual mitral cells is currently unknown, it is possible that it differs depending on the receptors they activate which could contribute to this differential aging of odor hedonic perception. Furthermore, as the DAergic system is aging, an additional,

nonexclusive possibility is that the activation of the olfactory pathway by a given odorant becomes ineffective in recruiting the reward system (Collier, Kanaan, and Kordower, 2017). Still ongoing aging process that would have spared LIM perceptive properties cannot be fully excluded, even though 22-month-old is an age very close to the end point of laboratory mice life span.

Finally, from an ecological perspective, we could hypothesize that odor familiarity could play a role. The repeated exposition to an odor could strengthen its neuronal processing thanks to plasticity mechanisms but how this could apply to laboratory animals is unclear.

The results of the optogenetic experiment also support the theory that combined OB-OT activity is sufficient to drive motivated behavior in older mice, while VTA activity is impaired. Indeed, the OB has been recently described as a core component of the reward system, driving motivated behavior (Midroit, Chalençon et al. 2021). Young adult mice are able to learn to self-stimulate their posterior OB. Here, we found that aged mice were also able to perform such OB self-stimulation. This conditioning induced an increase of OT activity compared to controls, indicating that the ventro-posterior OB was still able to recruit the reward system to drive motivated behavior. Remarkably, in aged mice the self-stimulation behavior is not accompanied by an increase of neural activity in the dopaminergic neurons of the VTA, revealing an only partial recruitment of the reward system. The lack of increased activation of the VTA in response to pleasant odorants or after optogenetic self-stimulation of the OB could reflect the overall degradation of the DAergic system that arises during aging. For instance, aging is accompanied by a reduction in the number of VTA neurons (Irwin et al. 1994; Barili et al. 1998; Norrara et al. 2018; Collier, Kanaan, and Kordower, 2017) or an impairment of DA synthesis (Araki et al. 1997; Irwin et al. 1994; Schuligoi et al. 1993; Barili et al. 1998; Watanabe, 1987; Norrara et al. 2018; Collier, Kanaan, and Kordower, 2017). Such alterations could lead to deficits in reward-related activity in the VTA. Nevertheless, self-stimulation remains effective in aged animals suggesting that the residual activity may be sufficient to drive attraction. In addition, we revealed that the OT showed increased activation during self-stimulation. As part of the ventral striatum, the OT is strongly inter-connected with the nucleus accumbens and sends projections to other structures of the reward system (e.g. orbitofrontal cortex et ventral pallidum) (Ikemoto, 2007; Wesson and Wilson, 2011; Xiong and Wesson, 2016). The activity of the OT could thus contribute to drive attraction as a gateway for soliciting the extended neural network that underlies motivation. In support of this view, the present study is the first to provide evidence that self-stimulation behavior can be achieved in aged animals despite the impairment of the VTA activation. It is unknown if this observation reflects a general pattern of the reward system activation in aging or if it is specific to OB self-stimulation. In any case, this observation and the finding that some odorants may keep their attractivity suggest that the olfactory pathway could be used in aging as an entry to stimulate and maintain the reward system.

5. Conclusions

The present study highlights the key role of OB-OT network in olfactory perception and odor-motivated behaviors. Aged mice provide an ecological model showing that attraction to pleasant odorants is impaired when activity in these structures is altered. In addition, it revealed new functional properties of the aged brain in which the OB-OT pathway is activated for odorants that are perceived as attractive and despite no increased activity in the VTA. This opens new avenues on the use of odorant training in the elderly to elicit motivation even when the DAergic system is altered as it is observed in normal and/or neurodegenerative aging (Araki et al. 1997; Irwin et al. 1994; Schuligoi et al. 1993; Barili et al. 1998; Watanabe, 1987; Norrara et al. 2018; Collier, Kanaan, and Kordower, 2017).

CRediT authorship contribution statement

Didier Anne: Writing – review & editing, Writing – original draft, Conceptualization. **Mandaïron Nathalie:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Forest Jérémy:** Investigation. **Richard Marion:** Writing – original draft. **Thevenet Marc:** Methodology, Formal analysis. **Breton Marine:** Investigation. **Athanassi Anna:** Formal analysis, Data curation. **Chalençon Laura:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Midroit Maëllie:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors have no actual or potential conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.neurobiolaging.2024.02.003](https://doi.org/10.1016/j.neurobiolaging.2024.02.003).

