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# Bases neurales de la valeur hédonique des odeurs et ses altérations au cours du vieillissement

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Laura Chalencon. Bases neurales de la valeur hédonique des odeurs et ses altérations au cours du vieillissement. Neurosciences. Université de Lyon, 2020. Français. NNT: 2020LYSE1321 . tel-03524858

**HAL Id: tel-03524858**

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N° d'ordre NNT : 2020LYSE1321

**THESE de DOCTORAT DE L'UNIVERSITE DE LYON**

opérée au sein de

**l'Université Claude Bernard Lyon 1**

**Ecole Doctorale Neurosciences et Cognition  
(ED 476 - NSCO)**

**Spécialité de doctorat : Neurosciences**

Soutenue publiquement le 17/12/2020, par :

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**Bases neurales de la valeur  
hédonique des odeurs et ses altérations  
au cours du vieillissement**

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# REMERCIEMENTS

Je tenais tout d'abord à remercier chaleureusement l'ensemble de mon jury de thèse qui a accepté d'évaluer mon travail de doctorat. Un grand merci au Dr. Philippe Faure, au Pr. Martin Giurfa, au Dr. Isabelle Caillé et au Pr. Anne Didier.

Je remercie également l'Université Claude Bernard Lyon 1 pour avoir financé mon doctorat.

Mes remerciements vont ensuite bien entendu à ma directrice de thèse, le Dr. Nathalie Mandairon. Nath, je ne saurais comment te remercier de m'avoir accompagné dans cette belle aventure que représente une thèse. Ton encadrement durant ma thèse a été génialissime ! Je n'aurais pas pu rêver avoir une meilleure directrice de thèse, qui me corresponde aussi bien en tout cas !

Un grand merci également à tous les membres de l'équipe Neuropop pour leur dynamisme, leur soutien et leur bonne humeur.

Je remercie également l'ensemble des personnes avec qui j'ai eu l'occasion de collaborer durant ma thèse. Merci à Norbert Noury pour m'avoir accueilli durant plusieurs mois dans son laboratoire à l'INSA pour que je puisse réaliser mes expériences comportementales chez l'Homme dans son living-lab. Merci également à Nicolas Renier pour m'avoir lui aussi accueilli dans son laboratoire afin que j'analyse les images obtenues après la clarification iDISCO. Merci à Olivier Raineteau pour m'avoir accordé de son temps pour m'aider à réaliser des nano injections dans le bulbe olfactif.



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# RÉSUMÉ

Les informations sensorielles que nous percevons suscitent des réponses émotionnelles qui guident notre comportement d'approche ou de retrait, c'est le reflet de ce que l'on appelle la valeur hédonique du stimulus sensoriel. La dimension hédonique est un paramètre dominant de la perception olfactive. En effet, notre première réaction face à une odeur est en général "j'aime" ou "je n'aime pas" avant même d'essayer de l'identifier.

Il est bien établi que la valeur hédonique des odorants possède une composante innée mais elle peut être également modifiée par un certain nombre de paramètres dont le vieillissement. L'altération par l'âge de la valeur hédonique des odorants n'est pas sans conséquence puisqu'elle peut affecter profondément la qualité de vie en modifiant les interactions sociales ou la prise alimentaire. **Pendant ma thèse, je me suis intéressée aux mécanismes neuronaux sous-tendant l'attraction induite par les odorants plaisants et à leurs altérations lors du vieillissement.**

L'équipe a mis en évidence dans une précédente étude que les odorants plaisants étaient représentés dans la région postérieure du bulbe olfactif, premier relai cortical de l'information olfactive. Ces données soulèvent la question de comment l'information hédonique présente dans le bulbe olfactif est traitée par le reste du cerveau pour générer un comportement d'attraction. Comme les comportements motivés sont connus pour reposer sur la mise en jeu du système de récompense, nous avons recherché dans une première étude le rôle de ce circuit dans l'attraction induite par les odorants plaisants. Nous avons tout d'abord mis en évidence que l'activation optogénétique du bulbe olfactif postérieur induit un comportement d'autostimulation intracérébrale, révélant la capacité de cette région à recruter le système de récompense. Ce conditionnement est accompagné d'une activation neurale de l'aire tegmentale ventrale et du tubercule olfactif. Cette dernière structure est particulièrement intéressante puisqu'elle fait partie du striatum ventral ce qui lui confère la particularité d'être au carrefour entre le système olfactif et celui de la récompense. Par la technique d'iDISCO, nous avons montré que le bulbe olfactif postérieur projette préférentiellement vers le tubercule olfactif. De plus, nous avons révélé que les odorants attractifs activent de façon spécifique le tubercule olfactif et induisent une préférence de place conditionnée sous contrôle du système dopaminergique. La mise en évidence du pouvoir récompensant de certains odorants avec une implication forte du tubercule olfactif a pu être étendue à l'Homme. En effet, grâce à des expériences menées en living lab et à l'IRMf, nous montrons que les odorants attractifs

induisent une préférence de place conditionnée et une augmentation du signal BOLD spécifiquement au niveau du tubercule olfactif.

Dans une deuxième étude, je me suis intéressée à l'impact de l'âge sur la perception des odeurs plaisantes et aux bases neurales qui la sous-tendent. Nous avons montré qu'en accord avec des études précédemment menées chez l'Homme, le caractère plaisant de certains odorants s'altère au cours du vieillissement chez la souris. Nous avons montré également que certains odorants restent encore attractifs et activent toujours le bulbe olfactif postérieur et le tubercule olfactif alors que ce pattern neural n'est plus observé pour les odorants qui ont perdu leur pouvoir attractif. De plus, nous avons révélé que l'activation optogénétique du bulbe olfactif postérieur chez la souris âgée est capable d'induire un comportement d'autostimulation intracérébrale. Enfin, ce conditionnement est accompagné d'une activation neurale du tubercule olfactif, sans activation de l'aire tegmentale ventrale, révélant un recrutement seulement partiel du système de récompense.

Enfin, dans une troisième étude, j'ai utilisé les propriétés motivationnelles des odeurs pour identifier de nouveaux paramètres comportementaux chez l'homme permettant d'évaluer la valeur hédonique des odorants. Pour cela, nous avons enregistré et mesuré de façon automatique le comportement des sujets, libres de leurs mouvements, pendant l'exploration de flacons odorants. Nous avons identifié cinq paramètres moteurs étroitement corrélés à la valeur hédonique des odeurs : la vitesse d'approche et de retrait du flacon odorisé, le nombre de snif, la distance entre le flacon et le nez lors du snif et la distance entre le nez et le flacon après le snif. Ainsi, nous avons pu mettre en évidence une nouvelle méthode non invasive et non verbale d'évaluation des odeurs chez l'Homme.

Ainsi, lors de ma thèse, j'ai pu mettre en évidence l'existence d'une voie d'entrée directe et privilégiée entre le système olfactif et celui de la récompense conférant à certaines odeurs un effet récompensant qui pourrait expliquer leur fort pouvoir attractif. De plus, j'ai montré une altération de cette voie qui pourrait être à l'origine de l'anhédonie olfactive sélective constatée au cours du vieillissement.

# ABSTRACT

Sensory information elicits emotional responses that drive our approach or withdrawal behavior, reflecting the hedonic value of the sensory stimulus. The hedonic value is a dominant parameter of olfactory perception. Indeed, our first reaction to an odorant is generally "I like" or "I don't like" before even try to identify it.

It is well established that the hedonic value of an odorant has an innate component but it can also be modified by a large number of parameters including aging. The age-related alteration of odor hedonics can profoundly affect the quality of life by modifying social interactions or food intake. **During my thesis, I investigated the neural mechanisms underlying the attraction induced by pleasant odorants and their alterations during aging.**

In a previous study, our group showed that pleasant odorants are represented in the posterior part of the olfactory bulb, the first cortical relay of olfactory information. These data raised the question of how the hedonic information present in the olfactory bulb is processed by the rest of the brain to generate an attractive behavior. As motivated behaviors are known to rely on the reward system, in a first study, we have investigated the role of this neural circuit in the attraction induced by pleasant odorants. We first demonstrated that optogenetic activation of the posterior olfactory bulb induced an intracranial self-stimulation behavior, revealing the capacity of this region to recruit the reward system. This conditioning is accompanied by neural activation of the ventral tegmental area and the olfactory tubercle. This last structure is part of the ventral striatum, giving it the particularity of being at the crossroads between the olfactory system and the reward system. Using the iDISCO technic, we showed that the posterior olfactory bulb projects preferentially to the olfactory tubercle. Furthermore, we revealed that attractive odorants specifically activate the olfactory tubercle and induce a conditioned place preference under control of the dopaminergic transmission. We extended the evidence of a rewarding power of some odorants with a strong involvement of the olfactory tubercle to humans. Indeed, thanks to experiments conducted in living labs and fMRI, we showed that attractive odorants induced conditioned place preference and an increase of the BOLD signal specifically in the olfactory tubercle.

In a second study, I investigated the impact of aging on the perception of pleasant odorants and the neural bases underlying it. We showed that, in accordance with previous studies in humans, the pleasantness of some odorants is altered during aging in mice. We have

also shown that some odorants remained attractive and still activated the posterior olfactory bulb and the olfactory tubercle, whereas this neural pattern was no longer observed for odorants that have lost their attractive power. Moreover, we revealed that optogenetic activation of the posterior olfactory bulb in elderly mice is able to induce intracranial self-stimulation behavior. Finally, this conditioning was accompanied by neural activation of the olfactory tubercle, without activation of the ventral tegmental area, revealing only partial recruitment of the reward system.

In a third study, we proposed to use the motivational property of odorants to identify new behavioral parameters to assess odor hedonic value in humans. We recorded and automatically analyzed freely moving human subjects exploring odorized flasks and revealed that five motor parameters are linked to odor hedonics: the approach and withdrawal speeds of the odorant to the nose, the distance between the flask and the nose, the number of sniff and distance of withdrawal corresponding to the maximal distance between the nose and the flask after odor sniffing. We thus highlighted new, non-verbal and non-invasive parameters to evaluate olfactory hedonics in humans based on the assessment of odor motivated behavior.

Thus, during my thesis, I demonstrated the existence of a direct and privileged pathway between the olfactory system and the reward system conferring to some odorants a rewarding effect that could explain their strong attractive power. In addition, I showed that an alteration of this pathway could be at the origin of the selective olfactory anhedonia observed during aging.

# LISTE DES ABRÉVIATIONS

BO	Bulbe Olfactif
MSN	Medium Spiny Neuron
NAc	Noyau Accumbens
OFC	Cortex Orbitofrontal
PirCX	Cortex Piriforme
aPirCX	Cortex Piriforme antérieur
pPirCX	Cortex Piriforme postérieur
TAAR	Trace amine-Associated Receptor
TMT	2,5-dihydro-2,4,5-trimethylthiazoline
TO	Tubercule Olfactif
VP	Palidum Ventral
VTA	Aire Tegmentale Ventrale



# **INTRODUCTION**

## ***I. Les pouvoirs de l'odeur***

Parmi les différents sens, l'odorat apparaît comme un sens mystique. Gaston Bachelard écrivait à propos des odeurs "À elles seules les odeurs préparent les mythes" (*Bachelard et Bachelard 1988*). Le mystère entourant les odeurs trouve notamment son fondement dans les croyances qui accompagnaient l'origine des parfums durant l'Antiquité. Dans la mythologie grecque, la rose aurait jailli du sein de Gaïa, la déesse Terre. Chez les égyptiens, l'encens proviendrait de la transpiration divine. De même, le lieu de production des parfums est empreint de mystère. Il s'agirait d'un lieu peuplé d'animaux ailés tels que le phénix pour les grecs et du "Pays du dieu" gardé par un serpent gigantesque au corps recouvert d'or et aux sourcils de lapis-lazuli pour les égyptiens (*Erman et Ranke 1994*). De nombreux pouvoirs surnaturels ont été attribués aux odeurs au cours de l'Histoire, tantôt moyen de communication avec les dieux, tantôt source du pouvoir des sorcières, tantôt origine de la propagation de la peste (*Le Guérier 2002*). Mais leur véritable pouvoir ne serait-il pas l'inéluctable pouvoir attractif qu'elles exercent sur nous ?

Que l'on en soit conscient ou non les odeurs sont présentes partout autour de nous. Notre recherche de plaisir olfactif nous a poussé à développer des pratiques plaçant les odeurs au cœur de notre vie quotidienne, et ce, depuis l'Antiquité. Dès le II<sup>ème</sup> siècle av. J.-C., toutes les grandes puissances de l'Antiquité désiraient acquérir l'encens d'Oman qui était considéré comme plus précieux que l'or (*Bouchard 2013*). L'encens d'Oman a ainsi édifié dès l'Antiquité un commerce mondial permettant de se procurer aux quatre coins du monde l'odeur tant convoitée. De façon similaire, durant la Rome antique, les romains faisaient parvenir par navire entier celle qu'ils considéraient comme la reine des fleurs : la rose (*Caissard, Baudino, et Hugueney 2009*). Lors des banquets, des jets d'eau de rose arrosaient les hôtes et dans les amphithéâtres, les places les plus nobles étaient protégées du soleil par des stores imprégnés de parfum de rose. Néron, empereur romain connu pour ses extravagances, faisait ruisseler des roses du plafond lors de ses banquets, une mise en scène spectaculaire pour le plaisir des yeux et de celui du nez. Aujourd'hui encore, les odeurs imprègnent notre vie quotidienne. Nous parfumons nos maisons, notre linge et même nous-même. Nous utilisons également des épices pour parfumer nos plats et des arômes pour nos boissons. Par ces pratiques, nous contrôlons notre environnement olfactif afin que celui-ci nous procure du plaisir : plaisir alimentaire, appréciation d'un parfum, délectation de l'odeur de l'être aimé ... L'ensemble des pratiques

que nous avons développées autour de l'odorat révèlent notre recherche constante de plaisir olfactif.

## ***II. Odorant ou odeur ?***

Une odeur, correspond à la perception sensorielle d'une molécule chimique odorante. Nous désignerons ainsi par "odorant" la molécule chimique et par "odeur" la perception olfactive.

### **1- Lien entre molécule chimique odorante et perception odorante**

Pour être odorante, une molécule chimique doit posséder une volatilité suffisante pour voyager dans l'air jusqu'aux cavités nasales et une solubilité suffisante pour pénétrer dans le mucus de l'épithélium olfactif. De nombreux facteurs physico-chimiques influencent le caractère odorant d'une molécule chimique : son nombre d'atomes de carbone, son nombre de liaisons doubles ou triples, la présence de certains groupements fonctionnels, leurs nombres et leurs positions, la structure de la chaîne carbonée ou encore la cyclisation de la molécule (*Johnson et Leon 2007*). La chiralité de la molécule chimique est également un paramètre important dans la perception de son odeur. En effet, deux énantiomères auront des odeurs perçues comme différentes alors que les molécules sont identiques et que seul leur arrangement dans l'espace change. Par exemple, le +limonène sent plutôt le citron jaune alors que le – limonène sent le citron vert. De la même façon le +carvone possède une odeur de carvi alors que le –carvone sent la menthe douce. Ainsi, deux molécules structurellement proches peuvent avoir des odeurs assez éloignées et à l'inverse deux molécules possédant des structures très éloignées peuvent avoir des odeurs très proches d'un point de vue perceptif (*Meierhenrich et al. 2004*). Le lien entre structure moléculaire de l'odorant et perception olfactive a fait l'objet de nombreuses recherches (*Chastrette 1997*). Les études les plus anciennes ont tout d'abord corrélé la perception olfactive à un nombre restreint de caractéristiques physico-chimiques de l'odorant tel que la taille de la molécule (*Amoore, Johnston, et Rubin 1964*), son nombre d'atomes de carbone, la nature et/ou à la position des groupements chimiques dont elle est composée (*Dore, Gordon, et Jaubert 1984; Laska 2002; Meiners, Wäckers, et Lewis 2002; Guerrieri et al. 2005*). Les études plus récentes ont quant à elles tenté d'appréhender la complexité moléculaire de l'odorant dans son ensemble en construisant un espace multidimensionnel où chaque dimension

représente une propriété physico-chimique de l'odorant (*Haddad et al. 2008*). Ces études ont notamment permis de mettre en évidence un lien entre le caractère plus ou moins plaisant d'un odorant et certaines de ses caractéristiques physico-chimiques (*Khan et al. 2007; Kermen et al. 2011*). Toutefois, du fait de la diversité des caractéristiques physico-chimiques des odorants et de la complexité de la perception olfactive, le code exact permettant de relier la structure moléculaire à la perception n'a pas encore été découvert. Ainsi, aucun scientifique ou parfumeur peut, aujourd'hui encore, prétendre prédire ce que sentira une nouvelle molécule chimique sur la base de ses caractéristiques physico-chimiques ou prédire la structure physico-chimique d'une nouvelle odeur (*Khan et al. 2007*).

## 2- Différentes dimensions olfactives

Le nez humain est capable de distinguer plus d'un trillion d'odorant différents (*Bushdid et al. 2014*). Bien qu'a priori simple, la perception d'une odeur est en réalité très complexe. Un exemple indéniable de cette complexité est l'existence de plus de 500 arômes différents qui aboutissent tous à la perception d'une odeur de "fraise". La perception olfactive est un élément complexe car composite. Tel un son qui est défini par son volume, sa tonalité, sa fréquence et sa durée, une odeur possède plusieurs caractéristiques. Les études de psychophysiques réalisées chez l'Homme ont attribué aux odeurs quatre caractéristiques principales: l'identité, l'intensité, la familiarité et la valeur hédonique. L'identité, ou qualité de l'odeur, correspond au nom qui lui est attribuée. Est-ce que l'odeur sent plutôt la rose, la fraise ou la banane ? L'intensité correspond à la concentration perçue de la molécule odorante. Plus une molécule odorante est concentrée dans l'air, plus son odeur sera perçue comme intense. La familiarité correspond au caractère plus ou moins connu d'une odeur. Enfin, la valeur hédonique correspond au caractère plus ou moins plaisant de l'odeur. Une odeur pourra ainsi avoir une valeur hédonique positive ou négative selon si elle est considérée comme agréable ou désagréable par la personne qui la sent. D'autres caractéristiques peuvent également être utilisées comme la comestibilité de l'odeur, son caractère irritant ou encore sa fraîcheur. Cependant ces caractéristiques ne sont pas entièrement indépendantes et varient généralement l'une en fonction de l'autre. La valeur hédonique de certaines odeurs a par exemple été corrélée avec leur intensité et leur familiarité (*Henion 1971; Distel et al. 1999*). De plus, la perception d'une odeur n'est pas immuable mais peut varier d'un individu à l'autre selon sa culture ou son expérience, ou pour un même individu en fonction de son âge (*Mantel et al. 2019; Engen 1991*).

Comme cela sera développé dans le prochain chapitre, la valeur hédonique apparaît comme étant la caractéristique principale d'une odeur. Ainsi, cette thèse procèdera à l'étude de la valeur hédonique des odeurs, de ses méthodes de mesure, de ses bases neurales et de leur possible altération.

### ***III. La valeur hédonique des odeurs***

L'hédonisme, du grec *hêdonê* qui signifie "plaisir", est un courant philosophique dont la doctrine considère le plaisir comme un bien essentiel et qui fait de sa recherche le but principal de l'activité humaine. La notion d'hédonisme a été reprise dans le domaine de la sensorialité où la recherche du plaisir procuré par un stimulus est essentielle. La notion de valeur hédonique correspond en neurosciences au caractère plus ou moins plaisant d'un stimulus et représente le paramètre dominant de la perception olfactive. L'importance que nous accordons aux odeurs et au plaisir qu'elles nous procurent révèle l'importance de notre hédonisme olfactif.

#### **1- La valeur hédonique, paramètre dominant de la perception olfactive**

Contrairement à Jean-Baptiste Grenouille, personnage principal du roman de Patrick Süskind *Le Parfum : histoire d'un meurtrier*, nous rencontrons de grandes difficultés à nommer les odeurs (*Cain et al. 1998; Jönsson, Olsson, et Olsson 2005; Lawless et Engen 1977*). Malgré nos excellentes, bien que très longtemps sous-estimées, capacités à détecter et discriminer les odorants, nous possédons de grandes difficultés à les identifier et à les nommer (*Shepherd 2004; Zelano et Sobel 2005*). Alors que la langue française comporte pourtant de nombreux termes permettant de désigner une odeur tels que "arôme", "bouquet", "effluve", "exhalaison", "fragrance", "fumet", "miasme", "puanteur", "relent", "remugle" ou "émanation", très peu de mots permettent d'en établir une description précise (*David 2002*). Cette difficulté à poser un nom sur une odeur pourrait être due d'un point de vue cérébral à un manque de communication entre les structures olfactives et les aires impliquées dans le langage. En effet, chez des sujets présentant une aphasie (perte de la capacité de parler), la perte du volume de substance grise au niveau du lobe temporal et du gyrus frontal inférieur est corrélée avec la réduction des capacités à nommer les odeurs (*Olofsson et al. 2013*). Face à un stimulus visuel par exemple, notre première réponse va être de le nommer. Contrairement aux autres sens, l'identité ne va pas être le paramètre dominant de la perception olfactive.

Lorsque l'on sent une odeur, notre première réaction va être "J'aime" ou "Je n'aime pas cette odeur" (*Richardson et Zucco 1989*). Cette réaction est le reflet de la valeur hédonique de l'odeur. En étant le premier élément spontanément énoncé en réponse à une odeur, la valeur hédonique dévoile son importance dans la perception olfactive. La valeur hédonique est également le premier critère de catégorisation des odeurs. En effet, si l'on demande à des participants de faire des groupes d'odeurs, ils vont spontanément regrouper les odeurs en fonction de leur valeur hédonique (*Berglund et al. 1973; Schiffman, Robinson, et Erickson 1977*). De plus, lorsque l'on propose aux participants un large panel de descripteurs verbaux permettant de caractériser les odeurs, la valeur hédonique apparaît comme étant la première dimension émergeant des analyses multidimensionnelles; c'est-à-dire que les descripteurs verbaux en lien avec la valeur hédonique sont les plus discriminants (*Khan et al. 2007; Moskowitz et Barbe 1977; Zarzo 2008*). Parmi les différentes dimensions de la perception olfactive telles que l'identité ou l'intensité, la valeur hédonique apparaît ainsi comme le paramètre dominant (*Yeshurun et Sobel 2010*).

## 2- Mesure de la valeur hédonique des odeurs

### a) Chez l'Homme

Chez l'Homme, les réponses induites par les odeurs sont exprimées à différents niveaux (e.g. verbalisation des émotions ressenties, changements dans l'état physiologique ou adaptation du comportement moteur), ainsi la valeur hédonique d'une odeur peut donc être évaluée par différentes méthodes en fonction du niveau exploré.

La mesure la plus directe de la valeur hédonique d'une odeur est son évaluation subjective par l'individu. Les participants notent sur des échelles le caractère plaisant ou attractif de l'odeur, le plus souvent de "très déplaisant" à "très plaisant", en passant par "neutre" (*Stevens 1958; Poncelet et al. 2010*). L'évaluation du sujet par les systèmes de notation permet donc une mesure quantifiable de la valeur hédonique mais elle ne laisse pas au sujet la liberté d'exprimer spontanément sa réponse verbale à l'odeur. En effet, le sujet est guidé par des consignes et contraint d'évaluer les odeurs selon certains critères. Afin de rendre au sujet sa liberté de verbalisation, certaines études actuelles ont fait le choix d'analyser le champ lexical utilisé par le participant lorsque celui-ci est libre d'exprimer sa réponse verbale à une odeur. Ces études ouvrent la voie à des méthodes d'analyse du jugement hédonique plus éthologiques.

La valeur hédonique est également évaluée indirectement par des mesures physiologiques réalisées sur les participants. En effet, les odeurs sont des stimuli sensoriels provoquant des émotions fortes (*Becker et al. 2019*). Ainsi, l'évaluation de la nature de l'émotion (positive ou négative) ressentie par le participant par la mesure de paramètres physiologiques tels que la fréquence respiratoire, l'amplitude de l'inspiration, la fréquence cardiaque ou la réponse électrodermale, permet de déterminer de façon indirecte la valeur hédonique de l'odeur (*Royet et al. 2003; Ferdenzi et al. 2017; Joussain et al. 2017; Seubert et al. 2009; Chrea et al. 2009; Rétiveau, Iv, et Milliken 2004; Schiffman, Suggs, et Sattely-Miller 1995*). Ainsi, il a été montré qu'une odeur plaisante génère un sniff plus important en terme de volume et de durée qu'une odeur déplaisante (*Bensafi et al. 2003; Bensafi, Sobel, et Khan 2007; Ferdenzi et al. 2015*). A l'inverse, le rythme cardiaque et la conductance électrodermale augmentent en réponse à une odeur déplaisante mais pas à une odeur plaisante (*Brauchli et al. 1995; Alaoui-Ismaili, et al. 1997; Alaoui-Ismaili, Vernet-Maury, et al. 1997; Bensafi et al. 2002*). Le réflexe de micro-sursaut induit par les odeurs déplaisantes est également utilisé comme mesure de la valeur hédonique (*Ehrlichman et al. 1995; 1997; Miltner et al. 1994*). De plus, il a été mis en évidence que lors du jugement de la valeur hédonique d'une odeur, le temps de réponse pour les odeurs désagréables est sensiblement plus court que pour les odeurs agréables (*Bensafi, Rouby, Farget, Vigouroux, et al. 2002*), suggérant que ce paramètre puisse être utilisé pour déterminer la valeur hédonique d'une odeur. De nouvelles méthodes ont également été développées afin de mesurer de façon non invasive la réponse du participant. Ces méthodes reposent sur la mesure de l'activité vasomotrice de l'individu grâce à une caméra capable de mesurer de faibles variations de couleur de la peau (*Bousefsaf, Maaoui, et Pruski 2016*). Bien que très prometteuses, ces méthodes ne sont pour l'instant pas assez efficaces pour être utilisées comme mesure fiable de la valeur hédonique d'une odeur.

Par ailleurs, il est également possible de mesurer les expressions faciales effectuées par un individu adulte en réponse à l'odeur. Ainsi, une odeur provoquant plus de mimiques relatives au dégoût sera considérée comme moins plaisante qu'une odeur provoquant des mimiques relatives à la satisfaction (*Li, Jia, et Wang 2020*). Chez le nouveau-né ces indices faciaux peuvent être mesurés afin d'évaluer le caractère hédonique d'une odeur. En effet, le nourrisson produit plus de mimiques de dégoût en sentant l'odeur de l'acide butyrique que celle de la vanille, respectivement évaluées comme déplaisante et plaisante par des adultes (*Soussignan et al. 1997; Schaal, Marlier, et Soussignan 2000*). L'analyse des mimiques permet donc d'obtenir des mesures de la valeur hédonique à un âge où le langage n'a pas encore été développé, mais

elle repose sur l'expertise de personnes capables de catégoriser comme positive ou négative les différentes expressions faciales.

Ainsi, l'évaluation verbale permet une mesure subjective alors que la mesure des paramètres physiologique du participant permet une évaluation objective de la valeur hédonique des odeurs. Cependant, ces mesures physiologiques sont invasives du fait de la présence de nombreux capteurs sur le corps et/ou le visage du participant. Elles ne permettent donc pas au participant d'exprimer une réponse comportementale spontanée en réponse à l'odeur. De façon analogue à l'analyse de la verbalisation libre du participant, il serait intéressant de développer de nouvelles méthodes d'analyse comportementales basées sur le comportement spontané du participant afin d'obtenir une mesure efficace, non invasive, sans consignes et donc plus éthologique de la valeur hédonique des odeurs.

#### *b) Chez l'animal*

Chez l'animal, l'absence de verbalisation contraint à effectuer des mesures comportementales indirectes de la valeur hédonique (*Doty 1986*). Selon sa valeur hédonique, un odorant va induire un comportement d'approche ou de répulsion. Ainsi, le niveau d'attractivité d'une odeur est mesurée classiquement chez l'animal par la durée de son temps d'exploration (*Kobayakawa et al. 2007; Mandairon, Poncelet, et al. 2009; Devore, Lee, et Linster 2013; Kermen et al. 2016; Jagetia et al. 2018*). Cette durée d'exploration a été corrélée avec l'évaluation du caractère plaisant de ces odeurs par l'Homme (Mandairon, Poncelet, et al. 2009a). La valeur hédonique d'une odeur peut également être mesurée chez certains vertébrés en quantifiant les comportements appétitifs et défensifs spécifiques à l'espèce induits par les odeurs (*Frank et al. 2019; Kermen, Darnet, et al. 2020; Mathuru et al. 2012; Yabuki et al. 2016*).

Plusieurs méthodes permettent donc de mesurer, de façon plus ou moins directe, la valeur hédonique des odorants que ce soit chez l'Homme ou chez l'animal. Chacune de ces méthodes possède des avantages et des inconvénients qui lui sont propres. Toutefois, quel que soit la méthode utilisée, il apparait clairement que les odorants présents dans notre environnement ont une influence sur notre humeur et sur nos comportements en fonction de leur valeur hédonique.

### 3- La valeur hédonique guide nos comportements



Les odorants sont des messagers porteurs de nombreuses informations sur notre environnement. En fonction de son caractère plaisant ou déplaisant, un odorant va induire un comportement d'approche ou de répulsion. Chez l'animal, tout comme chez l'Homme, la valeur hédonique des odorants guide les comportements essentiels à la survie, en participant notamment à l'évitement des dangers, à l'alimentation et aux interactions sociales (*Stevenson 2010; Walliczek-Dworschak et Hummel 2017*). Le pouvoir attractif ou répulsif des odeurs permet ainsi de s'approcher des stimuli indispensables à la survie et au contraire d'éviter les stimuli représentant des dangers. Les odorants sont des stimuli connus pour induire des émotions fortes et la valeur hédonique est un paramètre déterminant dans le traitement des affects positifs et négatifs induit par les odeurs (*Becker et al. 2019*).

#### a) *Évitement des dangers*

Les stimuli considérés comme des dangers induisent des émotions négatives fortes médiées par le système limbique (*LeDoux 2012*). Le traitement de ces stimuli permet à l'animal d'adopter un comportement pertinent vis-à-vis du danger. Le comportement le plus souvent adopté afin de favoriser la survie de l'animal est celui de la fuite. L'odorat est l'un des sens permettant la détection et la reconnaissance des dangers. Les odorants indicateurs d'éléments nuisibles pour la santé et/ou la survie ont une valeur hédonique négative et induisent donc une forte répulsion. Parmi ces odorants, on compte notamment chez l'animal les molécules chimiques odorantes émanant des prédateurs. Par exemple, la molécule de 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), composé odorant présent dans les fèces de renard, induit chez les souris et les rats qui n'ont pourtant jamais été en contact avec un renard ou tout autre prédateur, des comportements de peur et de défense (*Rosen, Asok, et Chakraborty 2015*). Pour aboutir à une réponse comportementale adaptée à sa signification éthologique pour l'animal, la TMT doit à la fois solliciter les systèmes olfactifs et limbiques, notamment l'amygdale, puis mettre en jeu le système moteur. De façon intéressante, la consommation de viande induit la production d'une molécule odorante typiques chez les carnivores et cet odorant est très répulsif chez les espèces considérées comme des proies telles que la souris ou le rat (*Ferrero et al. 2011*). Ceci suggère un rôle des odeurs dans la relation prédateur/proie basée sur le régime alimentaire.

L'odorat permet également l'évitement d'autres dangers en induisant une répulsion vis-à-vis d'autres odeurs telles que celle du dioxyde de carbone (*Hu et al. 2007; Li et Liberles 2015*) et des cadavres d'animaux en décomposition (*Hussain et al. 2013*) chez l'animal ou encore du feu, de la fumée, des produits chimiques toxiques et des aliments avariés chez l'Homme (*Santos et al. 2004; Wisman et Shrira 2015*). Nous pouvons par exemple détecter le mercaptan, la

molécule odorante du qui est utilisé pour donner une odeur au gaz, à une concentration infime de  $0,2 \times 10^{-6}$  g/L (*Yeshurun et Sobel 2010*).

#### b) Alimentation

Le sens de l'odorat est fortement lié à celui de la gustation. Alors que le système gustatif détecte le goût salé, sucré, amer, acide ou umami, l'odorat traite l'arôme des aliments. Ces deux sens sont souvent confondus car l'arôme (ou odeur) d'un aliment est majoritairement perçu lorsque l'aliment est en bouche. En effet, les odorants présents dans l'air environnant atteignent l'épithélium olfactif par la voie dite orthonasale (par les narines) alors que les molécules odorantes libérées par la mastication de l'aliment passent majoritairement par la voie dite rétronasale (par le pharynx). Il est établi qu'environ 80% de la saveur d'un aliment dépendrait en réalité à l'odorat (*Murphy, Cain, et Bartoshuk 1977*). Le plaisir ressenti par la consommation de nourriture serait ainsi principalement dû à la valeur hédonique positive de l'odeur des aliments. Le traitement de la valeur hédonique olfactive et gustative possèdent également des bases neurales communes telles que l'insula et le cortex orbitofrontal (*Rolls 2005*).

Chez l'Homme comme chez l'animal, la valeur hédonique des odeurs est une source d'information cruciale pour l'alimentation, permettant notamment de distinguer les aliments palatables des aliments avariés (*Demattè, Endrizzi, et Gasperi 2014; Takahashi, Nagayama, et Mori 2004; Kobayakawa et al. 2007; Li et Liberles 2015*). En effet, les odorants provenant d'aliments palatables vont induire chez l'animal un comportement attraction favorisant ainsi la prise alimentaire. A l'inverse, les odorants provenant d'aliment avariés tels que les amines vont induire une forte répulsion permettant ainsi d'éviter l'ingestion de toxines produites par les micro-organismes. Chez l'Homme, une odeur alimentaire plaisante va induire des réponses motrices viscérales traduisant l'appétence pour un plat (e.g. salivation ou augmentation de la mobilité gastrique) (*Proserpio et al. 2017*). De plus, la valeur hédonique d'un odorant va se modifier en fonction de l'état de satiété (*Rolls et Rolls 1997*). L'odeur d'un aliment sera en effet considérée comme plaisante au début de la consommation du dit aliment puis deviendra de moins en moins plaisante au cours de sa consommation, conditionnant ainsi l'arrêt de la prise alimentaire. Tout comme les chiens utilisant leur odorat pour pister une proie, il a été montré que l'attraction que possède l'Homme pour l'odeur du chocolat lui permet de suivre l'odeur à la trace (*Porter et al. 2007*). En effet, des participants ont été capables de suivre une piste olfactive (l'odeur de chocolat) sur une distance d'environ 10 mètres en utilisant seulement leur odorat.

### c) *Interactions sociales*

La valeur hédonique des odeurs possède un rôle clef dans les interactions sociales. D'un point de vue sociologique, elle possède notamment une place de premier choix dans la séduction. Les noms donnés aux parfums dans le domaine de la parfumerie de luxe évoquent en effet la sensualité (e.g. "Allure Sensuelle" de Chanel, "Eau Sensuelle" de Rochas), la passion (e.g. "Sì Passione" d'Armani, "Baiser volé" de Cartier, "Déclaration d'un soir" de Cartier), l'amour (e.g. "Amor Amor" de Cacharel, "Love Story" de Chloé, "Loverdose" de Diesel) et même le mariage (e.g. "Marry Me !" de Lanvin). L'utilisation de parfum dans les rituels liés à la séduction n'est pas exclusive à la culture occidentale. Chez les berbères par exemple, les hommes brûlent les racines de sarghine et s'imprègnent des fumigations afin que le parfum qui en émane favorise la séduction du partenaire (*Gobert 1961*). Les femmes de l'île de Nauru possèdent quant à elles un rituel complexe pour se parfumer aussi bien de l'extérieur que de l'intérieur afin d'attirer les partenaires (*Petit-Skinner 1981*). Dans ces pratiques, les parfums permettent de masquer ou modifier l'odeur corporelle afin de la rendre plus agréable. Toutefois, l'odeur corporelle elle-même possède un rôle important dans les interactions sociales et notamment dans le choix du partenaire sexuel. En effet, la concentration de certains composés volatiles odorants présents dans la transpiration humaine varie entre les hommes et les femmes (*Troccaz et al. 2009*). De façon intéressante, les femmes jugent les odorants présents dans la transpiration d'homme moins désagréables que les odorants présents dans la transpiration de femme (*Troccaz et al. 2009*). De plus, ces odorants présents dans la transpiration masculine sont perçus comme plus intenses chez les femmes étant dans la phase fertile de leur cycle menstruel (*Ferdenzi et al. 2019*). Ceci suggère un mécanisme d'attraction pour les personnes du sexe opposé basé sur l'odeur corporelle. Chez l'animal, une attraction spontanée vers les odeurs émises par les congénères du sexe opposé est également observée (*Xu et al. 2005; Kang, Baum, et Cherry 2009*), favorisant ainsi la reproduction.

L'attraction olfactive est impliquée dans la reproduction mais également dans la relation privilégiée entre la mère et le nouveau-né. Dès le plus jeune âge, les ratons présentent une forte attirance pour l'odeur de leur mère (*Porter, Cernoch, et McLaughlin 1983; Schneider et al. 2016*) et pour celles présentes dans le nid (*Porter et Etscorn 1974*). Cette attraction pour l'odeur maternelle est médiée par le système olfactif (*Schneider et al. 2016*). Chez l'Homme aussi, l'odeur maternelle est très attractive pour les nouveaux nés et les mères sont capables de reconnaître l'odeur de leur propre enfant parmi celle d'autres enfants dès six heures après leur naissance (*Porter, Cernoch, et McLaughlin 1983*). Ces interactions sociales basées sur l'odorat

sont indispensables à la survie du nouveau-né, lui permettant l'alimentation et la prédication de soins qui lui sont nécessaires.

La valeur hédonique guide également la reconnaissance olfactive de l'autre. Certaines ethnies telles que les Esquimaux, les Samoans, les Maoris et les Philippins se frottent ou se reniflent le visage pour se saluer. Le caractère plus ou moins hédonique de l'odeur corporelle permettrait ainsi de déterminer l'appartenance ou non au groupe (*Largey et Watson 1972*). Dans la culture occidentale, il a également été montré que de façon inconsciente nous approchons notre main de notre nez après avoir serré la main d'un individu afin d'en extraire des informations olfactives le concernant (*Frumin et al. 2015*). De manière intéressante, ce comportement n'est plus observé lorsque l'individu porte un gant, la poignée de main ne permettant alors plus la transmission des informations olfactives. Cette reconnaissance de l'autre sur la base de la valeur hédonique de son odeur semble posséder un intérêt évolutif majeur. En effet, un des exemples les plus notable est le rôle que la valeur hédonique joue dans l'évitement de la consanguinité. Les odeurs corporelles des membres de la famille sont jugées spontanément comme désagréables, suggérant un mécanisme olfactif de prévention contre la consanguinité (*Weisfeld et al. 2003*).

#### *d) Régulation des émotions*

Il est essentiel d'indiquer qu'au-delà des comportements essentiels à la survie et à la perpétuation de l'espèce, la valeur hédonique des odeurs intervient également dans la régulation des émotions. Par le terme d'émotion, nous désignons les réactions affectives que sont par exemple la joie, la colère, la peur, le dégoût, la tristesse ou la surprise. Les odorants sont des stimuli sensoriels connus comme provoquant des émotions fortes chez l'animal (*Ueno et al. 2019; Rosen, Asok, et Chakraborty 2015; Ferrero et al. 2011*) tout comme chez l'Homme (*Royet et al. 2003; Ferdenzi et al. 2017; Jousain et al. 2017; Seubert et al. 2009; Chrea et al. 2009; Rétiveau, Iv, et Milliken 2004; Schiffman, Suggs, et Sattely-Miller 1995*). L'aspect positif ou négatifs des émotions induites par les odeurs dépend notamment du caractère agréable ou désagréable de celles-ci (*Becker et al. 2019*). Par exemple, l'exposition quotidienne à des odeurs agréables (application d'eau de Cologne ou de crème parfumée) est susceptible d'améliorer significativement l'humeur (*Schiffman, Suggs, et Sattely-Miller 1995; Schiffman et al. 1995; Abriat et al. 2007*). Ainsi, lorsque l'on demande à des participants d'évaluer grâce à des échelles leurs émotions avant et après utilisation de ces composés odorants, on observe que les odeurs plaisantes induisent des émotions positives telles que la diminution du niveau d'anxiété, la réduction du stress ou des symptômes dépressifs ainsi qu'une augmentation de la joie, de la

sérénité ou encore de l'amusement. A l'inverse, la présence de mauvaises odeurs dans notre environnement tel qu'à l'abord des usines par exemple est une cause d'inconfort mesurable (*Sucker et al. 2008*).

Les odeurs sont également particulièrement efficaces pour se remémorer des souvenirs, notamment les souvenirs empreints d'émotions. Il a été mis en évidence que les souvenirs évoqués par les odeurs sont plus émotionnels que ceux évoqués par des stimuli verbaux, visuels, tactiles ou musicaux (*Herz 1998*). Telle l'odeur de la madeleine qui évoqua des souvenirs de son enfance au héros du roman *Du côté de chez Swann* de Marcel Proust, les odeurs induisent un rappel de la mémoire autobiographique puissant permettant de se rappeler des expériences personnelles et émotionnelles passées (*Chu et Downes 2002; Larsson et Willander 2009; Zucco et al. 2012*). Par exemple, des participants se rappelant des événements passés évoquent spontanément un pourcentage plus important de souvenirs joyeux en présence d'une odeur plaisante qu'en présence d'une odeur déplaisante (*Ehrlichman et Halpern 1988*).

Le lien étroit entre les odeurs et les émotions pourraient être dû au fait qu'elles possèdent des bases neurales communes. En effet, le traitement de l'information olfactive s'effectue notamment dans les aires cérébrales dévouées au traitement des émotions : le système limbique (*LeDoux 2012*). Par exemple, l'amygdale, qui est une structure clef du système limbique en étant impliquée notamment dans les réponses de peur, est également une structure olfactive dite secondaire impliquée dans le traitement de l'information olfactive. Parmi les différents systèmes sensoriels, l'odorat semble posséder une voie privilégiée vers le système limbique. En effet, la voie partant de l'épithélium olfactif est plus directe que celle partant par exemple de la peau (*Lledo, Gheusi, et Vincent 2005*). A partir des études d'imagerie cérébrales réalisés chez l'Homme est apparu l'hypothèse que la spécificité de l'olfaction à susciter des émotions serait plus attribuable à une implication précoce du système limbique plutôt qu'à un mode de traitement conceptuellement différent des autres informations sensorielles (*Lledo, Gheusi, et Vincent 2005*).

Ainsi, la valeur hédonique des odeurs guide indéniablement nos comportements. Toutefois, il est nécessaire de s'interroger quant à l'origine de cette valeur hédonique. En d'autres termes, le caractère plus ou moins plaisant d'une odeur est-il prédéterminé avant la naissance ou la valeur hédonique dépend-t-elle exclusivement des expériences vécues par l'individu ?