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-ANNEXE 3-

Combining a Breath-Synchronized Olfactometer with Brain Simulation to Study the Impact of Odors on Corticospinal Excitability and Effective Connectivity

(publié)

Combining a Breath-Synchronized Olfactometer with Brain Simulation to Study the Impact of Odors on Corticospinal Excitability and Effective Connectivity

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Abstract

It is widely accepted that olfactory stimulation elicits motor behaviors, such as approaching pleasant odorants and avoiding unpleasant ones, in animals and humans. Recently, studies using electroencephalography and transcranial magnetic stimulation (TMS) have demonstrated a strong link between processing in the olfactory system and activity in the motor cortex in humans. To better understand the interactions between the olfactory and the motor systems and to overcome some of the previous methodological limitations, we developed a new method combining an olfactometer that synchronizes the random order presentation of odorants with different hedonic values and the TMS (single- and dual-coil) triggering with nasal breathing phases. This method allows probing the modulations of corticospinal excitability and effective ipsilateral connectivity between the dorsolateral prefrontal cortex and the primary motor cortex that could occur during pleasant and unpleasant odor perception. The application of this method will allow for objectively discriminating the pleasantness value of an odorant in a given participant, indicating the biological impact of the odorant on brain effective connectivity and excitability. In addition, this could pave the way for clinical investigations in patients with neurological or neuropsychiatric disorders who may exhibit odor hedonic alterations and maladaptive approach-avoidance behaviors.

Introduction

It is widely accepted that olfactory stimulation elicits automatic reactions and motor behaviors. For example, in humans, the existence of an avoidance motor response (leaning away from the odor source) occurring 500 ms after

negative odor onset has been recently demonstrated¹. By recording freely moving human participants exploring odors emanating from flasks, Chalençon et al. (2022) showed that motor behaviors (i.e., speed of approach to the nose and withdrawal of the flask containing the odorant) are closely linked to odor hedonics². Moreover, a close link between processing in the olfactory system and activity in the motor cortex has been recently demonstrated in humans by using electroencephalography¹. Specifically, approximately 350 ms after the onset of negative odors, a specific mu rhythm desynchronization, known to reflect action preparation processes, was observed over and within the primary motor cortex (M1), shortly followed by a behavioral backward movement¹. Strengthening the idea of a relationship between the olfactory and motor systems, another recent study showed that exposure to a pleasant odorant increased corticospinal excitability compared to a no-odor condition³. In this study, single-pulse transcranial magnetic stimulation (spTMS) was applied to M1 to evoke a motor-evoked potential (MEP) in a target hand muscle, recorded peripherally with electromyography (EMG) during odor perception. Exposure to the pleasant odorant was provided passively by paper strips sodden with pure bergamot essential oil and placed on a metal holder under the nose³. In this context, it remains unclear whether the facilitation of the corticospinal excitability is due to the pleasant odorant stimulation or to unspecific behavioral effects such as sniffing and teeth clenching^{4,5}. Furthermore, it is still unknown how an unpleasant odorant modulates M1 excitability probed by TMS.

In summary, this highlights the need to develop a method that offers the following advantages over existing techniques used in previous studies^{3,6}: (1) randomizing the presentation of different odor conditions (pleasant/unpleasant/no-odor) within the same experimental phase, (2)

precisely synchronizing odorant presentation and TMS timing according to the human nasal breathing phases (inspiration and expiration) when studying the motor system.

TMS can also be used as a tool to investigate cortico-cortical interactions, also called effective connectivity, between multiple cortical areas and M1 with a high temporal resolution^{7,8,9,10,11,12}. Here, we use a dual-site TMS (dsTMS) paradigm, in which a first-conditioning stimulation (CS) activates a target cortical area, and a second-test stimulation (TS) is applied over M1 using another coil to evoke an MEP. The effect of the CS is evaluated by normalizing the amplitude of the conditioned MEP (dsTMS condition) to the amplitude of the unconditioned MEP (spTMS condition)¹³. Then, negative ratio values indicate suppressive cortico-cortical interactions, while positive ratio values indicate facilitatory cortico-cortical interactions between the two stimulated areas. The dsTMS paradigm thus provides a unique opportunity to identify the nature (i.e., facilitatory or suppressive), the strength, and the modulations of the effective connectivity between the preactivated area and M1. Importantly, cortico-cortical interactions reflect a complex balance of facilitation and suppression that may be modulated in different timing and mental states or tasks^{7,14}.

To our knowledge, the relatively new dsTMS paradigm has never been used to investigate cortico-cortical interactions during odor perception with different hedonic values. However, neuroimaging studies have shown that exposure to pleasant and unpleasant odorants induces connectivity changes in areas involved in emotion, decision-making, and action control, including the supplementary motor area, the anterior cingulate cortex, and the dorsolateral prefrontal cortex (DLPFC)^{15,16}. Indeed, the DLPFC is a key node mediating emotional control, sensory processing, and

higher-level aspects of motor control, such as preparatory processes^{17,18,19}. In addition, both human and animal studies have provided evidence that the DLPFC has diverse neuronal projections to M1^{17,18,20,21,22}. Depending on the context, these DLPFC projections can either facilitate or inhibit M1 activity^{7,19,20}. Thus, it seems possible that the effective connectivity between DLPFC and M1 is modulated during odor presentation and that pleasant and unpleasant odors recruit separated cortical networks, leading to a differential effect on DLPFC-M1 connectivity.