#### 4- Valeur hédonique innée, valeur hédonique acquise

La valeur hédonique d'une odeur est en partie innée mais elle dépend également de nos expériences olfactives qui peuvent la modifier tout au long de notre vie (*Engen 1991*). Bien qu'étant prédéterminée, la valeur hédonique serait donc également fortement plastique.

##### *a) Composante innée de la valeur hédonique*

La valeur hédonique possède une composante innée. En effet, une attirance ou une répulsion pour certaines odeurs peut être observée sans apprentissage préalable chez l'Homme tout comme chez l'animal (*Mandairon et al. 2009; Kermen et al. 2016; Jagetia et al. 2018*). De plus, la valeur hédonique d'une même odeur semble être déterminée dès le plus jeune âge (*Soussignan et al. 1997*) et être commune entre certaines espèces (*Keller et Vosshall 2007; Mandairon, Poncelet, et al. 2009*). La valeur hédonique négative de la TMT apparaît notamment comme étant innée car les animaux présentent spontanément un comportement de répulsion vis-à-vis de l'odorant alors qu'ils ne l'ont jamais senti auparavant (*Rosen, Asok, et Chakraborty 2015*). De plus, l'existence d'une relation prédateur/proie basée sur le régime alimentaire et sous-tendu par l'odorat corrobore l'hypothèse d'une composante innée de la valeur hédonique des odeurs (*Ferrero et al. 2011*). De la même manière, la valeur hédonique spontanément négative des odeurs corporelles des membres de la famille permettant d'éviter la consanguinité s'avère également être innée (*Weisfeld et al. 2003*).

L'hypothèse de l'existence d'une composante innée de la valeur hédonique est également supportée par l'existence d'un code de cette valeur hédonique dans la structure même de la molécule chimique odorante. En effet, le caractère agréable d'un odorant dépend de la complexité de sa structure moléculaire (*Kermen et al. 2011; Zarzo 2011*). De plus, il est possible de prédire la valeur hédonique d'une molécule odorante selon sa structure chimique (*Khan et al. 2007; Joussain et al. 2011; Genva et al. 2019*). Cette prédétermination de la valeur hédonique d'un odorant selon sa structure observée chez l'Homme semble également être présente chez la souris (*Mandairon, Poncelet, et al. 2009; Joussain et al. 2011*). L'existence d'une valeur hédonique innée aurait donc un intérêt évolutif majeur permettant aux odeurs d'induire une réponse comportementale appropriée et universelle via des réseaux neuronaux spécifiques et prédéterminés.

##### *b) Modulation de la valeur hédonique*



La valeur hédonique des odeurs peut être modulée chez l'Homme par la culture (*Seo et al. 2011; Ferdenzi et al. 2017; Ferdenzi et al. 2019*). L'odeur de fromage par exemple est très appréciée par les français alors qu'elle dégoûte la plupart des autres nationalités. Cette différence interculturelle est également mise en exergue par l'odeur du durian. Le durian est un fruit dont la consommation est très appréciée en Asie alors qu'il possède la particularité d'avoir une odeur extrêmement déplaisante pour les personnes qui n'y sont pas familières. Une étude a également montré que l'odeur de menthe est jugée comme plus plaisante par des Français d'origine Algérienne où la consommation de thé à la menthe est précoce et fréquente, que par des Français originaires d'Europe qui n'ont pas cette culture (*Poncelet et al. 2010*). De manière intéressante, la valeur hédonique de l'odeur de rose, odeur qui possède une influence culturelle équivalente entre les deux groupes, est jugée comme similaire.

La valeur hédonique des odeurs est également modulée par l'apprentissage, c'est-à-dire que nous pouvons changer notre appréciation d'une odeur en fonction de la bonne ou de la mauvaise expérience qui lui a été associée, c'est ce que l'on appelle un apprentissage associatif. Dans un apprentissage de type associatif, une odeur est associée à une récompense ou à une punition et va ainsi acquérir la valeur hédonique positive ou négative de la récompense ou de la punition qu'elle prédit. Chez l'Homme, une odeur peut par exemple être rendue plus agréable par son association avec un goût sucré (*Yeomans et al. 2006; Barkat et al. 2008*). Cette modulation de la valeur hédonique peut avoir lieu dès le plus jeune âge. Les nouveaux nés ont en effet une préférence olfactive pour les aliments consommés par leur mère durant la grossesse (*Schaal, Marlier, et Soussignan 2000*). Cela suggère des mécanismes de plasticité cérébrale pouvant avoir lieu dès le plus jeune âge, permettant ainsi de moduler la perception hédonique de l'odeur. Chez l'animal, les apprentissages associatifs odeur/récompense ou odeur/punition ont beaucoup été étudiés chez l'adulte comme chez le nouveau-né. Les rats sont notamment capables d'associer une odeur à une récompense ou à une punition dès le troisième jour après leur naissance (*Rudy et Cheatle 1977; Johanson et Hall 1979*). Cependant, ces apprentissages associatifs ont très largement été utilisés comme outils permettant d'analyser les mécanismes mnésiques (*Wilson et Linster 2008; Wilson et Sullivan 1994; Wilson et Stevenson 2003; Martin et al. 2004; Doucette et Restrepo 2008; Doucette et al. 2011; Fletcher 2012; Ross et Fletcher 2019; Grelat et al. 2018; Roesch, Stalnaker, et Schoenbaum 2007*) mais n'ont que très rarement été étudiés pour leurs spécificités en terme d'apprentissage. En effet, peu d'auteurs ont pris en compte la nature de cet apprentissage, c'est-à-dire l'acquisition d'une nouvelle valeur hédonique (*Yuan et al. 2002; Calu et al. 2007; Gadziola et al. 2015*).

## 5- Conclusion générale

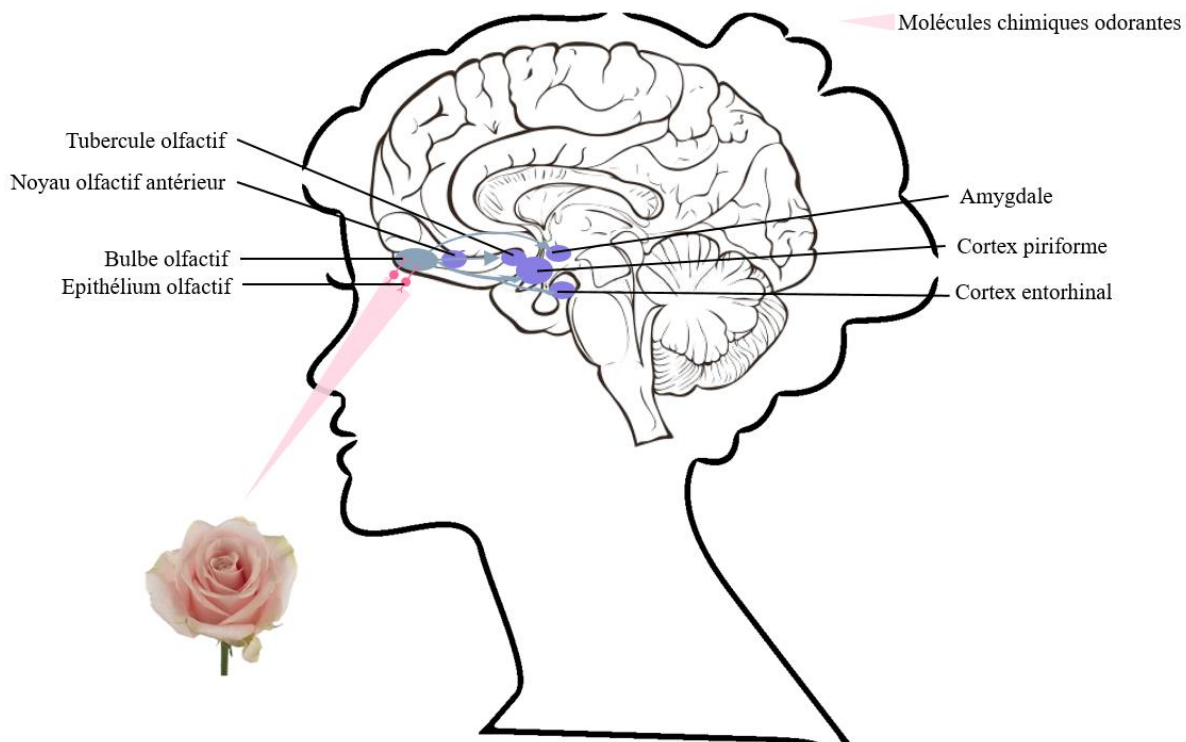
Qu'elle soit innée ou acquise par apprentissage, la valeur hédonique est un paramètre primordial qui permet à l'individu d'exprimer une réponse comportementale appropriée à l'odeur présente dans son environnement. Afin de comprendre comment les odorants sont capables d'induire un comportement d'approche ou d'évitement selon leur valeur hédonique, il apparaît comme nécessaire de décrypter comment ce paramètre perceptif est représenté au niveau cérébral. Cette thèse a donc pour ambition de faire le lien entre les éléments présents dans la littérature et d'apporter de nouvelles connaissances quant aux mécanismes neuronaux responsables de la valeur hédonique des odeurs.

## ***IV. Structures impliquées dans le codage de la valeur hédonique des odeurs***

Plusieurs structures cérébrales ont été désignées comme intervenant dans le codage de la valeur hédonique des stimuli sensoriels. Une des hypothèses relatives au codage de la valeur hédonique est l'existence de sous-circuits distincts codant pour les stimuli positifs ou négatifs (*Yamaguchi 2017*). Un certain nombre d'étude d'imagerie réalisés chez l'Homme corroborent en effet cette hypothèse en montrant une activation spécifique de certaines structures ou sous-structure cérébrales en réponse aux stimuli plaisants et une activation de structure différentes en réponse aux stimuli déplaisants. Ces structures appartiennent à la fois aux systèmes sensoriels relatifs aux stimuli étudiés et aux systèmes auxquels ils sont connectés comme le système limbique ou certaines structures corticales (*Zatorre 2015; Nadal et Skov 2018; Croy et al. 2016; Huang et al. 2016; Tiedemann et al. 2020; Wassiliwizky et al. 2017*).

Dans le cas des stimuli olfactifs, les structures impliquées dans le codage de la valeur hédonique des odeurs appartiennent principalement au système olfactif. Chez les mammifères, il existe deux systèmes olfactifs distincts. Le système olfactif dit principal correspond à l'ensemble des structures permettant la détection et le traitement des odorants. Parmi ses structures on compte l'épithélium olfactif qui détecte les molécules odorantes, le bulbe olfactif principal (BO) et les structures olfactives supérieures ou secondaires (Figure 1). Ces structures font parties du système limbique.





*Figure 1 : Système olfactif principal.*

*Les molécules chimiques odorantes se lient aux récepteurs olfactifs présents sur les cils des neurones sensoriels olfactifs présents dans l'épithélium olfactif (rose). Les neurones sensoriels olfactifs envoient leurs axones au niveau du bulbe olfactif (bleu), qui distribue ensuite l'information dans les structures olfactives secondaires (violet) : le cortex piriforme, le noyau olfactif antérieur, l'amygdale, le cortex entorhinal et le tubercule olfactif.*

Le système olfactif dit secondaire permet quant à lui la détection et le traitement des phéromones. Les phéromones sont des molécules chimiques odorantes qui ont la particularité de ne pas être, ou d'être très peu, volatiles. Contrairement aux odeurs qui se répandent dans l'air, les phéromones sont quant à elles majoritairement détectées sur des surfaces telles que le sol ou à la surface du corps d'un individu. Les phéromones sont des molécules permettant la communication intra ou inter-espèce. Elles sont détectées au niveau de l'organe voméronasal situé dans la cavité nasale puis l'information est transmise au niveau du bulbe olfactif accessoire. Chez l'Homme, l'existence de phéromones tout comme d'un organe voméronasal fonctionnel est sujet à controverses. Certains supposent que notre passage de quadrupède à bipède ayant éloigné notre nez du sol, nous aurions délaissé ce système de communication basé sur les phéromones au cours de l'évolution (*Aiello et Dean 1991*) (mais voir aussi *Troccaz et*

*al. 2009; Ferdenzi et al. 2019*). Ici nous avons choisi de restreindre notre étude aux odorants qui sont véhiculés par l'air et donc d'exclure les phéromones.

Ainsi, l'étude des bases neurales de la valeur hédonique des odeurs sera réalisée au sein des structures du système olfactif principal -l'épithélium olfactif, le BO, le cortex piriforme (PirCX), l'amygdale et le tubercule olfactif (TO)- ainsi que dans les systèmes avec lequel il est connecté tel que le système de récompense.

## 1- L'épithélium olfactif

### a) *Les neurones sensoriels olfactifs*

L'épithélium olfactif se situe dans la partie postéro-dorsale de la cavité nasale. Sa forme repliée le distingue de la muqueuse nasale et permet d'augmenter considérablement la surface de détection des odorants. L'épithélium olfactif est constitué de trois types de cellules : les cellules de soutien qui permettent la cohésion de l'épithélium olfactif, les neurones sensoriels olfactifs qui captent et transmettent l'information olfactive et les cellules basales qui sont des cellules souches permettant le renouvellement des neurones sensoriels olfactifs. La surface de l'épithélium olfactif est tapissée de mucus sécrété par les glandes de Bowman.

Nous possédons environ 12 millions de neurones sensoriels olfactifs au sein de notre épithélium olfactif (*Breer 2003*). A l'extrémité de la dendrite des neurones sensoriels olfactifs se trouvent des cils qui baignent dans le mucus olfactif et dont la membrane exprime les récepteurs olfactifs. Ainsi, les neurones sensoriels olfactifs lient les odorants au niveau de leurs cils, transforment le message chimique en message électrique et transmettent l'information olfactive via leur axone jusqu'à l'encéphale. Plus précisément les axones des neurones sensoriels olfactifs se rassemblent en faisceaux, les nerfs olfactifs, et passent la lame criblée du crane pour projeter au niveau du BO.

Les neurones sensoriels olfactifs ont la particularité d'être renouvelés tout au long de la vie d'un individu à partir des cellules basales (*Yu et Wu 2017*). Les neurones sensoriels olfactifs sont les seuls neurones du corps humain à être en contact direct avec le milieu extérieur et par conséquent ils sont facilement altérables. Le phénomène de régénération des neurones sensoriels olfactifs permet de maintenir l'intégrité de l'épithélium olfactif tout au long de la vie. L'ensemble des neurones sensoriels olfactifs sont renouvelés tous les 30 à 90 jours selon l'espèce (*Mackay-Sim et Kittel 1991*).

## b) Les récepteurs olfactifs

Lorsque nous inspirons, les molécules chimiques présentes dans l'air autour de nous vont pénétrer dans la cavité nasale et se fixer au niveau des récepteurs olfactifs présents sur les neurones sensoriels olfactifs. La découverte de gènes à l'origine de l'expression des récepteurs olfactifs par Linda Buck et Richard Axel leur a valu un prix Nobel de Physiologie et Médecine en 2004 (*Buck et Axel 1991*). De par la forte diversité des récepteurs olfactifs, les gènes permettant leur codage correspondent à environ 3% du génome chez la souris. Les récepteurs olfactifs sont composés de sept domaines transmembranaires capables de reconnaître spécifiquement un odorant par un système comparable à celui d'une clef et d'une serrure. Ainsi, seul un faible nombre d'odorant sera capable de se lier à un type de récepteur donné selon sa structure moléculaire. Les récepteurs olfactifs sont couplés à des protéines G spécifiques du système olfactif ( $G_{olf}$ ). La fixation de l'odorant sur son récepteur va induire l'activation de la protéine  $G_{olf}$  qui va conduire à une cascade de signalisation intracellulaire permettant la transformation de l'information chimique en information électrique par les neurones sensoriels olfactifs (*Kleene 2008*). Un neurone sensoriel olfactif n'exprime qu'un seul type de récepteur olfactif (*Touhara et al. 1999; Malnic et al. 1999*) et un type de récepteur est exprimé par 5 000 à 10 000 neurones sensoriels olfactifs (*Mobley et al. 2014*).

Nous possédons environ 390 récepteurs olfactifs différents (*Saito et al. 2009*), mais le nombre d'odorant que nous pouvons détecter est beaucoup plus considérable (*Bushdid et al. 2014*), suggérant ainsi qu'un même récepteur serait capable de lier plusieurs molécules odorantes. Ceci a été mis en évidence par une étude de Malnic et al. (*Malnic et al. 1999*) qui a montré in vitro qu'un même neurone sensoriel olfactif répond à plusieurs odorants et donc que le type de récepteur olfactif présent à sa surface est capable de reconnaître plusieurs odorants. Le code combinatoire permettant de déterminer quels odorants sont capables de se lier à quels récepteurs reste encore inconnu, mais sa modélisation fait l'objet de nombreuses recherches (*Bernabei et al. 2013*). Par ailleurs, chacun des gènes des récepteurs olfactifs existe sous forme d'allèles différents dans la population, et chaque allèle code pour une séquence protéique spécifique avec des propriétés de liaison différentes (*Malnic et al. 1999*). Cette variabilité génomique explique la variabilité de perception interindividuelle constatée pour certaines molécules. Par exemple, le composé stéroïde de l'androsténone, phéromone présente chez le porc, est apprécié de manière différente selon l'allèle du récepteur olfactif exprimé (*Keller et al. 2007*). En effet, la majorité des français possèdent une aversion forte pour l'odeur de l'androsténone qu'ils décrivent comme une odeur de sueur ou d'urine. A l'inverse, les anglais

qui présentent une mutation du gène OR7D4 codant pour le récepteur de l'androsténone décrivent l'odeur de l'androsténone comme sucrée ou florale. De cette différence génétique résulte la castration systématique des porcs en France, et non en Angleterre, pour éviter l'imprégnation de cette odeur dans la viande de porc.

*c) Codage spatial de la valeur hédonique au sein de l'épithélium olfactif*

Il existe plusieurs zones au sein de l'épithélium olfactif exprimant des récepteurs, et donc liant potentiellement des odorants différents (*Ressler, Sullivan, et Buck 1993; Vassar, Ngai, et Axel 1993; Strotmann et al. 1994*). Selon leur valeur hédonique les odorants ne possèderaient pas la même structure chimique (*Kermen et al. 2011; Zarzo 2011; Khan et al. 2007; Joussain et al. 2011; Genva et al. 2019*). Les odorants plaisants possèderaient notamment une structure moléculaire plus complexe que les odorants déplaisants (*Kermen et al. 2011*). Du fait de leur taille plus imposante les odorants plaisants ne pénétreraient donc pas de la même façon et pas aussi profondément dans la cavité nasale que les odorants déplaisants qui eux sont plus simples. De plus, le sniff est également un paramètre pouvant moduler la zone de liaison des odorants au sein de l'épithélium olfactif. Le volume et la durée du sniff ne sont en effet pas les mêmes selon la valeur hédonique de l'odeur, les odorants plaisants étant sentis plus amplement que les odorants déplaisants (*Bensafi et al. 2003; Bensafi, Sobel, et Khan 2007; Ferdenzi et al. 2015*). Dans ce contexte, existe-t-il donc des zones de l'épithélium olfactif spécialisées dans la liaison d'odorants plaisants et d'autres dans la liaison d'odorants déplaisants ?

L'existence des Trace amine-Associated Receptor (TAAR) qui lient spécifiquement les odorants aversifs tels que l'odeur de prédateur ou de nourriture avariée (*Johnson et al. 2012; Ferrero et al. 2011; Takahashi, Nagayama, et Mori 2004*) ainsi que leur localisation majoritaire dans la partie dorsale de l'épithélium olfactif (*Johnson et al. 2012; Ferrero et al. 2011*) corrobore l'hypothèse d'un codage spatiale de la valeur hédonique des odeurs au niveau de l'épithélium olfactif. Ces récepteurs TAAR sont retrouvés dans l'épithélium olfactif (Figure 2) de nombreuses espèces dont l'Homme (*Liberles 2015*). Par ailleurs, l'enregistrement direct chez l'Homme de l'activité des neurones sensoriels olfactifs présents dans l'épithélium olfactif révèle que certaines localisations de l'épithélium olfactif répondent préférentiellement aux odeurs plaisantes alors que d'autres répondent préférentiellement aux odeurs déplaisantes (*Lapid et al. 2011*). Ces données vont dans le sens d'un codage de la valeur hédonique des odeurs dès l'épithélium olfactif.

De façon intéressante, la valeur hédonique des odeurs pourrait également être codée dans d'autres structures de la cavité nasale en dehors de l'épithélium olfactif. Les ganglions de Grueneberg sont des structures olfactives présentes à l'entrée de la cavité nasale et donc distinctes de l'épithélium olfactif (Figure 2). Or il a été montré que les cellules des ganglions de Grueneberg sont capables de lier certains odorants aversifs tels que les odeurs de prédateurs ou les signaux d'alarme olfactifs émis par les congénères afin de prévenir d'un danger (Brechtbühl, Klaey, et Broillet 2008; Brechtbühl et al. 2013). Les ganglions de Grueneberg représentent donc des sous-régions de la cavité nasale qui permettraient de coder la valeur hédonique spontanément répulsive des odorants.

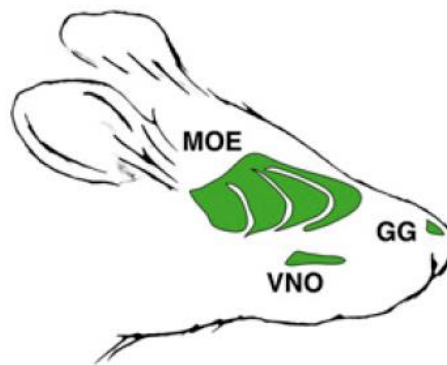


Figure 2 : Systèmes olfactifs au sein de la cavité nasale.

*La cavité nasale comporte trois sous-systèmes olfactifs : l'épithélium olfactif (MOE), l'organe voméronasal (VNO) et les ganglions de Grueneberg (GG) (Brechtbühl et al. 2013).*

#### *d) Conclusion*

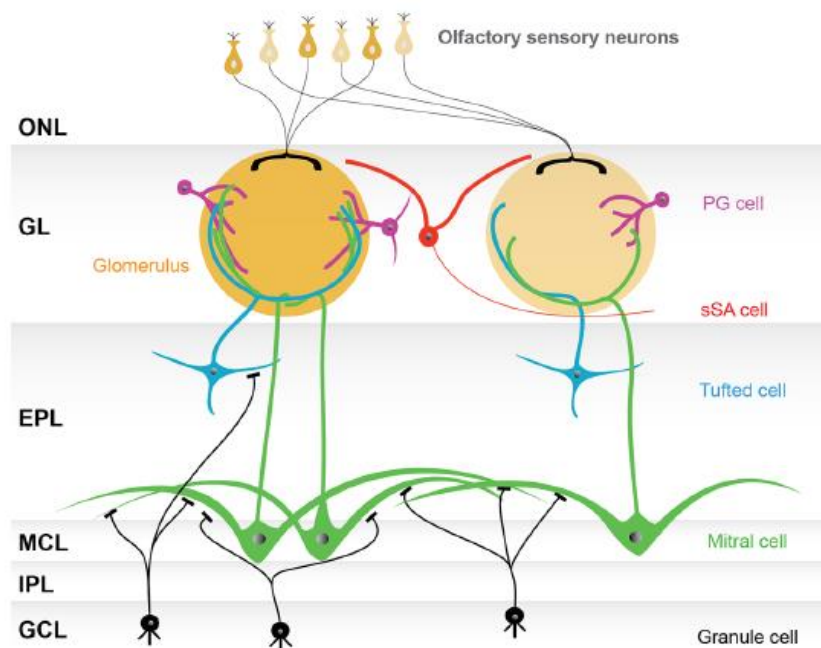
Comme nous l'avons vu, plusieurs sous-systèmes olfactifs sont présents dans la cavité nasale, l'épithélium olfactif lui-même même est composé de plusieurs zones liants des odorants différents. Un codage spatial de la valeur hédonique des odeurs semble être présent dans ces différentes zones de la cavité nasale. Ainsi, selon leurs caractéristiques physico-chimiques et donc leur caractère plus ou moins plaisant, les odorants pourraient se lier sur des récepteurs olfactifs présents dans des zones différentes. Cependant du fait du nombre restreint d'étude portant sur le sujet, il est à ce jour encore impossible de déterminer avec précision comment est représentée topographiquement la valeur hédonique des odeurs au sein de la cavité nasale. Cartographier plus précisément le lieu de liaison des odorants selon leur valeur hédonique ainsi

que leur activité évoquée dans les neurones sensoriels olfactifs pourrait permettre de mieux appréhender les lois régissant la fixation d'un odorant à son récepteur.

## 2- Le bulbe olfactif

### a) Structure, transmission et traitement de l'information olfactive

Le BO est un paléocortex composé de six couches : la couche des nerfs olfactifs, la couche glomérulaire, la couche plexiforme externe, la couche des cellules mitrales, la couche plexiforme interne et la couche granulaire (*Kosaka et Kosaka 2009*) (Figure 3).



*Figure 3 : Structure laminaire du bulbe olfactif.*

*Le BO est composé de la couche des nerfs olfactifs (ONL), de la couche glomérulaire (GL), de la couche plexiforme externe (EPL), de la couche des cellules mitrales (MCL), de la couche plexiforme interne (IPL) et de la couche granulaire (GCL) (Nagayama, Homma, et Imamura 2014).*

L'information olfactive entre dans le BO par le biais des axones des neurones sensoriels olfactifs. L'ensemble des axones des neurones sensoriels olfactifs constitue la couche des nerfs olfactifs. Ces axones contactent les cellules principales du BO au niveau de la couche glomérulaire. La couche glomérulaire tient son nom des structures ovoïdes dont elle est composée : les glomérules. Plus précisément, c'est au sein des glomérules que les axones des



neurones sensoriels olfactifs font synapse avec les dendrites des cellules de projections du BO. Les glomérules sont entourés d'interneurones GABAergiques et dopaminergiques : les cellules périglomérulaires et cellules superficielles à axone court. Chaque glomérule reçoit des connexions des neurones sensoriels olfactifs de l'épithélium olfactif exprimant le même type de récepteur (*Buck 2000; Mori et Sakano 2011*). Ceci révèle que l'information portée par un odorant emprunte une voie définie entre l'épithélium olfactif et les glomérules du BO. Cette voie se poursuit au niveau des cellules de projections du BO. Il a en effet été mis en évidence que deux cellules de projections voisines vont connecter le même glomérule et établissent des jonctions gap intra-glomérulaires (*Toshio Kosaka et Kosaka 2004*) qui favorisent leur synchronisation au sein de ce glomérule (*Schoppa et Westbrook 2001*). De plus, les cellules de projections voisines, donc potentiellement connectées au même glomérule, ont une activité similaire par rapport aux cellules de projections distantes (*Buonviso et Chaput 1990*). Cette organisation permet l'établissement d'un réseau spécifique pour chaque odorant, de l'épithélium olfactif jusqu'au BO, nommé colonnes d'activité (*Guthrie et al. 1993; Willhite et al. 2006; Cummings et Belluscio 2008*). Les colonnes d'activités observées au sein du BO sont semblables aux colonnes de dominance oculaires du cortex visuel ou aux barrels du cortex somato-sensoriel (*Lledo, Gheusi, et Vincent 2005*) et constitueraient une représentation topographique de l'identité de l'odeur au sein du BO. Les axones des cellules de projections du BO transitent au sein de la couche granulaire qui contient le dernier type d'interneurones du BO (les cellules granulaires) et se regroupent ensuite en tractus olfactif latéral pour transmettre l'information olfactive jusqu'aux structures olfactives secondaires. Jusqu'à présent, les colonnes d'activité codant pour l'identité de l'odeur n'ont pas été retrouvées dans les structures olfactives secondaires (*Sosulski et al. 2011; Igarashi et al. 2012*).

Il existe deux types de cellules de projections dans le BO : les cellules mitrales dont les corps cellulaires sont localisés dans la couche des cellules mitrales et les cellules à panache dont les corps cellulaires sont localisés dans la couche plexiforme externe. Les cellules mitrales et les cellules à panache sont toute deux glutamatergiques. Une étude de reconstruction 3D des projections bulbaires réalisée par Igarashi et al. (*Igarashi et al. 2012*) suggère que les cellules à panache projettent majoritairement dans les structures olfactives situées à l'avant de l'encéphale (e.g. noyau olfactif antérieur, partie cap du TO) alors que les cellules mitrales transmettraient l'information olfactive moins vite que les cellules à panache mais dans des structures plus postérieures de l'encéphale (e.g. TO cortical, PirCX, amygdale) (Figure 4). De par leurs différences en termes de latence de réponse et de cibles, les cellules mitrales et les

cellules à panache possèderaient un rôle différent dans le traitement de l'information olfactive. Les cellules à panache coderaient pour la concentration de l'odeur alors que les cellules mitrales ne répondraient qu'aux concentrations importantes et permettraient la discrimination et l'identification des odeurs présent dans un mélange (Igarashi et al. 2012).

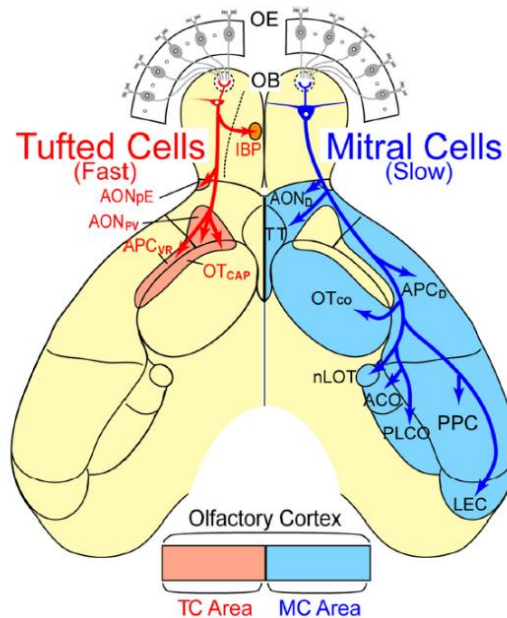


Figure 4 : Différence de projections des cellules du bulbe olfactif dans les structures olfactives secondaires.

*Les cellules mitrales du BO projettent dans des structures olfactives plus postérieures que les cellules à panache (Igarashi et al. 2012).*

L'information olfactive est modulée à l'entrée et à la sortie du BO respectivement par les cellules périglomérulaires et les cellules granulaires. Les cellules périglomérulaires modulent l'entrée de l'information olfactive en se connectant à la fois aux axones des neurones sensoriels olfactifs et aux dendrites des cellules mitrales et des cellules à panache. Les cellules périglomérulaires projettent sur plusieurs glomérules (Pinching et Powell 1972; Schneider et Macrides 1978) permettant ainsi de contrôler l'activité des colonnes d'activité bulbaire. En inhibant les glomérules adjacents qui sont moins activés par l'odeur, les cellules périglomérulaires permettent l'augmentation du ratio signal/bruit et sont donc considérées comme des normalisateurs de l'entrée sensorielle. Cette normalisation serait requise pour la représentation de l'identité de l'odeur indépendamment de sa concentration (Aungst et al. 2003; Cleland et Sethupathy 2006; Cleland et al. 2007). Les cellules superficielles à axone court ont



quant à elles pour rôle de favoriser l'activité des cellules périglomérulaires (*Pinching et Powell 1971*). Les cellules granulaires modulent la sortie de l'information olfactive du BO en connectant les axones des cellules de projections au niveau de la couche plexiforme externe. Les cellules granulaires représentent 90% des cellules du BO. L'activation des cellules de projections du BO induit une activation des cellules granulaires qui les inhibent en retour, formant ainsi une boucle de rétroaction (*Schoppa et al. 1998*). Cette boucle permet l'inhibition latérale des cellules de projections, favorisant ainsi la conservation des colonnes d'activités dans le BO (*Mori et Yoshihara 1995*). Les cellules granulaires jouent également un rôle dans la genèse des oscillations rythmiques au sein du réseau bulbaire qui participe au codage de l'odeur (*Gervais et al. 2007; Lazarini et Lledo 2011*). Par ailleurs, le BO constitue un des sites d'intégrations des neurones issus de la neurogenèse adulte dans plusieurs espèces (*Altman 1969; Lledo et Valley 2016*). Les cellules souches en prolifération dans la zone sous-ventriculaire des ventricules latéraux donnent des neuroblastes qui migrent le long du flux rostral migratoire pour s'intégrer dans le BO. Les neuroblastes issus de la neurogenèse adulte se différencient en interneurons périglomérulaires et granulaires et jouent notamment un rôle dans la mémoire et les apprentissages olfactifs (*Sultan et al. 2011; Moreno et al. 2012; Mandairon et al. 2018; Forest et al. 2019; Forest et al. 2020*). Le BO reçoit des projections massives provenant de la majorité des systèmes neuromodulateurs (e.g. acétylcholine, noradrénaline et sérotonine) (*Macrides et al. 1981; Kiselycznyk, Zhang, et Linster 2006; Linster et Cleland 2016*). Ces afférences ont des effets à la fois sur l'activité du réseau bulbaire et sur un large éventail de comportements olfactifs tels que la discrimination et les apprentissages olfactifs. Contrairement à d'autres structures sensorielles, le BO ne reçoit pas de projections dopaminergiques extrinsèques, depuis l'aire tegmentale ventral, mais il contient des neurones dopaminergiques intrinsèques dans la couche glomérulaire (*Linster et Cleland 2016*).

#### *b) Codage de la valeur hédonique des odeurs dans le bulbe olfactif*

Au-delà de son rôle connu chez l'animal dans la discrimination et la mémoire olfactive (*Wilson et Sullivan 1994; Wilson et Linster 2008; Tong, Peace, et Cleland 2014; Brennan et Keverne 2015; Mandairon et al. 2018; Forest et al. 2019; Forest et al. 2020*), le BO intervient également dans le codage de la valeur hédonique des odeurs.

Un codage spatial de la valeur hédonique des odeurs a été retrouvée dans le BO. En effet, il a été montré chez la souris que l'odeur d'urine d'un congénère du sexe opposé induit une activation de la partie ventrale du BO (*Xu et al. 2005*). L'odeur d'urine est considérée comme un mélange complexe, contenant à la fois des odeurs et des phéromones, qui est attractif

pour l'animal lorsque celui-ci provient d'un émetteur du sexe opposé au récepteur. Cette activation de la partie ventrale du BO n'est pas retrouvée en réponse à l'odeur d'urine de prédateur (*Kang, Baum, et Cherry 2009*). A contrario, les odeurs aversives telles que l'odeur de prédateur ou de nourriture avariée, est codée par la partie dorsale du BO. En effet, l'activation optogénétique de glomérules dorsaux répondant au TMT est suffisante pour induire un comportement de peur chez l'animal (*Saito et al. 2017*), tandis que leur l'inactivation altère le comportement aversif de l'animal (*Cho et al. 2011; Saito et al. 2017*). De plus, des animaux transgéniques présentant une déplétion des glomérules de la partie dorsale du BO perdent le comportement inné d'aversion pour les odeurs de prédateur et de nourriture avariée alors qu'ils sont capables de les détecter (*Kobayakawa et al. 2007*). De façon intéressante, *Kobayakawa et al.* ont montré que les animaux transgéniques étaient tout de même capables d'apprendre par apprentissage associatif la valeur hédonique négative de ces odorants. Cela suggère que le codage de la valeur hédonique est différent selon son caractère inné ou acquis. L'ensemble de ces études suggèrent l'existence d'un axe dorso-ventral de codage de la valeur hédonique des odeurs ayant une pertinence éthologique. La partie ventrale coderait ainsi pour les odorants ayant un intérêt éthologique positif alors que la partie dorsale coderait pour les odorants ayant un intérêt éthologique négatif. L'étude de *Kobayakawa et al.* (*Kobayakawa et al. 2007*) suggère également que ce codage serait différent dans un contexte d'apprentissage.

Lorsque l'on s'intéresse aux odorants ne semblant pas avoir de pertinence éthologique particulière pour l'animal, on s'aperçoit que le codage de la valeur hédonique s'effectuerait plutôt selon l'axe antéro-postérieur du BO. Une étude de l'équipe a comparé les cartographies d'activation du BO en réponse à des odeurs considérées comme plaisantes ou déplaisantes de façon innée (sans apprentissage) (*Kermen et al. 2016*). Les résultats révèlent que la partie antérieure du BO code préférentiellement pour les odeurs plaisantes alors que la partie postérieure code pour les odeurs déplaisantes. Cette représentation topographique est retrouvée au niveau des glomérules mais également au niveau de la couche granulaire du BO. De plus, lorsque ces patterns d'activités sont inhibés par optogénétique, on observe une inversion de la valeur hédonique des odeurs. Ainsi l'inhibition des cellules granulaires de la partie postérieure du BO induit une forte attraction pour les odorants déplaisants alors que l'inhibition des cellules granulaires de la partie antérieure induit une répulsion pour les odorants plaisants. Cette étude révèle donc que la valeur hédonique innée des odeurs sans pertinence éthologique particulière est codée spatialement selon l'axe antéro-postérieur du BO.

De plus, des enregistrements électrophysiologiques réalisés dans un contexte d'apprentissage associatif odeur/récompense ou odeur/punition ont mis en évidence une modification de la représentation spatio-temporelle de l'odeur au sein du BO. En effet, l'acquisition de la nouvelle valeur hédonique de l'odeur induit à la fois une modification des potentiels de champ locaux du BO (*Martin et al. 2004*) et de l'activité des cellules mitrales (L. M. Kay et Laurent 1999; Doucette et Restrepo 2008; Doucette et al. 2011a; Liu et al. 2017), et à la fois une modification des cartographies d'activation de la couche granulaire du BO en réponse à l'odeur (*Yuan et al. 2002; Salcedo et al. 2005; Busto et al. 2009*). Ainsi la représentation de valeur hédonique des odeurs au sein du BO semble être plastique. De plus, il a été montré que l'activité des cellules granulaires issues de la neurogenèse adulte serait impliquée dans la facilitation de l'apprentissage de l'association entre l'odeur et la récompense (*Grelat et al. 2018*). En effet, la stimulation optogénétique de ces interneurons récemment intégrés dans le réseau bulbaire facilite l'association entre odeur/récompense ainsi que l'inversion de cet apprentissage.

#### *c) Etudes chez l'Homme*

Toutes les études portant sur l'implication du BO dans le traitement de l'information olfactive ont jusqu'à présent seulement été réalisées chez l'animal. Le BO est en effet trop petit et localisé trop proche des sinus pour permettre son analyse par les techniques d'imagerie par résonance magnétique fonctionnelle (IRMf) classiques. Cependant, une étude récente a réussi à imager par IRMf l'activité du BO en réponse à l'odeur grâce à l'utilisation d'une antenne adaptée au visage du participant permettant d'obtenir une meilleure résolution au niveau du BO (*Fournel et al. 2020*). Cette étude ouvre une nouvelle voie dans l'étude du rôle du BO chez l'Homme, notamment dans le codage de la valeur hédonique des odeurs.

#### *d) Conclusion*

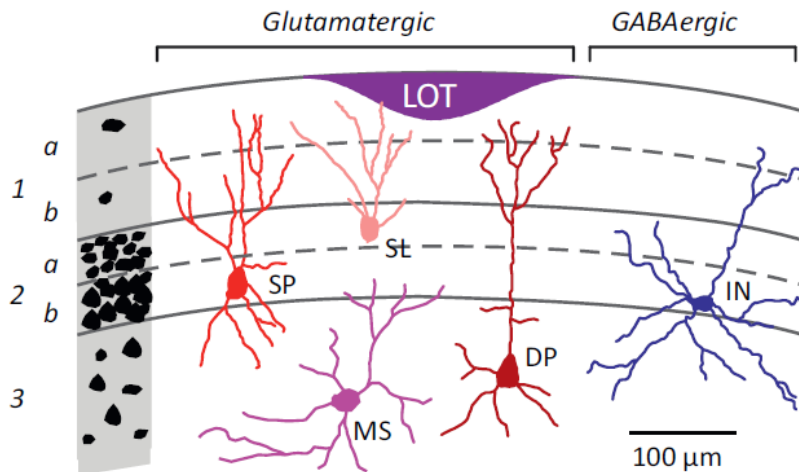
Les études réalisées jusqu'à ce jour chez l'animal ont permis de mettre en évidence que plusieurs représentations topographiques de la valeur hédonique des odeurs semblent coexister dans le BO notamment en fonction du type d'odeur (avec ou sans pertinence éthologique). Ainsi l'axe dorso-ventral ainsi que l'axe antéro-postérieur du BO semblent être impliqués dans le codage de la valeur hédonique des odeurs. Ces représentations bulbaires sont également fortement plastiques et peuvent être modulées notamment dans un contexte d'apprentissage associatif olfactif. Les représentations topographiques présentes dans le BO pourraient provenir d'un codage de la valeur hédonique dès l'étage périphérique qui seraient ensuite transmises

jusqu'au BO. Il est en effet très bien documenté que les récepteurs olfactifs répartis le long de l'axe dorsomedian/ventrolateral dans l'épithélium projettent sur les glomérules répartis le long de l'axe dorso/ventral dans le BO (*Touhara et al. 1999; Kobayakawa et al. 2007; Cho et al. 2011*). De plus, les récepteurs TAAR et les cellules des ganglions de Grueneberg codant pour les odorants aversifs projettent majoritairement sur la partie dorsale du BO (*Johnson et al. 2012; Ferrero et al. 2011; Brechbühl et al. 2013*). Cependant du fait du peu d'étude ayant porté sur le sujet, aucun gradient de projection de l'axe antéro-postérieur de l'épithélium olfactif sur l'axe antéro-postérieur du BO n'est observé (*Saucier et Astic 1986*). Les systèmes neuromodulateurs projetant sur le BO pourraient également jouer un rôle dans la signature neurale de la valeur hédonique au sein du BO. Le BO révèle donc son rôle déterminant dans le codage de la valeur hédonique des odeurs, mais comment cette information est-elle transmise et traitée par les structures en aval du BO ?

### 3- Le cortex piriforme

#### *a) Structure, transmission et traitement de l'information olfactive*

Parmi les différentes structures olfactives secondaires, le PirCX est considéré comme le cortex olfactif principal car il reçoit le nombre le plus important de projections bulbaires via le tractus olfactif latéral (*Nagayama et al. 2010*). Le PirCX est un paléocortex comprenant trois couches (Figure 5). La couche la plus superficielle (1) contient principalement les afférences bulbaires et les dendrites des neurones du PirCX. La couche de cellules denses (2) contient les corps cellulaires des neurones de projections du PirCX. La couche profonde (3) contient également des neurones principaux mais en beaucoup plus faible quantité. Les différents types de neurones de projection du PirCX sont des cellules glutamatergiques qui envoient leurs prolongements pour moduler l'activité des cellules à l'intérieur du PirCX ou pour contacter d'autres structures cérébrales. Différents types d'interneurones GABAergiques sont dispersés uniformément dans toutes les couches du PirCX, permettant ainsi une modulation de l'entrée ou de la sortie de l'information olfactive.