Here, we propose a new method suitable for the methodologically rigorous study of the modulations of corticospinal excitability and effective connectivity that might occur during the perception of pleasant and unpleasant odors, all delivered in synchrony with human nasal breathing.

Protocol

All experimental procedures described in the following sections have been approved by an Ethics Committee (CPP Ile de France VII, Paris, France, protocol number 2022-A01967-36) in accordance with the Declaration of Helsinki. All participants provided written informed consent before study enrollment.

1. Participant recruitment

1. Inclusion/exclusion criteria.
 1. Include adult (> 18 years) participants. Screen all participants for any contraindications to TMS according to international expert guidelines²³.
 2. Exclude participants with implanted medical devices (e.g., cochlear implant, cardiac pacemaker, etc.), a personal or family history of seizure, headache, brain trauma, and neuroactive medication. Exclude

participants considered "anosmic" according to the European Test of Olfactory Capabilities²⁴.

2. Handedness: Check for right-handedness as assessed by the Edinburgh Handedness Inventory questionnaire²⁵.
NOTE. It is highly recommended to recruit only right-handed participants in studies assessing corticospinal excitability and effective connectivity in the motor system^{26,27}.
3. Information and informed consent: Give all participants basic information about the study objectives, procedures, and risks approved by the Ethics Committee and ask them to sign written informed consent.

2. Experimental procedure

1. Patient installation: Ask the participant to sit on a comfortable chair (dental chair type) with both hands relaxed and pronated. Position the participant's head on a chin rest to minimize head movement during stimulation.
2. Electromyography recordings
 1. Prepare the participant's skin before electrode application using an exfoliant scrub to lightly abrade the areas and clean the areas using alcohol pads where electrodes will be applied.
 2. Apply two silver/silver chloride disposable recording electrodes with a belly-tendon montage of the first dorsal interosseous (FDI) muscle. Add the ground electrode to the styloid process of the ulna (**Figure 1**).
 3. Connect the electrodes to the amplifier with cables and the data acquisition system.

4. Record the EMG signal using an analog-to-digital (AD) conversion system. Amplify and filter EMG signals (gain = 1000) using a bandwidth frequency between 10 Hz and 1 kHz. Digitize at a sampling rate of 2,000 Hz and store each EMG file for offline analysis.
 5. Check the quality of the signal displayed on the computer screen connected to the data acquisition system.
3. TMS coil_{M1} position.
 1. Connect this coil to the A stimulator (**Figure 1**).
 2. Place a tight-fitting cap over the participant's head. Use a tape measure to perform nasion-inion, tragus-tragus, and head-circumference measurements based on standard cranial landmarks. Identify and mark with a pen the scalp vertex at the intersection of the mid-sagittal (nasion-inion) and interaural (tragus-tragus) lines²⁸.
 3. Place tangentially to the scalp the first small figure-of-eight coil (inner diameter: 40 mm) over the presumed hand area of the left M1 (coil_{M1}), which is 5 cm lateral from the vertex, with the handle pointing backward and laterally at a 45° angle to the midsagittal line, resulting in a posterior-anterior current flow (monophasic current waveform). This orientation corresponds to a maximum induced current flowing within M1 within M1²⁹.
 4. Ensure that the placement of the coil_{M1} is optimal, in accordance with the most recent international recommendations³⁰. Start by delivering a few single pulses at 30% of the maximum stimulator output (%MSO) and check that the stimulation produces an MEP as recorded by the EMG system and displayed on the computer screen connected to the data acquisition system.
 1. If there are no visible responses, gradually increase the stimulation intensity (5 %MSO increments) until MEPs are observed. Then, test four spots around the first site by delivering multiple pulses. Determine the mean peak-to-peak MEP amplitude for each site.
 2. Select the location where the average peak-to-peak MEP amplitude is the highest. This is the so-called hotspot location for the participant³⁰. Mark the coil_{M1} location on the cap to ensure proper coil placement throughout the experiment.
 4. Resting motor threshold (rMT) and TMS intensities
 1. Determine the resting motor threshold (rMT) defined as the TMS intensity that produces a 50% probability of eliciting an MEP^{23,30}.
 1. Use the available online freeware (TMS Motor Threshold Assessment Tool, MTAT 2.1), which is based on a maximum-likelihood parameter estimation using a sequential testing strategy²⁹. The stimulation sequence always starts with the intensity set at 37 %MSO.
 2. Let one experimenter hold the coil_{M1} while another indicates whether the MEP amplitude is > 0.05 mV. The predictive algorithm then determines the next stimulation intensity to be delivered and is stopped after 20 stimulations, which provides sufficient accuracy for the rMT estimation according to previous studies³¹⁻³⁴.