*Figure 5 : Structure du cortex piriforme.*

*Le cortex piriforme est composé de trois couches : la couche superficielle (1), la couche des cellules denses (2) et la couche profonde (3). Les cellules semilunaires (SL) et les cellules pyramidales superficielles (SP) ont leurs corps cellulaires condensés respectivement dans les couches 2a et 2b. Les corps cellulaires des cellules pyramidales profondes (DP) et les cellules épineuses multipolaires (MS) se trouvent dans la couche 3. (Bekkers et Suzuki 2013).*

Le tractus olfactif latéral projette dans la couche 1a du PirCX alors que les fibres associatives et commissurales projettent sur les couche 1b, 2 et 3 (Bekkers et Suzuki 2013). De par des différences à la fois anatomiques, physiologiques et fonctionnelles, le PirCX est communément divisé selon son axe antéro-postérieur (Gottfried 2010). La partie antérieure du PirCX (aPirCX) reçoit la majorité des projections du BO (Schwob et Price 1978). A l'inverse, la partie postérieure (pPirCX) reçoit majoritairement des connexions du reste de l'encéphale, notamment du cortex préfrontal, de l'amygdale et du cortex entorhinal (Luskin et Price 1983; Johnson et al. 2000). Le aPirCX semble avoir une sensibilité plus importante que le pPirCX à l'activité afférente bulbaire et jouerait ainsi un rôle dans la détection des odorants et le codage de leur identité chimique (Lynch et Granger 1989). Le pPirCX, quant à lui, en recevant à la fois des informations du aPirCX et d'autres aires cérébrales aurait moins un rôle de cortex primaire mais plus d'aire associative. Il permettrait d'associer à la fois les informations olfactives et les informations issues d'autres systèmes sensoriels, des systèmes limbiques ou encore moteurs (Johnson et al. 2000). L'association de l'ensemble de ces informations permettrait au pPirCX de jouer un rôle dans la reconnaissance de l'odeur en tant qu'objet et dans sa mémorisation (Wilson et Sullivan 2011).

De manière générale, le PirCX a un rôle à la fois dans le traitement de l'information olfactive et dans la mémoire olfactive (*Nemitz et Goldberg 1983*). Le PirCX contribuerait à la reconnaissance de l'odeur (son identité), aux mécanismes d'habituation olfactive, à la mémoire olfactive et à l'apprentissage olfactif (*Wilson 1998; Barkai et Saar 2001; Mouly et al. 2001; Kadohisa et Wilson 2006*).

*b) Codage de la valeur hédonique des odeurs dans le cortex piriforme*

Concernant le codage spatial de la valeur hédonique dans le PirCX, il apparaît que les projections du BO vers le PirCX sont très diffuses (*Sosulski et al. 2011; Igarashi et al. 2012*), ne permettant pas la conservation de l'organisation en colonnes d'activité du BO. Ainsi, aucune organisation topographique, ni de l'identité ni du caractère hédonique de l'odeur, n'a été mise en évidence au sein du PirCX (*Stettler et Axel 2009*). Cependant, des études d'électrophysiologie ont mis en évidence que le taux de décharge des neurones du PirCX est modulé par un apprentissage associatif impliquant un indiciage olfactif, suggérant ainsi un codage temporel de la valeur hédonique des odeurs dans un contexte d'apprentissage (*Wilson et Linster 2008; Wilson et Sullivan 1994; Wilson et Stevenson 2003*). Ces études ont notamment montré que le PirCX possède plusieurs catégories de neurones codant pour des éléments différents de l'apprentissage. En effet, certains neurones du pPirCX ont une fréquence de décharge plus importante en réponse aux odeurs plaisantes et d'autres en réponse aux odeurs déplaisantes, ils coderaient ainsi la valeur hédonique de l'indice olfactif (*Calu et al. 2007*). D'autres neurones du PirCX auraient quant à eux un taux de décharge différent selon si l'odeur est associée à une récompense ou à une punition, ils seraient ainsi plutôt impliqués dans le codage de l'association odeur/récompense ou odeur/punition (*Roesch, Stalnaker, et Schoenbaum 2007; Calu et al. 2007*). Ces neurones sont présents dans le aPirCX et le pPirCX mais la modification de la fréquence de décharge semble être plus importante dans les neurones du pPirCX (*Calu et al. 2007*). De plus, l'apprentissage associatif odeur/récompense entraîne une augmentation durable de l'amplitude du potentiel de champ évoqué et cette modification est limitée à la partie postérieure du PirCX (*Mouly et al. 2001*). Par ailleurs, il a été montré par microdialyse que le pPirCX synthétise du glutamate et du GABA durant l'acquisition de l'association odeur/punition. Durant le sommeil lent, le PirCX semble alors se "déconnecter", afin de faciliter la consolidation de l'association odeur/punition comme le montre la corrélation entre la diminution de l'activité du PirCX et l'augmentation du comportement de peur exprimé par l'animal (*Barnes et al. 2011*).

L'implication du PirCX dans le codage de la valeur hédonique après apprentissage a également été mis en évidence dans l'attraction des nouveaux nés pour les mamelles de leur mère. Bien qu'étant principalement innée, l'attraction des nouveaux nés pour l'odeur de leur mère est également acquise par l'association entre l'odeur maternelle et les soins maternels durant la période périnatale. Ainsi, l'introduction d'une nouvelle odeur (différente de l'odeur maternelle) et son association aux soins maternels ou à des stimuli sensoriels imitant les soins maternels (c'est-à-dire la stimulation tactile ou le lait) induit une attraction pour la nouvelle odeur comparable à l'attraction de l'odeur maternelle (*Roth et Sullivan 2005; Raineki, Moriceau, et Sullivan 2010*). Cette nouvelle odeur qui a acquis les caractéristiques de l'odeur maternelle induit une activation du PirCX.

#### *c) Etudes chez l'Homme*

Chez l'Homme, les études en IRMf ont révélé une implication du PirCX dans le codage de la valeur hédonique acquise dans un contexte d'apprentissage associatif (*Gottfried 2010*). En effet, durant d'apprentissage associatif, l'activité du PirCX varie en fonction de la valeur hédonique des odeurs (*Gottfried et al. 2002; Gottfried, O'Doherty, et Dolan 2002*). En parallèle, des études mesurant l'activité du PirCX en réponse à des odeurs spontanément plaisantes ou déplaisantes (sans apprentissage associatif préalable) ont observé une activation préférentiel du PirCX en réponse aux odeurs déplaisantes (*Royet et al. 2003; Bensafi, Sobel, et Khan 2007*). Cependant le PirCX semble également coder pour l'intensité (*Anderson et al. 2003*) et pour la saillance émotionnelle du stimulus (*Royet et al. 2003*) induit par les odeurs. Le changement d'activité du PirCX observé en réponse aux odeurs déplaisantes pourrait ainsi être dû à une saillance plus importante des odeurs désagréables et non à un codage de la valeur hédonique des odeurs par le PirCX (*Royet et al. 2003*).

#### *d) Conclusion*

Comme nous l'avons vu, la présence de projections diffuses entre le BO et le PirCX ne permettrait pas la conservation de la représentation topographique de la valeur hédonique observée dans le BO au niveau du PirCX. Toutefois, le PirCX est tout de même impliqué dans le codage de la valeur hédonique des odeurs et dans sa modulation, notamment lors d'un apprentissage associatif. La partie antérieure et la partie postérieure du PirCX ne semblent pas être impliquées dans les mêmes processus. En effet, le aPirCX semble plutôt coder pour la valeur hédonique initiale de l'odeur alors que le pPirCX semble quant à lui coder la modulation de la valeur hédonique par apprentissage. Chez l'Homme, le rôle du PirCX est encore sujet à



controverse. Des études supplémentaires pourraient permettre de mieux appréhender les fonctions exactes du PirCX.

#### 4- L'amygdale

##### *a) Structure, transmission et traitement de l'information olfactive*

L'amygdale est constituée de plusieurs noyaux rassemblés en 3 groupes : l'amygdale corticale, l'amygdale basolatérale et l'amygdale centromédiane. Ces groupes ont été établis sur une base à la fois structurale et fonctionnelle (*Swanson et Petrovich 1998*).

L'amygdale est une structure limbique qui joue un rôle clef dans les émotions (*Gallagher et Chiba 1996*). Elle est impliquée à la fois dans le traitement des stimuli induisant de la peur que dans le traitement des stimuli motivationnels, induisant ainsi des comportements de fuite ou d'approche. Le codage par l'amygdale de ces comportements pourtant opposés dépend des circuits intra ou inter-structures sollicités (*Janak et Tye 2015*). L'amygdale fait également parti du système olfactif, elle est considérée comme une structure olfactive secondaire. L'amygdale reçoit les connexions bulbaires au niveau postéro-latéral de la région corticale (*Sosulski et al. 2011*). Cette région reçoit aussi l'information olfactive en provenance du PirCx (*Price 1973*).

##### *b) Codage la valeur hédonique des odeurs dans l'amygdale*

Les projections du BO vers l'amygdale ne seraient pas diffuses contrairement au PirCX, l'organisation topographique du BO serait ainsi conservée au niveau de l'amygdale. En effet, chaque glomérule du BO projettent sur des points spatialement différents de l'amygdale corticale (*Sosulski et al. 2011*). Plus précisément, les cellules de projections du BO projetant sur l'amygdale corticale proviendraient préférentiellement des glomérules localisés dans la partie dorsale du BO (*Miyamichi et al. 2011*). Or les glomérules de la partie dorsale du BO codent pour l'aversion pour les odorants ayant un intérêt éthologique négatif pour l'animal (e.g. nourriture avarié, prédateur). Par ailleurs, il a été montré que l'amygdale est activée en réponse à l'odeur de TMT (*Takahashi 2014*), que l'inhibition par optogénétique de la voie BO-amygdale corticale induit une altération de la répulsion innée des rongeurs pour l'odeur de prédateur et que l'activation des neurones de l'amygdale corticale répondant aux odeurs est suffisante pour rétablir la valeur hédonique spontanément négative de l'odeur (*Root et al. 2014*). Ainsi, l'existence d'une voie privilégiée entre la partie dorsale du BO et l'amygdale corticale



permettrait une réponse comportementale adaptée et universelle aux odeurs représentant un danger pour la survie de l'animal. A contrario, la partie médiale de l'amygdale ainsi que la voie BO-amygdale médiale sont activées en réponse à l'odeur congénère du sexe opposé mais pas à celle de congénère du même sexe ou de prédateur (*Westberry et Meredith 2016; Kang, Baum, et Cherry 2009*).

De plus, un certain nombre d'études s'est intéressé aux rôle de l'amygdale dans un contexte d'apprentissage associatif odeur/choc électrique, le potentiel de champ local de l'amygdale corticale augmente en réponse à l'odeur devenue répulsive alors que l'amygdale basolatérale ne répond pas à l'odeur mais seulement au choc électrique (*Sevelinges et al. 2004*). De plus, durant ce même type d'apprentissage conditionné de peur, l'amygdale synthétise du glutamate et du GABA et cette synthèse s'effectue avant la synthèse observée dans le PirCX (*Hegoburu et al. 2009*). Ces résultats suggèrent que l'amygdale serait impliquée en amont du PirCX dans l'acquisition du comportement. L'amygdale est également fortement impliquée dans la mise en place de la valeur hédonique au cours du développement (*Perry et al. 2016*). Chez les nouveaux nés la lésion de l'amygdale altère l'acquisition par apprentissage associatif de la valeur hédonique des odeurs (*Sullivan et Wilson 1993*).

### c) Etudes chez l'Homme

Chez l'Homme, alors que certaines études réalisées en tomographie à émission de positons (TEP) ou en IRMf s'accordent sur une activation de l'amygdale en réponse aux odeurs déplaisantes (*Zald et Pardo 1997; Royet et al. 2003*). Certaines études accordent également à l'amygdale un rôle dans le codage de la valeur hédonique positive des odeurs. En effet, il a été montré que le caractère plaisant ou déplaisant d'une odeur serait latéralisé chez l'Homme. Les odeurs déplaisantes activeraient préférentiellement l'amygdale de l'hémisphère droit alors que les odeurs plaisantes activeraient préférentiellement l'amygdale gauche (*Patin et Pause 2015*). De plus, une augmentation d'activité de l'amygdale est observée en réponse aux odeurs plaisantes dans un contexte d'apprentissage (*Gottfried, O'Doherty, et Dolan 2002*). D'autres encore suggèrent que l'amygdale ne serait pas impliquée dans le codage de la valeur hédonique (*Gottfried et al. 2002; Anderson et al. 2003; Zelano et al. 2007*) mais seulement dans celui de l'intensité de l'odeur (*Anderson et al. 2003; Rolls, Kringelbach, et de Araujo 2003*). Plus récemment, une étude soutient que l'amygdale posséderait la capacité de coder la totalité de la dimension hédonique indépendamment de l'intensité. En effet, le codage des odeurs plaisantes et déplaisantes se feraient tous deux dans l'amygdale mais selon des patrons d'activité spatio-

temporelle différents, le caractère déplaisant étant codé plus précocement et dans une localisation différente du caractère plaisant (*Jin et al. 2015*).

#### *d) Conclusion*

La représentation topographique dorso-ventrale de la valeur hédonique des odeurs observée dans le BO semble être ainsi conservée au niveau de l'amygdale. La valeur hédonique des odeurs ayant une pertinence éthologique pour l'animal serait codée spatialement au sein de l'amygdale, la partie corticale codant pour la valeur hédonique négative (avec ou sans apprentissage) et la partie médiale pour la valeur hédonique positive. Toutefois, l'amygdale ne semble pas particulièrement répondre aux odeurs ne possédant pas de forte signification éthologique pour l'animal (odeurs autres que celles de prédateur ou de congénère du sexe opposé). Dans les études d'imagerie chez l'Homme, l'absence de consensus quant à l'activation de l'amygdale en réponse aux odeurs plaisantes ou déplaisantes pourrait en partie provenir du manque de résolution spatiale de ces techniques, ne permettant pas de distinguer de sous-systèmes au sein de l'amygdale codant soit pour les odeurs plaisantes soit pour les odeurs déplaisantes. De plus, comme le suggère l'étude de Jin et al. (*Jin et al. 2015*), il serait intéressant de prendre en compte la composante temporelle de l'activation de l'amygdale afin de mieux appréhender ce processus dans sa complexité.

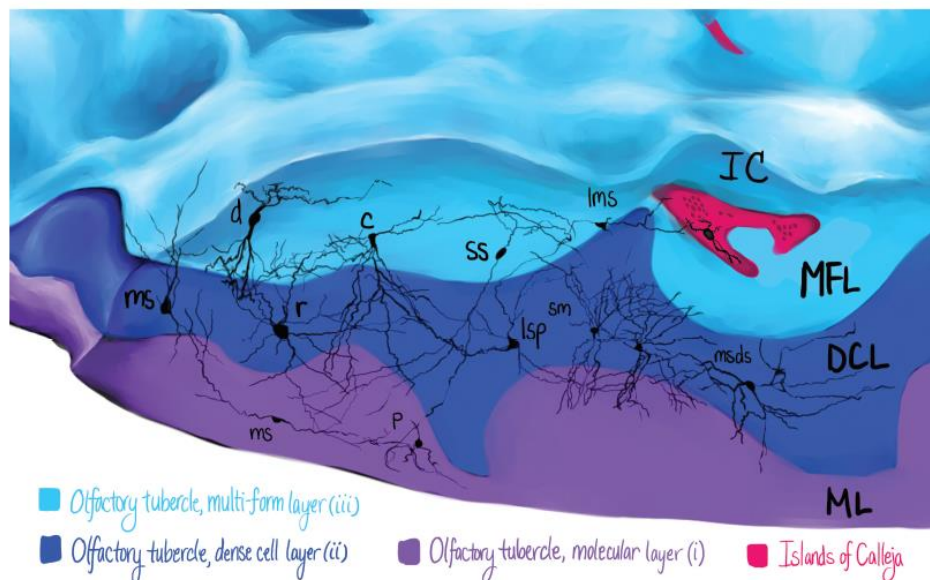
## 5- Le tubercule olfactif

### *a) Structure*

Le TO est une structure du prosencéphale basal. De par son origine embryonnaire, son anatomie et sa composition en neurotransmetteur, le TO a la particularité d'être considéré comme faisant partie du striatum ventral (*Alheid et Heimer 1988; Voorn et al. 2004*).

Comme l'ensemble du striatum, le TO est majoritairement composé de cellules GABAergique (*Cansler et al. 2020*). Le TO est divisé en deux parties principales, la partie cap du TO qui ne présente pas d'organisation laminaire et la partie corticale. Dans la partie corticale du TO, les cellules sont réparties en trois couches (Figure 6). La couche I ou couche moléculaire, la plus ventrale et donc la plus superficielle du TO, permet une modulation locale de l'information notamment par les cellules piales. La couche II ou couche des cellules denses, est composée majoritairement des cellules de projections du TO, les Medium Spiny Neurons (MSN). Les MSN sont présents dans l'ensemble du TO mais sont fortement condensés dans la

couche II. Ils envoient de longs prolongements à l'extérieur du TO afin de notamment connecter les autres structures du striatum et le mésencéphale. La couche III ou couche multiforme est quant à elle la couche la plus dorsale. D'autres sous-régions sont également présentes dans le TO, il s'agit des îlots de Calleja. Les îlots de Calleja sont des agrégats de cellules GABAergiques présents dans l'ensemble du TO.



*Figure 6 : Structure du tubercule olfactif.*

*Le tubercule olfactif est composé de trois couches : la couche moléculaire (I), la couche des cellules denses (II) et la couche multiforme (III) (Xiong et Wesson 2016).*

Le TO est communément divisé en TO médian et TO latéral (Alheid et Heimer 1988; Voorn et al. 2004), le TO médian étant dans l'alignement de l'amygdale.

#### *b) Connexions avec le système olfactif*

Le TO est une structure multi-sensorielle qui reçoit majoritairement des connexions du système olfactif (White 1965), mais aussi de la rétine (Mick, Cooper, et Magnin 1993) et du cortex auditif (Budinger et al. 2006). Ici, nous nous focaliserons sur les connexions sensorielles du TO avec le BO et le PirCX (Figure 7).

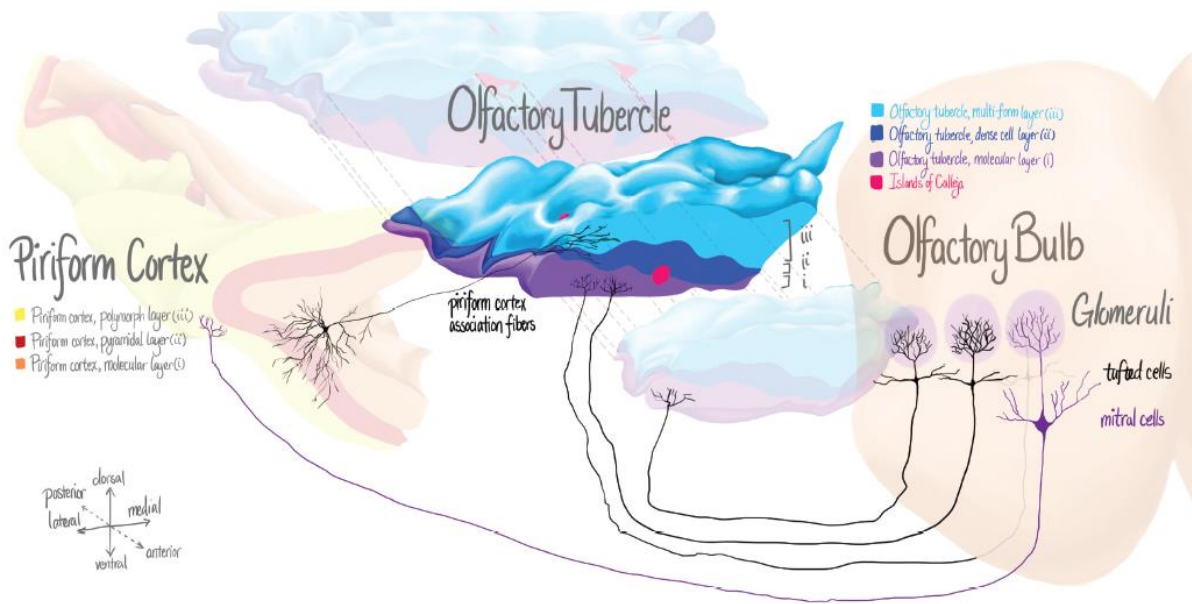


Figure 7 : Connexions directes et indirectes du bulbe olfactif sur le tubercule olfactif.

Les axones des cellules mitrales et des cellules à panache du BO projettent de façon directe sur la couche I du TO. Le BO projette également sur les cellules pyramidales du PirCX qui projettent à leur tour sur la couche II du TO (Xiong et Wesson 2016).

Le TO est une structure olfactive secondaire, donc par définition il reçoit des projections directes du BO. Plus précisément, ce sont les axones des cellules mitrales et des cellules à panaches du BO qui empruntent le tractus olfactif latéral pour projeter sur le TO (Xiong et Wesson 2016; Zhang, et al. 2017). Les cellules mitrales et à panaches connectent les cellules de la couche moléculaire du TO. Environ 10% des projections du BO connectent le TO, et majoritairement sa partie latérale (Price 1973; Schwob et Price 1984; Shipley 1985). Une étude basée sur la date de naissance des neurones montre que le TO est innervé par les cellules mitrales du BO se formant tardivement (E12) lors de l'embryogenèse. Or les cellules mitrales de la partie postérieure du BO se forment plus tardivement (E12) que les cellules mitrales de la partie antérieure. Ainsi, ces données suggèrent que les connexions bulbaires du TO proviendraient majoritairement de la partie postérieure du BO (Imamura et al. 2011). De façon intéressante, l'équipe a montré que la partie postérieure du BO code pour la valeur hédonique spontanément positive des odeurs (Kermen et al. 2016). L'ensemble de ces données permet d'émettre l'hypothèse d'une connexion privilégiée entre les cellules mitrales de la partie postérieure du BO et le TO pour sous tendre la valeur hédonique positive des odeurs.

Le TO reçoit également des projections indirectes du BO via le PirCX. En effet, les cellules mitrales du BO projettent majoritairement sur les cellules pyramidales du PirCX qui projettent à leur tour sur le TO (*Xiong et Wesson 2016; Johnson et al. 2000*). Tout comme les projections directes, les connexions indirectes du BO via le PirCX innervent principalement la partie latérale du TO. Cependant, les cellules pyramidales du PirCX ne projettent pas sur la couche I comme les cellules mitrales du BO mais directement sur la couche II du TO, permettant ainsi une activation des MSN. Une étude a montré l'implication fonctionnelle de la voie indirecte BO-TO via le PirCX en mettant en évidence la présence d'activité bi-phasique au sein du TO qui proviendrait d'une stimulation directe depuis le BO puis d'une stimulation indirecte via le PirCX (*Carriero et al. 2009*). Le TO est donc innervé par le BO par des connexions à la fois directes et indirectes.

En recevant des informations à la fois du BO et du PirCX, le TO apparaît comme étant fortement interconnecté avec le système olfactif. Cependant, le TO est une structure olfactive particulière qui comme nous l'avons vu précédemment tient sa singularité de son appartenance au striatum ventral. Le TO est ainsi considéré comme un carrefour entre le système olfactif et le système de récompense. Afin de comprendre comment le TO traite l'information relative au caractère plus ou moins plaisant de l'odeur, il est donc nécessaire de détailler les connexions du TO avec le système de récompense et de façon plus générale de décrire les mécanismes neuronaux sous-tendant les récompenses.

### *c) Connexions avec le système de récompense*

#### *Notion de récompense*

Une récompense est définie en neurobiologie comme un stimulus qui suscite et renforce le comportement d'approche (*Schultz 1998*). Les récompenses vont tout d'abord initier de la part de l'individu un comportement d'approche. Elles vont ensuite augmenter la fréquence et l'intensité du comportement conduisant à leur obtention. Enfin, elles vont empêcher l'extinction de ce comportement. On dit alors que les récompenses renforcent positivement le comportement à l'origine de leur obtention. Plusieurs stimuli sont connus comme étant des récompenses chez l'Homme, c'est notamment le cas du goût sucré, de l'argent, d'un visage souriant, de l'art, de la musique, des relations sexuelles ou de l'amour (*Berridge, Robinson, et Aldridge 2009; Pessiglione et al. 2007; Salimpoor et al. 2011; Ferreri et al. 2019; Lacey et al. 2011; Fisher, Aron, et Brown 2005; Zeki 2007*).

#### *Dopamine, récepteurs dopaminergiques et tubercule olfactif*

La dopamine est le neuromodulateur sous-tendant l'effet renforçateur des récompenses. La dopamine est une monoamine synthétisée à partir des acides aminés tyrosine ou phénylalanine. On dénombre deux sources principales de dopamine dans l'encéphale : la substance noire et l'aire tegmentale ventrale (VTA) (*Fallon et Moore 1978*). Historiquement, il a tout d'abord été mis en évidence que la dopamine sécrétée par la substance noire joue un rôle dans les comportements moteurs (*Berke 2018*). Le rôle de la dopamine dans la motivation a quant à lui été révélé plus tard par des enregistrements électrophysiologiques de la VTA chez le singe (*Schultz et Romo 1990*). Ainsi la dopamine synthétisée et sécrétée par la VTA est le substrat neurobiologique sous-tendant le pouvoir récompensant des stimuli naturels tels que la nourriture ou l'accouplement, ou moins naturels tels que les drogues (*Imaizumi, Takeda, et Fushiki 2000; Domínguez-Salazar, Naser, et Velázquez-Moctezuma 2014; Robinson et Berridge 2003; Ikemoto et Bonci 2014*).

Il existe cinq types de récepteurs dopaminergiques : D1, D2, D3, D4 et D5. Ces récepteurs sont répartis en deux familles selon la protéine G à laquelle ils sont couplés. La famille des récepteurs de type D1 comprend les récepteurs D1 et D5 qui sont couplés à des protéine G de type activatrice ( $G_s$ ). A l'inverse, la famille des récepteurs de type D2 est composée des récepteurs D2, D3 et D4 qui sont quant à eux couplés à des protéine G de type inhibitrice ( $G_i$ ). Les différentes familles de récepteurs peuvent être exprimées au sein d'une même structure cérébrale. De façon globale, il est admis que les récepteurs de type D1 auraient plutôt un rôle dans le renforcement positif alors que les récepteurs de type D2 auraient plutôt un rôle dans le renforcement négatif. Cependant, la fixation de la dopamine sur un récepteur de type D1 ou de type D2 peut avoir des effets opposés mais également synergiques selon la structure ciblée (*Steinberg et al. 2014*). Les cellules du TO expriment des récepteurs dopaminergiques de type D1, D2 et D3 mais les MSN expriment seulement les récepteurs de type D1 et D2 (*Murata et al. 2015*). Les récepteurs dopaminergiques présents à la surface des cellules du TO permettent à la VTA de moduler l'activité du TO.

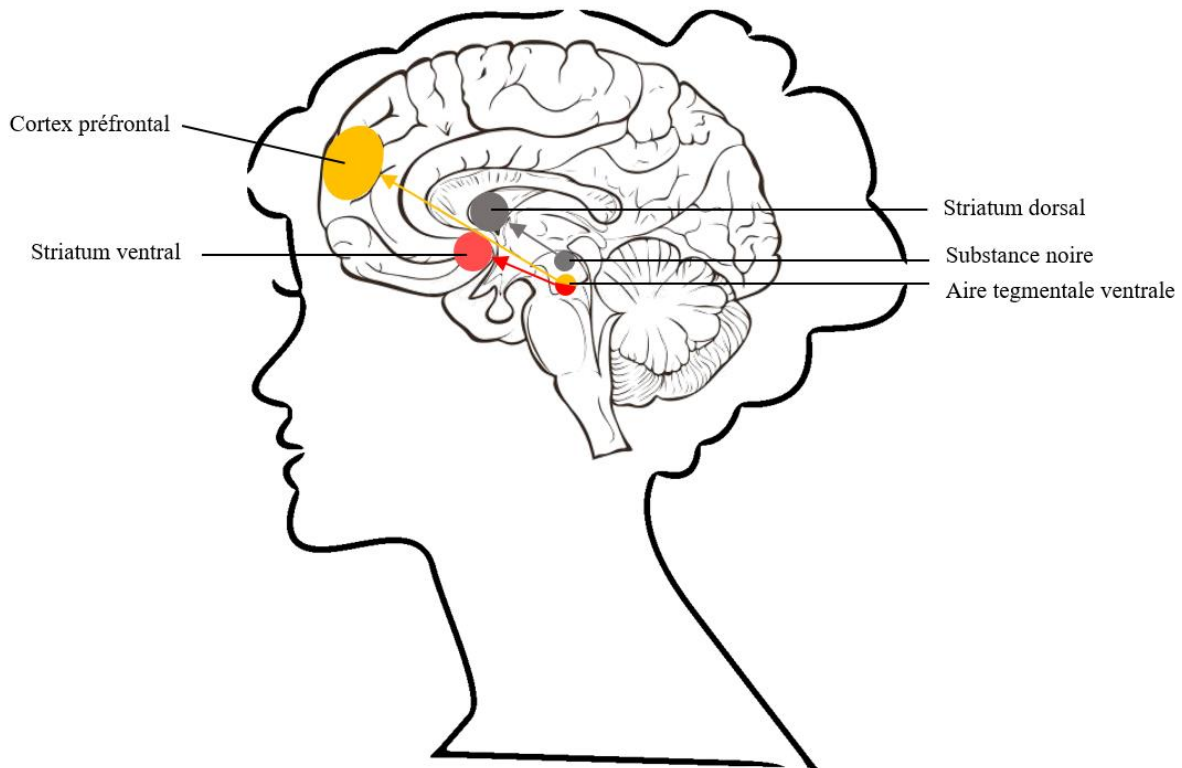
D'un point de vu anatomo-fonctionnel, le TO fait partie intégrante du système de récompense. Quels sont les arguments anatomiques et fonctionnels qui ont mené à cette observation ?

#### *Le tubercule olfactif, une structure du système de récompense : arguments anatomiques*

Il existe trois principales voies dopaminergiques : la voie méso-limbique, la voie méso-corticale et la voie nigro-striée (Figure 8). Le tubercule olfactif fait partie de la voie méso-



limbique qui est constituée des corps cellulaires de neurones dopaminergiques de la VTA situés dans le mésencéphale et de leurs projections au niveau du pallidum ventral et du striatum ventral dont font partis le noyau accumbens (NAc) et TO. Plus précisément, la VTA postéro-médiale, qui semble être la plus impliquée dans les comportements récompensants, projette sur le TO médian et la partie médiale du NAcShell alors que la VTA latérale projette sur le IOT, le NAcCore et la partie latérale du NAcShell (Ikemoto 2007; Zhang, et al. 2017).



*Figure 8 : Les voies dopaminergiques.*

*La voie méso-limbique est constituée des projections dopaminergiques de l'aire tegmentale ventrale vers le striatum ventral (en rouge). La voie méso-corticale est constituée des projections dopaminergiques de l'aire tegmentale ventrale vers le cortex préfrontal (en jaune). La voie nigro-striée est constituée des projections dopaminergiques de la substance noire vers le striatum dorsal (en gris).*

De par son appartenance au striatum ventral, le TO est fortement interconnecté avec le NAc et le pallidum ventral (VP) (Xiong et Wesson 2016). Les MSN du TO et du NAc forment ce que l'on appelle un "pont striatal", permettant au TO de moduler l'activité du NAc et inversement (Figure 9).

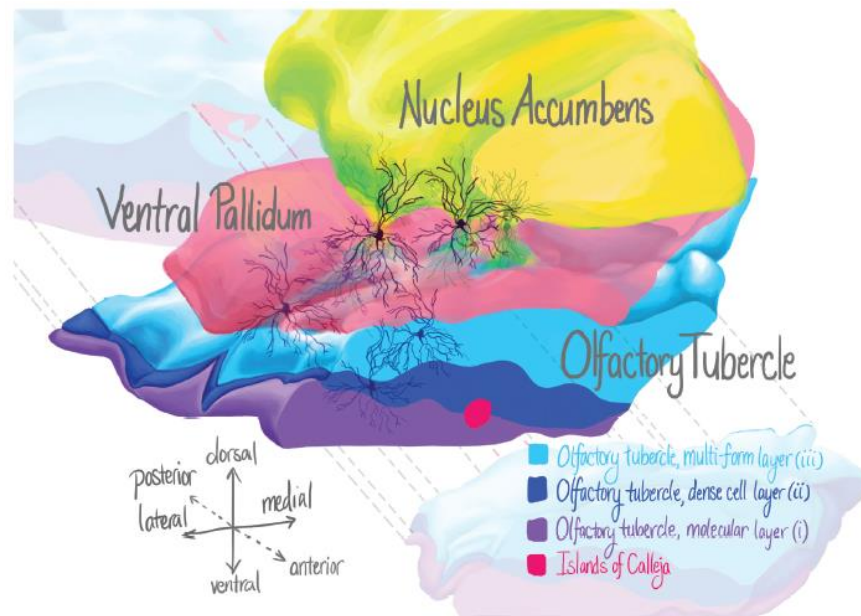


Figure 9 : Connexions du tubercule olfactif avec le noyau accumbens et le pallidum ventral.

*Les MSN du TO, du NAc et du VP sont fortement connectés. Les connexions entre le TO et le NAc forment le "pont striatal". (Xiong et Wesson 2016).*

De par ses afférences dopaminergiques provenant de la VTA et son interconnexion avec le NAc et le VP, le TO fait donc partie intégrante du système de récompense d'un point de vue anatomique.

*Le tubercule olfactif, une structure du système de récompense : arguments fonctionnels*

L'existence d'un réseau dédié au codage de l'effet renforçateur des récompenses a été mis en évidence dans les années 50 par les expériences dites d'autostimulation intracérébrales. Ces expériences réalisées chez le rongeur consistaient à implanter une électrode dans une aire cérébrale et à donner la possibilité à l'animal de s'auto-délivrer une stimulation électrique dans ladite structure en appuyant sur un levier. Ces expériences sont des conditionnements de type opérant lors desquels l'animal apprend que l'appuie sur le levier conduit à la délivrance de la stimulation intracérébrale. Olds et Milner (*Olds et Milner 1954*) et de nombreux autres après eux ont mis évidence que lorsque la stimulation était délivrée le long de ce que l'on nomme le "medial forebrain bundle", les animaux appuyaient de plus en plus sur le levier afin d'obtenir la stimulation électrique, présentant ainsi un comportement d'autostimulation intracérébrale. Depuis leurs débuts, les expériences d'autostimulations intracérébrales ont permis de révéler les structures appartenant au système de récompense (*Olds et Fobes 1981; Bielajew et Harris*



1991). En effet, une autostimulation intracérébrale est notamment observée dans la VTA, le NAc et le VP. Plusieurs études ont également tenté d'appliquer les expériences d'autostimulation intracérébrale à l'Homme (*Bishop, Elder, et Heath 1963*).

L'implication fonctionnelle du TO dans le système de récompense a également été révélée par les expériences d'autostimulation intracérébrale. En effet, la stimulation directe du TO ainsi que la stimulation de la voie VTA-TO médian induit un comportement d'autostimulation intracérébrale (*Kornetsky, Huston-Lyons, et Porrino 1991; Fitzgerald, Richardson, et Wesson 2014; Zhang, et al. 2017*). De plus, le TO constitue également un site d'auto-administration de drogues. En effet, les animaux sont capables de se conditionner pour s'auto-administrer de la morphine, de la cocaïne ou encore de l'amphétamine ainsi que ses dérivés spécifiquement dans le TO (*Kornetsky, Huston-Lyons, et Porrino 1991; Ikemoto 2003; Ikemoto et Donahue 2005; Ikemoto, Qin, et Liu 2005; Sellings, McQuade, et Clarke 2006; 2006; Shin et al. 2008*).

#### *d) Codage la valeur hédonique des odeurs dans le tubercule olfactif*

Alors que le rôle de la partie cap du TO n'est à ce jour pas très bien définie, une représentation topographique de la valeur hédonique des odeurs a été observé au sein du TO cortical. Il a en effet été montré qu'après un apprentissage associatif odeur/sucre ou odeur/choc électrique, les odeurs devenues plaisantes activent les MSN de la partie médiale du TO alors que les odeurs devenues déplaisantes activent les MSN de la partie latérale du TO et que les récepteurs de type D1 du TO semblent plus impliqués que les récepteurs de type D2 quelque que soit l'apprentissage (*Murata et al. 2015*). Il apparaît que la partie médiale du TO semble également être plus impliquée dans le codage de la valeur hédonique positive innée des odeurs ayant une pertinence éthologique pour l'animal que la partie latérale. En effet, une lésion ou une inhibition de la partie médiale, et non de la partie latérale, du TO induit une altération du traitement des signaux olfactifs d'attraction pour le sexe opposé (*DiBenedictis et al. 2014; DiBenedictis et al. 2015; Agustín-Pavón, Martínez-García, et Lanuza 2014*). La valeur hédonique des odeurs serait ainsi codée spatialement au sein du TO, la partie médiale codant pour la valeur hédonique positive et la partie latérale pour la valeur hédonique négative.

En parallèle, des études chez l'animal ont montré que la stimulation directe du TO ou la stimulation de la voie VTA-TO médian altère les préférences olfactives (*Fitzgerald, Richardson, et Wesson 2014; Zhang, Liu, et al. 2017*). De plus, après un apprentissage associatif entre une odeur et une récompense, l'acquisition de la nouvelle valeur hédonique de l'odeur

induit une activation du TO (*Gadziola et al. 2015*). En effet, il a été observé que les neurones du TO augmentent leur taux de décharge de façon robuste et flexible en réponse à l'odeur prédictive de récompense et que bien qu'apparaissant lors de la stimulation olfactive, cette modulation d'activité coderait la réponse comportementale qui s'en suivait.

*e) Etudes chez l'Homme*

Peu d'étude ont analysé l'activité du TO en IRMf de par sa petite taille et sa forme très variable entre les individus (*Zelano et al. 2005*). De ce fait, le rôle du TO dans le codage de la valeur hédonique reste controversé chez l'Homme. En effet, il a été montré que l'activité de structures olfactives dans ou à proximité du TO est positivement corrélée avec le jugement hédonique des participants (*de Araujo et al. 2005*). Cependant, Zelano et al. suggèrent que l'activité du TO serait associée au type odorant (olfactif ou trigéminal) mais pas à la valeur hédonique de l'odeur (*Zelano et al. 2007*).

*f) Conclusion*

Le TO possède donc une signature neurale de la valeur hédonique des odeurs. Les études menées jusqu'à ce jour ont porté soit sur les odorants ayant une signification éthologique pour l'animal soit sur des odorants placés dans un contexte d'apprentissage et donc induisant un fort comportement d'approche ou de répulsion. Qu'en est-il des odorants ne possédant pas de signification éthologique particulière mais induisant tout de même de façon spontanée un comportement d'approche ou de répulsion? Chez l'Homme, du fait du peu d'étude ayant étudié l'activité de TO en réponse à une odeur, il n'est à ce jour pas encore possible d'étendre les mécanismes observés chez l'animal à l'Homme. De par sa position au carrefour entre le système olfactif et le système de récompense, le TO posséderait donc une localisation privilégiée pour convertir la valeur hédonique spontanément positive codée dans le BO en comportement motivé d'attraction pour l'odeur. La particularité de l'odorat à connecter rapidement le système de récompense fait-il de cette perception sensorielle une voie d'entrée privilégiée pour stimuler le système de récompense ?

## 6- Autres structures possédant un rôle dans le codage de la valeur hédonique des odeurs

D'autres structures ont été identifiées comme jouant un rôle dans le codage de la valeur hédonique des odeurs. C'est notamment le cas du cortex orbitofrontal (OFC). L'OFC est une

sous-région du cortex préfrontal considérée comme une structure associative. L'OFC reçoit notamment des projections du PirCX et est considéré de par cette connexion comme une structure olfactive tertiaire. Chez l'animal, il a été montré que l'OFC est impliqué dans le codage de la valeur hédonique acquise des odeurs. En effet, lors d'un apprentissage associatif avec un indiçage olfactif, l'activité de l'OFC varie au cours de l'acquisition de la nouvelle valeur hédonique (*Roesch, Stalnaker, et Schoenbaum 2007*). Le rôle de l'OFC a plus amplement été mis en évidence chez l'Homme par des études en IRMf. Ces études semblent s'accorder sur une représentation spatiale de la valeur hédonique des odeurs dans l'OFC. La valeur hédonique positive serait codée dans la partie médiale de l'OFC alors que la valeur hédonique négative dans la partie latérale (*Rolls, Kringelbach, et de Araujo 2003; Anderson et al. 2003; Grabenhorst et al. 2007; Gottfried et al. 2002*). Une étude suggère cependant que la partie médiale de L'OFC représenterait la valeur hédonique absolue de l'odeur alors que la région antéro-latérale permettrait l'évaluation d'une différence d'hédonicité entre les odeurs (*Grabenhorst et Rolls 2009*).

Les études effectuées en IRMf chez l'Homme révèlent également, mais de manière plus sporadique, un rôle de l'insula, du cortex cingulaire antérieur, de la VTA et du NAc dans le codage de la valeur hédonique des odeurs. L'insula, qui est le cortex gustatif primaire, semble répondre préférentiellement aux odeurs déplaisantes en lien avec la notion de dégoût (*Royet et al. 2003; Grabenhorst et Rolls 2009*). Il a également été observé que l'insula antérieure serait effectivement impliquée dans la représentation des odeurs déplaisantes mais que la partie postérieure de l'insula répondrait également aux odeurs plaisantes (*Wicker et al., 2003*). Le cortex cingulaire antérieur répondrait quant à lui plutôt aux odeurs ayant une valeur hédonique positive (*Bensafi et al. 2012*). Dans un contexte d'addiction, la VTA et le NAc répondent aux odeurs des stimuli addictifs (e.g. nourriture, alcool) (*Kareken et al. 2004; Bragulat et al. 2008; Bragulat et al. 2010*). De façon intéressante, une activation de la VTA a également été observée en réponse à un mélange d'odeurs agréables en dehors de tout contexte d'addiction (*Bensafi et al. 2012*).

Ainsi des structures extérieures au système olfactif, mais y étant connectées, semblent également être impliquées dans le codage de la valeur hédonique des odeurs.