2. Set the %MSO for the conditioning and the test pulse stimulation. Use the previously determined rMT value of the participant.

NOTE: Here, the intensity for the first conditioning stimulation (coil_{DLPFC}) was set to 110% of the rMT^{19,20}. The intensity of the test stimulation (coil_{M1}) was set at 120% of the rMT, an intensity that differs slightly from previous studies that used a TS intensity that evoked a MEP of ~1 mV in all participants^{19,20}. This fixed peak-to-peak intensity occurs at very different points on the input-output recruitment curves due to the high inter-subject variability in motor output³⁵. Therefore, the stimulation intensity could be optimized using 120% RMT intensity across individuals.

5. TMS coil_{DLPFC} positioning

1. Connect this coil to the B stimulator (**Figure 1**).
2. Use the recently updated scalp heuristic to locate the region of the scalp corresponding to the left DLPFC^{36,37} to estimate the position of the second small figure-of-eight coil (internal diameter: 40 mm) over the DLPFC (coil_{DLPFC}). Download the online Excel Spreadsheet Calculation Tool³⁶ and enter the nasion-inion and tragus-tragus distances and the head circumference in centimeters as inputs. Report the X_{LA} and the Y_{LA} distances directly on the participant's head.
3. Place tangentially to the scalp the coil_{DLPFC} over the presumed left DLPFC location, with the handle pointing downward and laterally at a -45° angle to the mid-sagittal line. Mark the coil_{DLPFC} placement on

the cap to ensure proper coil placement throughout the experiment.

NOTE: This scalp-based targeting method for both coil_{M1} and coil_{DLPFC} locations is not optimal. In fact, it is known to be less accurate than the neuronavigation method used to target the brain areas of interest based on individual T1 anatomical magnetic resonance imaging (MRI)³⁸.

6. Delay between the conditioning and test pulses: Set this delay to 10 ms on the pulse generator device.

NOTE: Here, the delay is fixed at 10 ms based on previous studies showing an inhibitory influence from the left DLPFC to the left M1 at this interval^{19,20}. This inhibitory effect observed at 10 ms is likely due to the activation of the basal ganglia via the DLPFC projections to the pre-SMA, thereby exerting an indirect influence on M1³⁹. The delay can be adjusted in the code according to the user's needs. For example, a longer interstimulation interval (i.e., 25 ms) could be used to investigate polysynaptic indirect cortico-subcortical-cortical circuits connecting DLPFC to M1¹⁹. Furthermore, differential facilitatory/inhibitory influences have been demonstrated using dual-site ppTMS between multiple cortical areas, with intervals ranging from 1 ms to 150 ms^{40,41}. Thus, the fact that the interval can be adjusted opens the way to a wide range of possibilities for future research studies.

7. Olfactometer settings

1. Select odorants with pleasant and unpleasant hedonic values. Dilute in advance the odorants individually in mineral oil to create iso-intense perception.

NOTE: Here, the selection and concentration of odorants (i.e., isoamyl acetate and butyric acid

diluted to 0.6% and 0.11% vol/vol concentrations, respectively) were based on previous studies by our group using the same olfactometer setup and odorants^{42,43}. A pilot study confirms that the positive and the negative odors did not differ in terms of intensity but were opposite in hedonic value. In the control condition (i.e., no odorant), only airflow is delivered to the participant.

2. Write the code to deliver the odorants. For each trial, indicate the total duration of the trial, the odorant to be delivered, the flow rate of the odorant controller (in milliliters per minute), the flow rate of the carrier air regulator (in milliliters per minute), and the flow rate of the suction regulator.

NOTE: The order of the odorant delivered can be randomized between positive, negative, and no-odor. Here, each trial has a duration of 12 s. The order of odor delivered was pseudo-randomized. In addition, based on a pilot experiment, the flow rate of the odor controller was set to 200 mL/min, the flow rate of the carrier air regulator at 500 mL/min, and the flow rate of the suction regulator at 100 mL/min.

3. Position the nasal cannula near the participant's nostrils to measure nasal breathing. Instruct the participant to breathe normally through the nose.
4. Turn on the portable air compressor, the olfactometer case, and the PC containing the software. Check all the cable connections (**Figure 1**).

NOTE: The olfactometer used in the current study has been described in detail in a previous publication⁴⁴ but has been modified here to allow TMS triggering with variable delays after inspiration

onset detection. Briefly, the device is composed of several modules, including 1) an air source and air treatment system coming from a portable air compressor, 2) a stimulation system including electronic and pneumatic devices, 3) a homemade mixing head coupled to a delivery system that allows the diffusion of odorants in the participant's nose, 4) a respiratory sensory system that triggers the olfactometer according to the nasal respiration measurement with a nasal cannula and 5) a software control system⁴⁴.