## 7- Conclusion générale

Une signature neurale de la valeur hédonique des odeurs est retrouvée dans un certain nombre de régions du système olfactif. Comme nous l'avons vu, le caractère plaisant ou déplaisant d'une odeur est sous-tendu par des réseaux neuronaux différents. Ces réseaux distincts peuvent à la fois correspondre à des structures du système olfactif différentes ou à des sous-régions d'une même structure. Cependant, l'ensemble des structures du système olfactif interagissent les unes avec les autres et avec d'autres systèmes auxquels elles sont reliées. Afin de comprendre comment est codée la valeur hédonique des odeurs et comment est traduite en action motrice la valeur hédonique positive, il apparaît donc comme nécessaire de ne pas limiter son étude au système olfactif. Une des particularités du système olfactif est de faire partie du système limbique. La valeur hédonique étant un paramètre perceptif fortement émotionnel, la majorité des études actuelles se sont intéressées au rôle du système limbique dans la perception hédonique des odeurs. Cependant, selon sa valeur hédonique, une odeur va induire un comportement motivé d'approche ou de répulsion, permettant de s'adapter à son environnement. Les comportements motivés étant sous-tendus par le système de récompense, la valeur hédonique de l'odeur pourrait donc être en partie codée par le système de récompense et notamment par le TO qui pourrait constituer une porte d'entrée pour les informations olfactives. Cette thèse a donc pour ambition d'étudier la valeur hédonique à travers le prisme de la motivation afin de déterminer comment le système de récompense peut participer à la réponse hédonique d'une odeur.

La valeur hédonique d'une odeur peut être modulée par l'expérience, les apprentissages mais également lors du vieillissement normal ou pathologique. Dans une dernière partie de ma thèse, je me suis plus particulièrement intéressée à la modulation de la valeur hédonique des odeurs au cours du vieillissement et à comment elle est représentée au niveau cérébral avec l'âge.

## ***V. Valeur hédonique des odeurs et vieillissement***

Le vieillissement normal est accompagné de déficits à la fois cognitifs (*Toepper 2017*) et sensoriels (*Allen et Eddins 2010; Desai et al. 2001; Werner, Peterzell, et Scheetz 1990*). Parmi les déficits sensoriels, des troubles de la perception des odeurs apparaissent au cours du vieillissement.

## 1- Les troubles olfactifs liés à l'âge

Des déficits olfactifs sont couramment observés chez les personnes âgées. Plus de 50% des 65-80 ans sont atteints de troubles de l'odorat et cette prévalence augmente avec l'âge, atteignant près de 80% chez les plus de 80 ans (*Seubert et al. 2017; Doty et Kamath 2014; Lafreniere et Mann 2009*).

On peut définir deux types de troubles olfactifs liés à l'âge : les troubles d'ordre quantitatifs et les troubles qualitatifs. Les troubles quantitatifs correspondent à la perte partielle (hyposmie) à totale (anosmie) de l'odorat. Ils sont caractérisés par un seuil de détection des odeurs plus important que la moyenne de la population. Ces troubles induisent également des difficultés dans la discrimination et l'identification des odeurs. Les troubles olfactifs d'ordre qualitatifs correspondent quant à eux à une altération de la qualité de l'odeur (dysosmie), c'est-à-dire que les odeurs sont perçues différemment. Parmi ces troubles qualitatifs, la parosmie correspond à une dépréciation des odorants, c'est-à-dire qu'un même odorant sera perçu comme moins plaisant par une personne parosmique que par une personne ne présentant pas de troubles de l'odorat. Ainsi la valeur hédonique des odeurs peut être altérée au cours du vieillissement.

Chez l'animal, des troubles olfactifs d'ordre quantitatif apparaissent au cours du vieillissement. Ces troubles concernent à la fois la détection et la discrimination olfactives. En effet, le seuil de détection des odorants est plus élevé chez la souris âgée que chez la jeune (*Bodyak et Slotnick 1999; Kraemer et Apfelbach 2004; Patel et Larson 2009*), mais la détection reste possible à des concentrations supralaminaires. Les animaux âgés sont également toujours capables de discriminer deux odeurs très éloignées d'un point de vue perceptif (*Kraemer et Apfelbach 2004; Moreno et al. 2014; Rey et al. 2012*), mais la discrimination de deux odeurs perceptivement proches est plus difficile (*Mandairon, Peace, et al. 2011; Moreno et al. 2014; Yoder et al. 2017*). Concernant les troubles d'ordre qualitatif, ces derniers restent peu étudiés chez l'animal. Il apparaît que l'apprentissage de la nouvelle valeur hédonique d'une odeur, acquise par apprentissage associatif odeur/récompense, est toujours effectif chez l'animal âgé mais que cet apprentissage est plus lent que pour les animaux jeunes (*Dardou, Datiche, et Cattarelli 2008; Kraemer et Apfelbach 2004; LaSarge et al. 2007*). Cependant, les animaux âgés ayant des troubles d'apprentissage assez généraux, il est difficile de conclure quant à une possible altération du caractère hédonique des odorants.

## 2- Altération de la valeur hédonique des odeurs au cours du vieillissement

### a) *Mise en évidence de l'altération de la valeur hédonique des odeurs chez l'Homme*

Plusieurs études ont mis en évidence une modification du jugement hédonique des odeurs par les sujets âgés, cependant leurs résultats ne sont pas consensuels. Une première étude a observé que l'odeur de lavande et celle de menthe verte sont jugées comme plus plaisantes par des sujets âgés que par des sujets jeunes (*Wang et al. 2005*). Une seconde étude utilisant un panel d'odeur plus important a montré que la valeur hédonique de certaines odeurs reste constante au cours du vieillissement, alors que certaines odeurs deviennent moins plaisantes et d'autres plus plaisantes (*Markovic et al. 2007*). Enfin, une étude plus récente prenant en considération la valeur hédonique initiale des odeurs a démontré que seule la valeur hédonique des odeurs initialement plaisante est altérée au cours du vieillissement (*Joussain et al. 2013*) (mais voir (*Vieillard et al. 2020*)). Ainsi la valeur hédonique des odeurs déplaisantes ne varie pas en fonction de l'âge alors que les odorants jugés plaisants par des sujets jeunes sont jugés comme moins plaisants par des sujets âgés.

### b) *Effet sur le bien-être*

Il est largement admis que les déficits olfactifs liés à l'âge induisent des effets néfastes sur le bien-être physique, la qualité de vie, la nutrition, le plaisir de manger et la sécurité des personnes âgées (*Doty et Kamath 2014; Attems, Walker, et Jellinger 2015; Lafreniere et Mann 2009*). En effet, la diminution des capacités olfactives est fortement reliée à l'augmentation du risque de malnutrition observée chez les personnes âgées (*Schiffman et Zervakis 2002; Murphy 2008*). De plus, les déficits olfactifs sont à l'origine de nombreux accidents domestiques car plus de 65% des personnes âgées, contre 10% des jeunes, ne sont pas capables de détecter l'odeur du gaz domestique (*Chalke, Dewhurst, et Ward 1958; Stevens et al. 1987*). De façon stupéfiante, une étude longitudinale réalisée sur 1 162 sujets âgés ne présentant pas de démence a montré que le risque de mortalité est 36% plus élevé chez les personnes présentant des troubles olfactifs (*Wilson, Yu, et Bennett 2011*).

De façon plus spécifique, l'altération de la valeur hédonique des odeurs induit des effets néfastes sur le bien-être, c'est notamment le cas dans la maladie d'Alzheimer, la maladie de Parkinson, les maladies liées aux troubles de l'humeur comme la dépression, la schizophrénie, mais aussi dans le vieillissement normal (*Joussain et al. 2016; Mrochen et al. 2016; Atanasova et al. 2010; Lombion-Pouthier et al. 2006; Moberg et al. 2003; Naudin et al. 2014; Walsh-*

*Messinger et al. 2018; Joussain et al. 2017*). L'altération de la valeur hédonique des odeurs a été reliée au statut nutritionnel des personnes âgées (*Joussain et al. 2017*). En effet, il est possible de prédire le statut nutritionnel d'un individu âgé grâce de sa réponse psychophysiological à une odeur. Ainsi, les personnes présentant une augmentation de la fréquence cardiaque en réponse aux odeurs aversives possèdent un meilleur statut nutritionnel et cette corrélation n'est pas observée chez les sujets jeunes. Cette étude met en évidence que la conservation d'un jugement hédonique correct au cours du vieillissement permettrait une meilleure sélection des aliments et améliorerait ainsi l'apport nutritionnel des personnes âgées.

### 3- Bases neurales de l'altération de la valeur hédonique des odeurs liée à l'âge

Un certain nombre de structures cérébrales ont été identifiées comme jouant un rôle dans le codage de la valeur hédonique des odeurs chez le sujet jeune. Quand est-il du sujet âgé ? La modulation de la valeur hédonique avec l'âge est-elle due à des altérations anatomo-fonctionnelles spécifiques de structures clés codant pour le caractère plaisant des odorants, à une modification globale du réseau ou à des atteintes d'ordre périphérique ? Très peu d'études se sont intéressées à cette problématique. Elles sont décrites ci-dessous.

#### *a) Rythmes beta et altération de la valeur hédonique des odeurs liée à l'âge*

L'étude EEG menée chez la personne âgée par *Joussain et al. (Joussain et al. 2013)* a mis en évidence que l'altération du caractère agréable des odeurs est accompagnée par une désynchronisation dans la bande de fréquence beta en réponse à ces odeurs. Chez l'Homme, la signification des rythmes EEG dans la modalité olfactive n'est pas bien connue. Cependant, le fait que la modulation dans la bande bêta soit observée à un stade précoce du processus olfactif (300 ms) suggère une éventuelle modulation au niveau des structures olfactives primaires et/ou secondaires. Par ailleurs, chez l'animal, les oscillations beta observées notamment dans le BO et dans les structures olfactives secondaires ont été reliées aux propriétés physiques de l'odorant ainsi qu'à différents processus olfactifs tel que la discrimination et l'apprentissage associatif (*Kay et al. 2009; Frederick et al. 2016; Courtiol, Buonviso, et Litaudon 2019*).

Ainsi, l'altération de la valeur hédonique observée au cours du vieillissement reste à ce jour très mal comprise et afin de mieux comprendre ses bases neurales, il est nécessaire de



s'intéresser au vieillissement des structures olfactives impliquées dans le codage de la valeur hédonique des odeurs.

*b) Vieillessement des structures impliquées dans le codage de la valeur hédonique des odeurs chez le jeune*

Tout comme l'ensemble des systèmes sensoriels, le système olfactif subit des altérations à la fois structurales et fonctionnelles au cours du vieillissement normal. Ces altérations sont observées à tous les niveaux du traitement de l'information olfactive, de l'épithélium olfactif, jusqu'aux structures olfactives secondaires et tertiaires, en passant par le BO.

*Vieillessement de l'épithélium olfactif*

Au sein de l'épithélium olfactif, le nombre de neurones sensoriels olfactifs diminue au cours du vieillissement. Cette diminution peut être causée par certains facteurs environnementaux mais également par la mort cellulaire plus importante des neurones sensoriels olfactifs (*Conley et al. 2003*) et la diminution de leur renouvellement due à un défaut de prolifération des cellules basales (*Weiler et Farbman 1997*). De plus, l'expression de certains gènes des récepteurs olfactifs diminue au cours du vieillissement (*Cavallin et al. 2010; Khan, Vaes, et Mombaerts 2013*). Toutefois, l'intégrité de la réponse des neurones sensoriels olfactifs subsistant dans l'épithélium olfactif des souris âgées semble maintenu (*Lee et al. 2009*).

Des études histologiques de l'épithélium olfactif humain ont également montré des changements dans sa nature et son intégrité, notamment une diminution du nombre de récepteurs, un amincissement de l'épithélium en général et un remplacement de l'épithélium olfactif par l'épithélium respiratoire (*Doty et Kamath 2014; Naessen 1971; Nakashima, Kimmelman, et Snow 1984; Morrison et Costanzo 1990; Paik et al. 1992*).

*Vieillessement du bulbe olfactif*

Plusieurs études ont montré une diminution du volume du BO au cours du vieillissement, et plus particulièrement au niveau de la couche glomérulaire, chez l'Homme tout comme chez l'animal (*Hinds et McNelly 1981; Bhatnagar et al. 1987; Yousem et al. 1998; Sama-ul-Haq, Tahir, et Lone 2008*). Cependant, une étude plus récente a montré chez la souris que le BO ne semble pas s'atrophier au cours du vieillissement mais que la densité synaptique au sein des glomérules diminue ce qui suggère une diminution des connexions entre les axones olfactifs et les cellules mitrales et/ou entre les axones olfactifs et les cellules mitrales ou encore entre les cellules mitrales et les cellules périglomérulaires (*Richard, Taylor, et Greer 2010*).



Par ailleurs, le phénomène de neurogenèse adulte qui se produit au sein du BO chez le rongeur semble également s'altérer au cours du vieillissement. De façon précoce, dès l'âge de 6 mois, la prolifération des progéniteurs de la zone sous-ventriculaire diminue fortement conduisant à une réduction de 65% de nouveaux neurones formés chez la souris (*Enwere et al. 2004; Rey et al. 2012; Moreno et al. 2009; 2014*). Plus tardivement, autour de 18 mois, le taux de différenciation et de survie des nouveaux neurones diminue également (*Rey et al. 2012*).

Aucune étude à ce jour n'a analysé finement les modifications liées à l'âge de la structure du BO en fonction de son axe dorso-ventral ou antéro-postérieur ni révélée de changement dans les représentations topographiques de la valeur hédonique des odeurs.

#### *Vieillesse des structures olfactives secondaires et tertiaires*

Les études d'imagerie réalisées chez le sujet âgé ont montré une diminution du volume de plusieurs structures olfactives secondaires dont le PirCX, l'amygdale et le cortex entorhinal (*Segura et al. 2013*). De plus, le PirCX, l'amygdale (*Wang et al. 2005*), mais aussi l'OFC (*Suzuki et al. 2001*), voient leur activité en réponse à une odeur diminuer au cours du vieillissement. De façon surprenante, le volume, la densité cellulaire et la densité axonale du PirCX semble pourtant rester relativement stable chez l'animal (*Curcio, McNelly, et Hinds 1985*). Cependant, une perte des récepteurs synaptiques AMPA est observée au sein du aPirCX, ce qui suggère qu'il pourrait y avoir des changements subtils dans la fonction synaptique glutamatergique au cours du vieillissement (*Gocel et Larson 2013*).

#### *Vieillesse du tubercule olfactif*

Au niveau du TO, des changements dans le nombre, le volume et la localisation des îlots de Calleja ont été observés au cours du vieillissement, suggérant une modification du traitement de l'information olfactive aussi bien à l'intérieur qu'à l'extérieur du TO (*Adjei et al. 2013*). Les îlots de Calleja étant majoritairement composés de cellules GABAergiques, un nombre réduit d'îlot rendrait le TO plus excitable et/ou incapable d'exercer une inhibition sur les structures auxquelles il est connecté. Ainsi, la réduction des îlots de Calleja observée au cours du vieillissement pourrait entraîner une instabilité importante au sein du système de récompense. Or, le système dopaminergique est très fortement dégradé chez le sujet âgé. En effet, une diminution de la quantité de dopamine, de ses enzymes de synthèse et de ses récepteurs est observée au niveau de l'aire tegmentale ventrale et du striatum (*Araki et al. 1997; Irwin et al. 1994; Schuligoi et al. 1993; Barili et al. 1998; Watanabe 1987; Norrara et al. 2018*). De plus, on observe une dégénérescence des neurones dopaminergiques au cours du vieillissement

normal, comme le montre notamment l'altération de la fonction protéasome/lysosome, les dommages oxydatifs et l'inflammation qui augmentent avec l'âge (*Collier, Kanaan, et Kordower 2017*). Par ailleurs, certains troubles olfactifs d'ordre quantitatif ont été corrélés avec la perte de substance grise dans le NAc (*Bitter et al. 2010*) et ont été reliés à un défaut de fixation de dopamine au niveau du striatum (*Larsson et al. 2009*).

Ainsi, le TO pourrait posséder un rôle majeur dans l'altération de la valeur hédonique liée à l'âge.

#### 4- Conclusion générale

Comme nous l'avons vu, la valeur hédonique des odeurs est fortement altérée au cours du vieillissement normal. Cette dépréciation des odeurs possède de nombreux effets néfastes sur le bien-être des personnes âgées, notamment sur leur alimentation. La détermination des mécanismes neuronaux responsables de cette altération de la valeur hédonique liée à l'âge représente donc un enjeu de santé public majeur.

A notre connaissance, seule une étude menée chez l'homme a essayé de déterminer les bases neurales de la dépréciation des odeurs observée au cours du vieillissement. Cette étude suggère que l'altération de la valeur hédonique serait liée à des modifications de l'activité des structures olfactives primaires et/ou secondaires. De nombreuses altérations à la fois structurales et fonctionnelles apparaissent au cours du vieillissement à tous les étages du système olfactifs et des structures supérieures étroitement connectées (épithélium olfactif, BO, PirCX, TO et OFC). Ces modifications pourraient toutes participer à l'altération de la valeur hédonique liée à l'âge et il serait intéressant de déterminer si tel est le cas.

## **OBJECTIFS DE LA THÈSE**

La valeur hédonique des odeurs est une composante principale de la perception olfactive. En effet, elle est un paramètre déterminant permettant de guider les comportements d'approche ou d'évitement. Ces comportements sont essentiels à la survie aussi bien chez l'Homme que chez l'animal en participant à l'alimentation, aux interactions sociales et à l'évitement des dangers. Toutefois, la capacité d'appréciation ou de dépréciation des odeurs peut être modifiée dans plusieurs conditions dont le vieillissement normal. Ainsi, l'objectif global de cette thèse est de déterminer les bases neurales de la valeur hédonique des odeurs et leurs altérations au cours vieillissement normal et de mettre à profit ces découvertes pour développer de nouvelles méthodes de mesure de la valeur hédonique.

Le BO est la première structure corticale du système olfactif. L'implication du BO dans le traitement du caractère hédonique des odeurs ne possédant pas de signification biologique particulière a été dévoilée par une étude précédente de l'équipe (*Kermen et al. 2016*). En effet, il a été mis en évidence que la valeur hédonique est représentée le long de l'axe antéro-postérieur du BO, les odorants plaisants étant représentés dans la partie antérieure et les odorant déplaisants dans la partie postérieure de la couche granulaire du BO. De plus, la modulation par optogénétique de ces représentations neurales induit une modification de la valeur hédonique initiale de l'odeur.

Le premier objectif de cette thèse était de faire le point sur l'état des connaissances actuelles quant au codage de la valeur hédonique des odeurs dans le BO. Indépendamment de la représentation topographique mis en évidence par Kermen et al. (*Kermen et al. 2016*), plusieurs autres représentations topographiques de la valeur hédonique des odeurs sont présentes au sein du BO. Ces représentations dépendent du type d'odorant (avec ou sans signification éthologique particulière) et sont fortement plastiques, pouvant ainsi être modulée par apprentissage ou par association avec d'autres odorants (**Revue 1**).

Le second objectif de cette thèse était de déterminer comment est traitée l'information hédonique dans les structures au-delà du BO. En effet, nous avons étudié comment la représentation hédonique de l'odeur présente dans le BO est transformée en comportement motivé d'approche de l'odeur. Ainsi, nous avons émis l'hypothèse que les odorants plaisants solliciteraient le système de récompense et décrypté les circuits neuronaux impliqués. Ainsi, nous avons combiné des approches optogénétique, iDISCO, de cartographie d'activité cellulaire, de comportement animal, et de pharmacologie chez la souris. Nous avons ensuite étendu nos recherches chez l'Homme grâce à des tests comportementaux en living lab et d'IRMf (**Etude 1**).

L'altération de la valeur hédonique des odeurs liée à l'âge possède de nombreux effets néfastes sur la santé, la qualité de vie et le bien-être des personnes âgées. La capacité à percevoir correctement le caractère plus ou moins plaisant d'une odeur a notamment été relié au statut nutritionnel des individus âgés. Or très peu d'éléments présents dans la littérature scientifique permettent d'expliquer cette altération. Ainsi, le troisième objectif de ce travail de doctorat était d'identifier les bases neurales de l'altération de la valeur hédonique liée à l'âge. Dans ce but, nous avons étudié chez la souris le l'effet de l'âge sur la perception de la valeur hédonique des odeurs ainsi que les bases neurales de ces altérations au niveau et au-delà du BO. Nous nous sommes concentrés sur les structures mises en évidence dans l'étude 1 (**Etude 2**).

La valeur hédonique des odeurs peut être mesurée chez l'Homme par un certain nombre de méthodes basées pour la plupart sur l'aspect émotionnel de la valeur hédonique des odeurs. Ces méthodes possèdent des avantages et des inconvénients qui leur sont propres. Toutefois, aucune méthode actuelle ne permet une mesure à la fois objective, non invasive et efficace de la valeur hédonique des odeurs. Enfin, le quatrième et dernier objectif de cette thèse était de prendre en compte la composante motivationnelle de la valeur hédonique que nous avons mise en évidence dans la première étude et que nous avons confirmé dans la deuxième, afin de développer de nouvelles méthodes de mesure du caractère hédonique d'une odeur (**Etude 3**).

**REVUE 1** : "Codage de la valeur hédonique des odeurs dans le bulbe olfactif des vertébrés." (publiée)



# Odor hedonics coding in the vertebrate olfactory bulb

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Received: 4 September 2020 / Accepted: 29 November 2020 / Published online: 30 January 2021  
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## Abstract

Whether an odorant is perceived as pleasant or unpleasant (hedonic value) governs a range of crucial behaviors: foraging, escaping danger, and social interaction. Despite its importance in olfactory perception, little is known regarding how odor hedonics is represented and encoded in the brain. Here, we review recent findings describing how odorant hedonic value is represented in the first olfaction processing center, the olfactory bulb. We discuss how olfactory bulb circuits might contribute to the coding of innate and learned odorant hedonics in addition to the odorant's physicochemical properties.