5. Calibration: Proceed to the calibration phase (about 20 s.), which allows for calibrating the participant's respiratory signal and adjusting the detection thresholds of the expiratory and inspiratory phases. In this software, the expiratory phase is positive, and the inspiratory phase is negative.
6. Odor hedonic and intensity ratings: Deliver the two odorants in a randomized order and ask participants to rate the hedonic value and intensity of each odorant on visual analog scales ranging from 1 "not at all pleasant" to 9 "extremely pleasant" and from 1 "not at all intense" to "extremely intense."

8. Combining olfactometer and TMS: Set the delay between the detection of the inspiration phase and the trigger for sending the TMS at 600 ms.

NOTE: The setting of the delay is important and has to be determined according to the literature and the needs of the user. In this protocol, the delay was set at 600 ms, which has been shown to be the maximum conscious perceptual representation of odors⁴⁵. For single-pulse TMS condition, this trigger immediately activates the A stimulator, and a pulse is delivered by the coil positioned

on the left M1 to evoke an unconditioned MEP. For dual-coil TMS condition, this trigger is sent to two different devices (via two coaxial cables connected by a T-connection): the first one immediately activates the B stimulator and a conditioning pulse is delivered by the coil

positioned on the left DLPFC; the second one is received by a pulse generator which makes it possible to induce a fixed delay before activating the A stimulator, thus delivering a test-stimulation through the coil positioned on the left M1 to evoke a conditioned MEP (**Figure 1**).

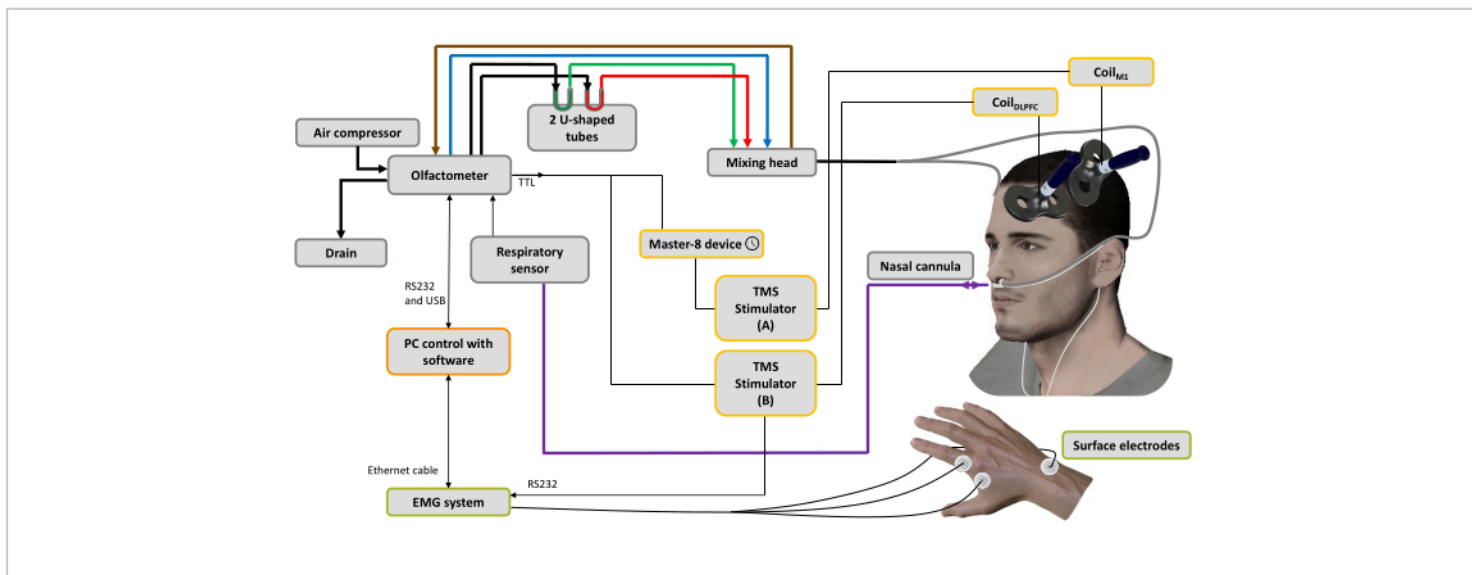


Figure 1: Experimental setup. The bold lines represent pneumatic connections. An air compressor is connected to the olfactometer to generate different air flows. A regulator controls the pressure, and the input air flow is directed to 3 channels (through 3 mass regulators): one for the air conveyor (blue line), one for the aspiration system (brown line) to clean and help control the stimulation time and the last one for the odorants⁴⁴. Two U-shaped tubes contain the odorants (green: pleasant; red: unpleasant) in which they are conditioned under pressure in the saturated steam state, ensuring an odorized air flow with stable intensity over time. The mixing head is used to mix the clean and odorized air streams. The airflow (odorized or pure) is delivered to the nostrils through two tubes (gray lines) attached to a nasal cannula, which is also used to record nasal breathing (purple line). Based on the respiratory signal, as soon as the inhalation phase is detected, for the spTMS condition a trigger is sent to a pulse generator device used to set a delay (here: 10 ms), then to a TMS stimulator A connected to Coil_{M1} applied over the left M1 hand muscle representation, while the TMS stimulator B is turned off. For the dsTMS condition, a trigger is immediately sent to the TMS stimulator B connected to the Coil_{DLPFC} applied over the left DLPFC, and the pulse generator device is used to set a delay (here: 10 ms) before triggering the TMS stimulator A connected to the Coil_{M1}. The respiratory signal and MEP amplitude acquired by the EMG system are recorded by software installed on a PC. [Please click here to view a larger version of this figure.](#)

3. Measurements

1. Run the custom-made coding script in the olfactometer software (see step 2.7.2) to deliver all combinations of spTMS and dsTMS with pleasant and unpleasant odors and no-odors occurring in a random order.

NOTE: Here, 20 trials were recorded for each condition (120 trials in total). The experiment was divided into 6 blocks of 20 trials each. The number of trials for each condition can be changed according to the user's needs.

4. Data analyses

1. For each participant, condition, and trial, extract the peak-to-peak MEP amplitude. This can be done using one of the open-source Toolboxes available online^{46,47}.
2. Normalize the data by calculating an MEP ratio expressing MEPs elicited by the test stimulation in dsTMS trials relative to MEPs elicited by the test stimulation in spTMS trials¹². Do this separately for each participant and for each odor condition (i.e., no-odor, positive odor, and negative odor). After this procedure,

interpret the results as follows: MEP ratios above 1 indicate a facilitatory influence of the DLPFC on M1, whereas MEP ratios below 1 indicate an inhibitory influence of the DLPFC on M1.

Representative Results

The representative data presented here reflect recordings from participants after completing the step-by-step protocol above to provide a preliminary insight into what we might expect.

Figure 2 shows an example of a representative participant's respiratory signals recorded with the olfactometer software. The expiratory and inspiratory phases are well detected when the thresholds are crossed. The odorant is triggered immediately after the expiration phase threshold and diffuses for 5 s. The TMS pulse is triggered with a delay (600 ms) after the inspiration phase threshold.

This result demonstrates that the method developed here can precisely synchronize odorant diffusion and TMS timing according to human nasal breathing phases.

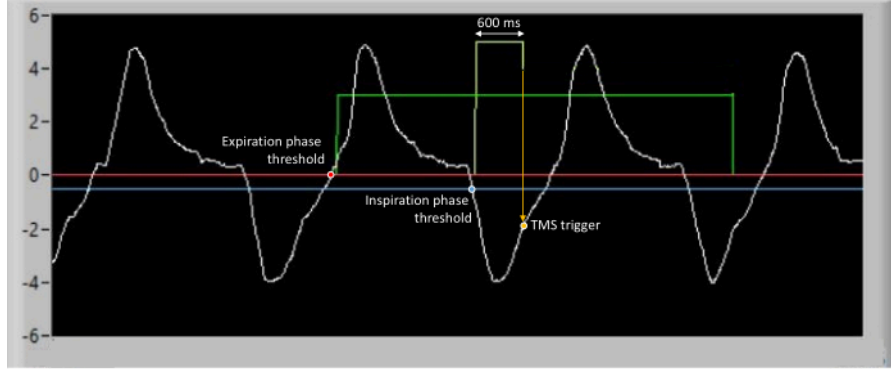


Figure 2: Example of raw data from respiratory recordings for a representative participant. The expiration phase is detected when a threshold (represented by the red line) is crossed. Inspiration phase is detected when a threshold (represented by the blue line) is crossed. The odorant is triggered immediately after the expiration phase threshold and diffuses for 5 s, as shown by the green line. The TMS pulse is triggered with a delay (600 ms) after the inspiration phase threshold. [Please click here to view a larger version of this figure.](#)

Figure 3 illustrates the results obtained from EMG data recordings from the right FDI muscle (MEP recordings), according to the conditions (spTMS and dsTMS) and the odor hedonic values (no-odorant, positive odorant and negative odorants) for a representative participant. The peak-to-peak amplitude of the MEPs evoked by spTMS (**Figure 3A**) and by dsTMS (**Figure 3B**) varied according to the hedonic value of the odorant. When the results are normalized (**Figure 3C**), all MEP ratios are below 1, indicating a suppressive effect

of the left DLPFC on the left M1. This result demonstrates that the method developed here allows the investigations of modulations of corticospinal excitability and effective connectivity that occur during pleasant and unpleasant odor perception, all delivered in a synchronized manner with human nasal breathing. These results are preliminary and deserve further investigation to conclude on the specific effects of odors hedonic values on corticospinal excitability and effective connectivity.

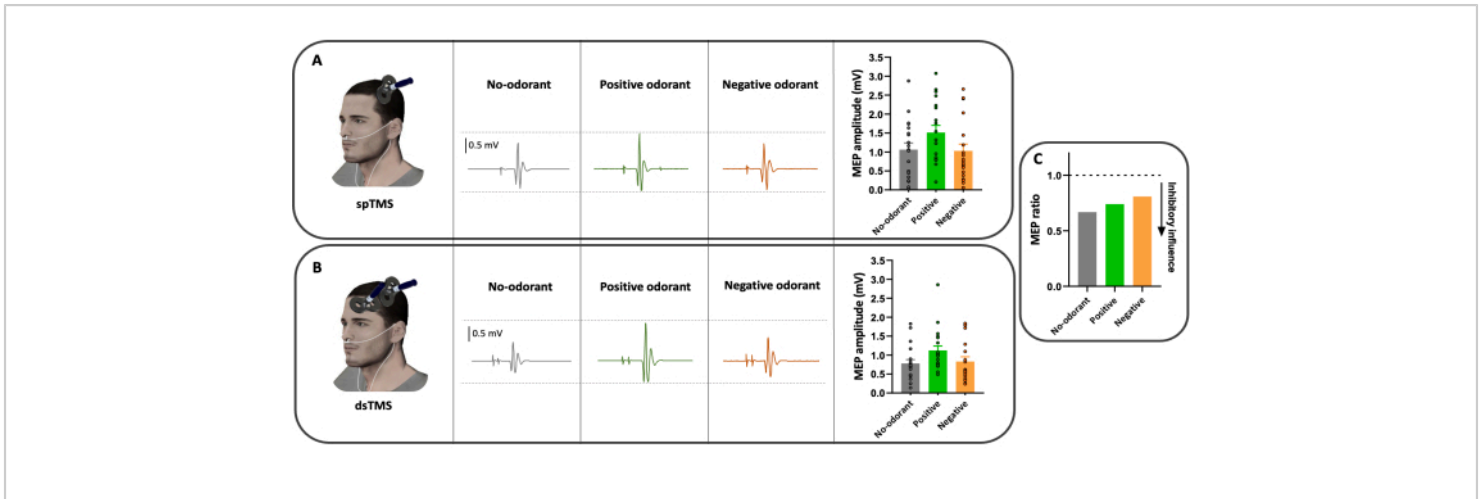


Figure 3: Example of typical raw recordings from the right FDI muscle of a participant. (A) spTMS condition with positive odorant (green), negative odorant (orange), and the no-odorant conditions (gray). **(B)** dsTMS condition, with positive odorant (green), negative odorant (orange) and the no-odorant conditions (gray). **(C)** MEP ratios obtained after the normalization procedure for a representative participant. The three MEP ratios are below 1, indicating an inhibitory influence of the DLPFC on M1. Raw MEP traces represent a single trial recording. Bar graphs show the mean, the standard deviation, and the individual MEP value of the 20 trials obtained in each condition. [Please click here to view a larger version of this figure.](#)

Discussion

The protocol above describes a novel method combining the use of a breath-synchronized olfactometer with single- and dual-coil TMS to investigate changes in corticospinal excitability and effective connectivity depending on the hedonic value of the odorants. This setup will allow for objectively discriminating the pleasantness value of an odorant in a given participant, indicating the biological impact of the odorant on brain effective connectivity and reactivity. The critical steps in this protocol involve both TMS parameters (placement, intensities) and olfactometer parameters (odorant selection, timing relative to respiratory phases).

This combination of spTMS and dsTMS with an olfactometer can be adapted in many ways, depending on the user's

needs, and has clear methodological advantages. As mentioned in the introduction, two methodological aspects seemed crucial for a more in-depth investigation of the mechanistic basis of the interactions between the olfactory and motor systems. The first was the possibility of presenting different odor conditions (pleasant/unpleasant/no odor) within the same experimental phase. This is now feasible because it is possible to specify on a trial-by-trial basis which odorant will be delivered to the subject at a constant intensity. This is a crucial point, as it allows us to eliminate the systematic intra-individual changes in MEPs amplitude within and between stimulus blocks observed in previous studies, even at relatively long interstimulus intervals^{48, 49}.

Indeed, the application of a TMS pulse to M1 allows the quantification of the observed changes in corticospinal

excitability with undeniable temporal accuracy. However, a very large number of factors can modulate corticospinal excitability, and these need to be controlled as much as possible. For example, the simple fact of voluntary inspiration or exhalation (a motor act) modifies the corticospinal excitability of non-respiratory finger muscles⁵⁰.

The second was the possibility to control and synchronize several factors with the respiratory phases. These include the precise duration and timing of odor diffusion to the participants and the timing of the TMS pulse. More importantly, these different parameters can be modified according to the user's needs, opening the way for future studies.

The method presented here opens the way for a wide range of future research and broader questions in the field of olfaction. First, no study has yet examined the temporal precision of the modulation of corticospinal excitability in response to an olfactory stimulus. Is this modulation very early (i.e., before the emergence of perceptual odor representations, estimated to be between 300 ms and 500 ms after odor onset⁴⁵) or later (i.e., when odor representations are extended to larger areas associated with emotional, semantic, and memory processing⁴⁵)? Is the timing of changes in corticospinal excitability the same depending on the hedonic value of the odor? Unpleasant odors, such as pain, often signal potential danger, elicit a faster response to quickly avoid or escape negative situations^{51,52}, and thus modulate corticospinal excitability earlier than positive odors. However, this remains speculative. By delivering the TMS pulse at different times after the onset of both positive and negative odors and comparing the changes in corticospinal excitability, the current protocol can address this question. Furthermore, although the focus of the present protocol was on the modulation of corticospinal excitability by targeting M1, the

TMS technique, due to its high temporal resolution, can also be used to investigate the causal brain-behavior relationships and the time course of other areas during olfactory processes, due to its high temporal resolution⁵³. Similarly, in the current protocol, we evaluated the effective connectivity between the DLPFC and M1 because there is evidence in the literature that modulations of this connectivity may occur during odor perception. However, other cortico-cortical or cortico-subcortical-cortical networks may be modulated during olfaction or motor control processes, and the connectivity within these networks can be easily assessed with this new method. The only change would then be the location of the coils toward the targeted cortical areas. For example, the orbitofrontal cortex has been shown to be involved in coding for odor hedonic value and odor perception⁵⁴, and a recent dual-site TMS study showed that this area has an inhibitory influence on M1 at rest¹². Probing changes in the effective connectivity between the orbitofrontal cortex and M1 during perception of positive and negative odors is an interesting avenue of study for a better understanding of the mechanisms behind the interactions between olfactory and motor systems.

In addition, this method proposes a new way to reliably assess odor hedonic perception in a non-verbal or conscious manner. This could pave the way for clinical investigations aimed at understanding abnormal interactions between processing in the olfactory and motor systems. For example, the current method could be used in patients with neuropsychiatric disorders such as major depressive disorder (MDD), which has been associated with alterations in olfactory function, including hedonic perception of odors and maladaptive approach and avoidance behaviors⁵⁵. Furthermore, as the left DLPFC has been shown to be hypoactive in MDD patients⁵⁶ and the DLPFC-M1 connectivity is modulated during approach-

avoidance behaviors¹⁹, the combination of TMS and an olfactometer may be a promising potential tool to elucidate neurophysiological indicators of dysfunctional connectivity between DLPFC and M1 in MDD patients. Neurophysiological findings can then be correlated with clinical symptomatology, such as the severity of depression or the olfactory anhedonia score, defined as the reduced ability to experience pleasure, found in patients with MDD⁵⁷. Finally, if abnormalities in effective connectivity are revealed in these patients using the method presented here and correlate with clinical symptoms, dual-site TMS could be used repeatedly to neuromodulate DLPFC-M1 connectivity and improve clinical symptoms, a protocol called paired-associative cortico-cortical stimulation^{58,59}.

Although the present method and results provide a proof of concept for future investigations into the neural mechanisms underlying the interactions between the olfactory and motor systems, some limitations and considerations must be mentioned. First, to increase the reliability and reproducibility of the measurements, the targeted brain areas should be precisely based on anatomical and functional areas (this is especially true for the DLPFC target). Second, as mentioned above and as demonstrated by E-field computational modeling, the scalp-based targeting method used to position the coils is suboptimal compared to MRI guidance⁶⁰. To maximize the accuracy and precision of TMS positioning, a neuronavigation system that co-registers the patient's head and structural magnetic resonance imaging (MRI) scan and provides real-time feedback on the coil position should be used³⁸. In addition, computational E-field dosimetry has been shown to provide more efficient and focused stimulation by determining the individual coil placement that maximizes E-field delivery to a specific brain target⁶¹. A third point to consider when interpreting the results

related to MEP amplitude. Indeed, MEP amplitude is known to reflect intrinsic different neural inputs to the corticospinal cells, including transcortical elements, and the activity of the spinal motoneuron pool^{62,63,64}. Therefore, the modulation of corticospinal excitability and effective connectivity observed during the exposure to a pleasant odor provides a partial picture of the more complex supraspinal and spinal networks that are likely to be involved in the modulation of the MEP amplitude. Results should be interpreted with caution.

Disclosures

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