**Keywords** Olfactory bulb · Hedonic value · Innate and learned valence · Odor preference · Behavior · Mouse · Rat · Zebrafish · Human

## Introduction

When we smell an odorant, our reaction is often that “I like it” or “I don't like it” (Richardson and Zucco 1989). This is known as odor hedonic value. A pleasant odorant has a positive hedonic value and may be predictive of a reward, whereas an unpleasant odorant has a negative hedonic value and may be predictive of a punishment. Odor hedonics can be unconditioned (innate) or learned and largely dominates olfactory perception. In addition to being the first aspect used to describe and categorize odors (Berglund et al. 1973; Schiffman et al. 1977), odor hedonic value is the most discriminating dimension in multidimensional analyses of odor verbal descriptors (Khan et al. 2007; Moskowitz and Barbe 1977; Zarzo 2008).

Odor hedonics is also the foundation of olfactory pleasure. Hence, deterioration in odor hedonic value in normal

aging (Joussain et al. 2013), parosmia (Walliczek-Dworschak and Hummel 2017) or neurodegenerative (Joussain et al. 2015; Mrochen et al. 2016) and neuropsychiatric conditions (Atanasova et al. 2010; Lombion-Pouthier et al. 2006; Moberg et al. 2003; Naudin et al. 2014; Walsh-Messinger et al. 2018) impairs the wellbeing of a significant number of the population. More generally, odor hedonic value governs approach/avoidance behavior in most vertebrates, crucially contributing to behaviors that are important for fitness and survival, such as feeding, social interaction and predator avoidance.

Odor hedonic value can be measured directly using subjective questionnaires in humans (Ferdenzi et al. 2016; Khan et al. 2007; Mandairon et al. 2009; Zarzo 2008) and/or indirectly by assessing odor-evoked autonomic responses (Alaoui-Ismaïli et al. 1997; Bensafi et al. 2002; Brauchli et al. 1995). In other vertebrates, odor hedonics is most commonly inferred from odorant investigation time (*rodents* (Jagetia et al. 2018; Kermen et al. 2016; Kobayakawa et al. 2007; Mandairon et al. 2009; Saraiva et al. 2016); *fish* (Hussain et al. 2013)), or from metrics quantifying species-specific appetitive and defensive behaviors (Frank et al. 2019; Kermen et al. 2020a; Mathuru et al. 2012; Yabuki et al. 2016). Despite some interindividual variability in odor hedonics in humans (Rouby et al. 2009), mice (Jagetia et al. 2018), and zebrafish (Kermen et al. 2020a), these psychophysical and behavioral approaches enabled species-specific odor preference gradients to be established at population level: from odorants with positive hedonic value (attractive, such as food odor)

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Nathalie Mandairon and Laura Chalençon contributed equally to this work

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to neutral and negative ones (aversive, such as rotten food, which is avoided), or danger signals (predator odor, which induces a panic response). Interestingly, odor preferences might be partially conserved among some vertebrates; for instance, odor investigation times in mice are positively correlated with pleasantness ratings in humans (Mandairon et al. 2009) (but see (Manoel et al. 2019)).

Given that odor hedonics is such a prominent aspect of olfactory perception in a broad range of species, abundant research has focused on understanding the neural underpinnings in the brain. Early research found neural representation of odor hedonics in the orbitofrontal cortex, insula, amygdala, and piriform cortex (Bensafi et al. 2007; Grabenhorst et al. 2007; Katata et al. 2009; Winston et al. 2005). More recently, a number of studies in human and animal models converged in showing that representations of odor hedonic value can be found at all levels of the olfactory system (reviewed in (Mantel et al. 2019)), including early levels, the olfactory epithelium (Lapid et al. 2011), and the olfactory bulb (OB) (Doucette et al. 2011; Kay and Laurent 1999; Kermen et al. 2016; Kobayakawa et al. 2007; Haddad et al. 2010).

In this review, we discuss how odor hedonics is represented and processed by the OB circuits. After briefly introducing the anatomical and functional features of the OB circuits, we discuss (1) a possible topographic OB representation of innate odor hedonic value, (2) how OB circuits represent and support learned odorant hedonic value, (3) how innate and learned odor hedonic values are integrated in OB circuits, and (4) the contribution of the OB in processing complex signals composed of odorants with different hedonic values.

## Olfactory bulb odor maps and plasticity

In vertebrates, information from olfactory sensory neurons (OSNs) located in the nose converges onto the OB. There, OSN axons contact OB output neurons—mitral/tufted cells in rodents and mitral/ruffled cells in fish—and local modulatory interneurons, within functional processing units called glomeruli (Nagayama et al. 2014; Satou 1990). As a result, odors are represented in the OB by odorant-specific spatiotemporal maps that are relatively consistent across individuals of the same species (Baier and Korsching 1994; Rubin and Katz 1999). These glomerular activity patterns are modulated by functionally diverse populations of pre- and post-synaptic OB interneurons. Periglomerular interneurons mediate interglomerular interactions, enabling olfactory contrast enhancement and input decorrelation (Cleland and Sethupathy 2006; Wanner and Friedrich 2020). Deeper in the OB, the odor pattern is regulated by feedback loops between output neurons and

granule cells that are involved in olfactory discrimination and memory (Grelat et al. 2018; Mori and Sakano 2011; Mori and Yoshihara 1995; Tan et al. 2010). Interestingly, approximately half of the bulbar interneurons are regenerated throughout life by adult neurogenesis, conferring additional plasticity to the system (Altman 1969; Imayoshi et al. 2008; Lledo and Valley 2016). In addition, bulbar odor activity patterns are further modulated via top-down input from brain regions involved in arousal, learning, and hedonics (Linster and Devore 2012; Padmanabhan et al. 2019).

The function of spatially organized OB activity maps and how they guide odor perception remain unclear. A coarse chemotopic organization is observed at local scale in zebrafish (Friedrich and Korsching 1998) and rodents (Chae et al. 2019; Rubin and Katz 1999). OB input and output patterns only partially relate to the odorants' physicochemical properties, which suggests they may also represent contextually and behaviorally relevant information, including fine odor discrimination (Linster et al. 2001) and odor hedonics (Chae et al. 2019; Haddad et al. 2010). Hence, both the topographical organization of odor maps and the wide range of neural computations performed by OB circuits are able to represent and impart meaning to an odor according to species-specific evolutionary constraints, olfactory context and past experience, before it is passed on to higher brain centers.

## Is there a topographic organization of innate odorant hedonics in the olfactory bulb?

In the fruit fly's antennal lobe, aversive odorants activate output neurons innervating glomeruli located more medially than those recruited by attractive odorants (Knaden et al. 2012; Seki et al. 2017). Hence, the hedonic value of an odorant is first represented in the antennal lobe by spatially segregated groups of glomeruli. A similar segregated arrangement of hedonic channels could also be present to some extent in the vertebrate OB (Fig. 1; Table 1).

The ventral domain of the vertebrate OB may be specialized in detecting appetitive and social odors. In the zebrafish, appetitive food-derived odors and attractive sex pheromones activate the ventrolateral and ventromedial OB, respectively (Kermen et al. 2020b; Yabuki et al. 2016; Yoshihara 2014). Mice of both sexes are strongly attracted to the odor of opposite-sex urine, which activates mitral cells located in the ventral OB (Kang et al. 2009; Xu et al. 2005). In rats, systematic analysis of hundreds of 2-deoxyglucose glomerular activation patterns revealed that floral, woody, fruity and herbaceous odorants, which are rated as pleasant by humans, preferentially activate the rat ventral OB (Auffarth et al. 2011).



In contrast, the dorsal OB, although it also responds to a large number of odorants with neutral hedonic value, plays a specific role in the processing of odorants signaling danger. The OSNs expressing trace amine-associated receptors, which mediate detection of spoiled flesh and/or predator odorants, project onto the dorsal OB in both zebrafish and mice (Dieris et al. 2017; Hussain et al. 2013; Pacifico et al. 2012). In the zebrafish, aversive odorants signaling decaying flesh and fear-inducing alarm odorants mostly activate the dorsal and dorsolateral OB domains, respectively (Dieris et al. 2017; Kermen et al. 2020a; Mathuru et al. 2012; Yoshihara 2014). Although spoiled food and predator odorants activate multiple glomeruli in both ventral and dorsal OB domains in rodents (Kobayakawa et al. 2007), the innate response to these odorants seems to rely solely on information conveyed by the dorsal domain. Optogenetic activation of a posterodorsal glomerulus responding to 2,3,5-trimethyl-3-thiazoline (TMT; a component of fox odor) is sufficient to induce freezing (Saito et al. 2017), whereas disrupting the function of dorsal TMT-responsive glomeruli impairs TMT-induced aversive behavior (Cho et al. 2011; Saito et al. 2017). Additionally, mutant mice devoid of dorsal glomeruli can detect the smell of spoiled food or predators, but do not display the same innate aversive response to these odors as their wild-type conspecifics (Kobayakawa et al. 2007).

In line with the role of the dorsal OB in mediating responses to aversive odorants with strong ethological relevance, unpleasant odorants described by human as “medicinal” (wintergreen, eucalyptus) or reminiscent of detergents strongly activate the dorsal OB in rats (Auffarth et al. 2011). Another study in mice showed that unlearned unattractive odorants with no particular ethological relevance activate the posterodorsal part of the glomerular layer to a greater extent than unlearned attractive odorants (Kermen et al. 2016). Furthermore, this study revealed an additional level of OB functional organization, in which odor hedonic information is represented along the anteroposterior axis of the ventral OB. The authors showed that unpleasant odorants evoked greater activity in the posteroventral OB, whereas pleasant odorants evoked greater activity in the anteroventral area. Manipulation of this OB hedonic signature using optogenetics reverted the initial odorant preference (Kermen et al. 2016).

Are these segregated hedonic OB representations preserved in the projections to higher brain centers? In mice, depending on their anteroposterior location in the OB, mitral cells differentially target the olfactory tubercle (Imamura et al. 2011; Midroit et al. 2020), a brain region known to code odor hedonic value (Gadziola et al. 2015; Midroit et al. 2020). The cortical amygdala, which is a brain region involved in innate olfactory behavior (Root et al. 2014), primarily receives dorsal OB input (Miyamichi et al. 2011).

Moreover, the anterior olfactory nucleus receives topographically organized projections from the dorsoventral axis of the OB (Miyamichi et al. 2011). These topographically organized cortical projections suggest that certain cortical OB targets might utilize the spatial segregation of hedonic information within the OB.

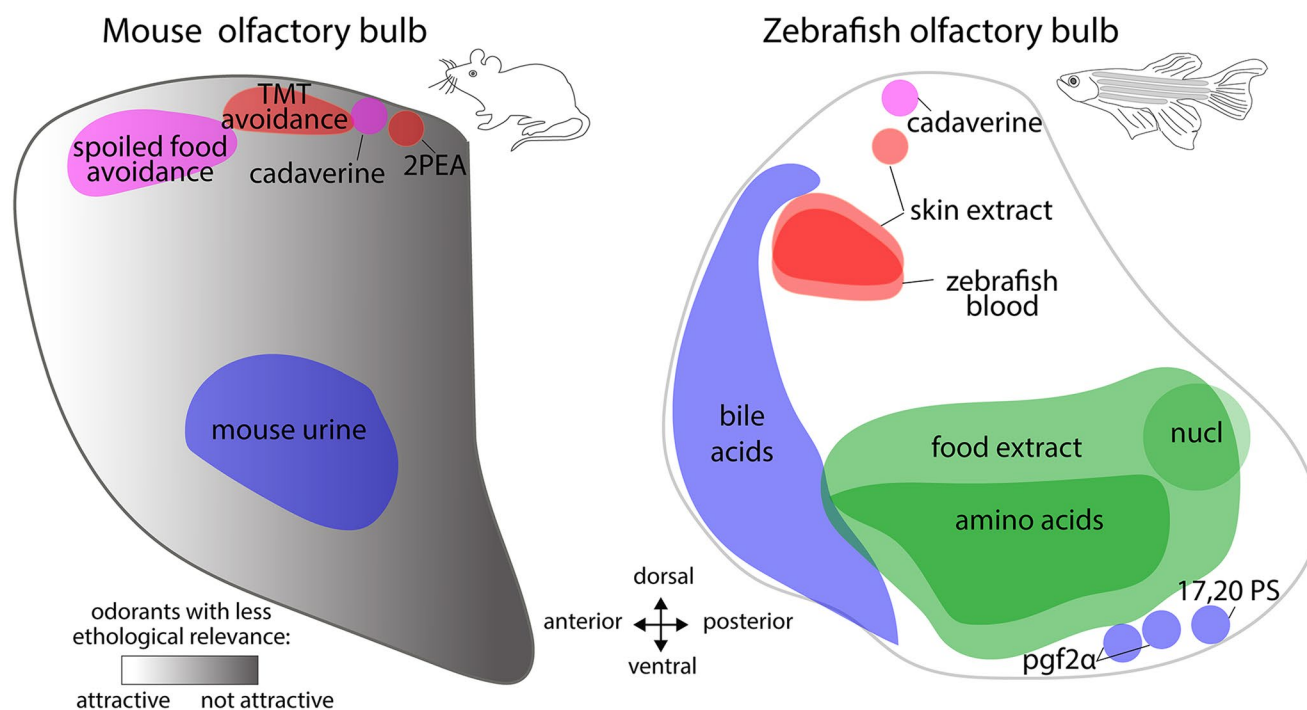
Taken together, the above findings suggest that the vertebrate OB is coarsely organized into nested axes that differentially mediate behavioral response to odorants, depending on hedonic value and ethological relevance. It would be interesting to determine whether these hedonic axes are conserved in the OB of other species than mice, rats and zebrafish, reflecting a general organizational principle in vertebrate olfaction. Additional systematic studies of downstream OB projection patterns would help in understanding how OB hedonic channels are distributed toward higher brain regions.

## Representation of learned hedonic value in olfactory bulb circuits

How does the OB network contribute to learned odor hedonics? The general contribution of OB circuits to different types of olfactory learning (habituation, perceptual, and associative learning) has been extensively reviewed elsewhere (Lledo and Valley 2016; Ross and Fletcher 2019; Tong et al. 2014; Wilson and Sullivan 1994). Here, we specifically discuss how bulbar activity patterns are modified when odorant hedonic value switches from neutral to appetitive or aversive, and the underlying mechanisms.

Early evidence, by unit recordings in the OB of anesthetized and awake mice performing an associative learning task, indicated that mitral cell firing rate is influenced by conditioned odor hedonic value (Doucette et al. 2011; Kay and Laurent 1999). Both appetitive and fear associative learnings durably modify behavioral response and odorant representation in the OB (at input, and possibly in the output layers) (Coopersmith et al. 1986; Fletcher 2012; Kass and McGann 2017; Sullivan and Leon 1987). Interestingly, fear learning—compared to appetitive learning—alters the animal’s defensive responses and the bulbar network in a way that is not odorant specific. Animals conditioned by foot shock developed a generalized fear response, not only to the learned odorants but also to odorants that were structurally unrelated (Kass and McGann 2017; Ross and Fletcher 2018). Paralleling this, olfactory fear learning enhances not only the representation of the learned odorant but also that of the unconditioned odorants in periglomerular interneurons (Kass and McGann 2017) and output neurons (Ross and Fletcher 2018).

The OB network has a specificity in rodents: it is the target of adult neurogenesis that has been shown to be



**Fig. 1** Spatial olfactory bulb domains activated by aversive and appetitive odors in the mouse and the zebrafish. Colored areas correspond to the olfactory bulb domain either responding to an odorant, or mediating the behavioral response to that odorant. Red areas represent domains responding to predator odors. Magenta areas represent domains responding to aversive spoiled food or decaying flesh. Green areas represent domains responding to food odors. Blues areas represent domains responding to putative social cues released by conspecifics. These maps are based on previously published works that are listed in

Table 1. In the mouse olfactory bulb, the grayscale gradient represents zones of the granule cell layer that are preferentially activated by attractive versus unattractive odors (Kermen et al. 2016). Note that on this lateral view of the olfactory bulb, no distinction is made between domains located in the medial and lateral halves. nucl, nucleotides; TMT, 2,3,5-trimethyl-3-thiazoline; 2PEA, 2-phenylethylamine; pgf2 $\alpha$ , prostaglandin F2 $\alpha$ ; 17,20 PS, 17 $\alpha$ , 20 $\beta$ -dihydroxy-4-pregnene-3-one-20-sulfate

involved in different types of olfactory learning. Adult neurogenesis underlies the acquisition and/or memory of associative appetitive learning (Alonso et al. 2012; Kermen et al. 2010; Mandairon et al. 2011; Mouret et al. 2009; Sultan et al. 2010) and fear conditioning (Valley et al. 2009). While a large number of studies focused on how adult-born neurons support olfactory learning, it remains unclear whether adult OB neurogenesis is involved in the acquisition of a new odor hedonic value after conditioning.

### Different olfactory bulb neuronal populations support innate and learned hedonic value, respectively

How are innate and learned odor hedonics represented in OB circuits? Recent studies indicate that innate responses to odors signaling danger and learned appetitive responses to novel odors might be mediated by developmentally distinct populations of OB neurons. Innate aversion to predator odors appears to depend on OB circuits formed in early development, around birth. In adult mice, fear response

to the predator odor TMT was abolished by inactivation of subsets of perinatally born, but not adult-born, olfactory neurons (Muthusamy et al. 2017; Sakamoto et al. 2011). Perinatal exposure to aversive odors disturbs aversion behavior later in life, which indicates that innately aversive odors exhibit a very restricted window of perinatal plasticity (Qiu et al. 2020a), after which their hedonic value cannot be altered. In contrast, detection of novel odors paired with a positive reward is resilient to early-born neuron silencing, but impaired by inactivation of subpopulations of adult-born granule cells (Muthusamy et al. 2017). Similar lines of evidence show that the activity of adult-born but not perinatally born granule cells contains information about an odor's learned positive hedonic value (Grelat et al. 2018). This differential involvement of early- and late-born neurons in learned odor hedonics could be explained by the fact that late-born but not early-born granule cells display a rapid form of structural plasticity (Breton-Provencher et al. 2016).

Taken together, these studies support the idea that innate (predator) odors are processed via hardwired circuits, established during early life stages and with a narrow window of plasticity, whereas the appetitive learning of novel

**Table 1** List of odorants and references used in Fig. 1

Odorants	Odor category	Species	References
Cadaverine	Aversive decay odor	Zebrafish	(Dieris et al. 2017)
Zebrafish blood	Alarm odor	Zebrafish	(Kermen et al. 2020a)
Zebrafish skin extract	Alarm cue	Zebrafish	(Chia et al. 2019; Diaz-Verdugo et al. 2019; Kermen et al. 2020a; Mathuru et al. 2012; Yoshihara 2014)
Amino acids	Attractive food odor	Zebrafish	(Friedrich and Korsching 1998; Koide et al. 2009; Tabor et al. 2004; Yaksi et al. 2009)
Food extract	Attractive food odor	Zebrafish	(Kermen et al. 2020b,a; Tabor et al. 2004)
Nucleotides	Attractive food odor		(Friedrich and Korsching 1998; Wakisaka et al. 2017)
Pgf2 $\alpha$ ; 17,20 PS	Reproductive pheromones	Zebrafish	(Dieris et al. 2017; Friedrich and Korsching 1998; Kermen et al. 2020b; Yabuki et al. 2016)
Bile acids	Putative social odor	Zebrafish	(Friedrich and Korsching 1998; Kermen et al. 2020a; Koide et al. 2009; Yaksi et al. 2009)
TMT	Predator odor	Mouse	(Cho et al. 2011; Igarashi et al. 2012; Kobayakawa et al. 2007; Saito et al. 2017)
2PEA	Predator odor	Mouse	(Ferrero et al. 2011)
Mouse urine	Attractive conspecific odor	Mouse	(Kang et al. 2009; Martel and Baum 2007; Martel et al. 2007; Schaefer et al. 2001; Xu et al. 2005)
Pentanal; 2-methylbutyric acid; alkylamines	Spoiled food	Mouse	(Igarashi et al. 2012; Kobayakawa et al. 2007; Takahashi et al. 2004)

Note that the list of bibliographic references used to map the OB domain activated by a given odorant is not exhaustive. TMT, 2,3,5-trimethyl-3-thiazoline; 2PEA, 2-phenylethylamine; pgf2 $\alpha$ , prostaglandin F2 $\alpha$ ; 17,20 PS, 17 $\alpha$ , 20 $\beta$ -dihydroxy-4-pregnene-3-one-20-sulfate

odors is flexibly supported throughout life via remodeling of OB response by plastic adult-born neurons.

### Odor hedonics in realistic conditions: olfactory bulb processing of hedonically complex odor blends

In natural situations, odors often consist of hedonically complex sensory signals comprising both positive and negative hedonic components. In order to survive, animals need to make rapid behavioral decisions based on these conflicting sensory inputs. For example, it might be evolutionarily advantageous to detect the odor of a predator even in presence of appetitive feeding or reproductive olfactory cues. Despite a few attempts at predicting the hedonic value of odorant mixtures in humans (reviewed in Thomas-Danguin et al. (2014)), surprisingly little is known regarding the integration of ethologically relevant odorants with contrasting hedonic values in the OB circuits.

The neural representation of complex odor blends at different stages of the olfactory system primarily involves component suppression, which can result in a new percept, qualitatively different from the individual components, or in the dominance of one odorant over the others (Thomas-Danguin et al. 2014). Although suppressive interactions between odorants can arise from peripheral effects in the nose, a large part originates from local OB circuit computations (Economo et al. 2016; Linster and Cleland 2004;

Qiu et al. 2020b; Tabor et al. 2004). In the OB, interactions have been documented between odorants of similar or opposite hedonic value. The odor of fennel or clove, which are spices commonly used by humans to mask the flavor of spoiled food, suppresses the activity of spoiled food odorant-responsive mitral cells in mice (Takahashi et al. 2004). In the zebrafish, appetitive food extracts suppress mitral cell response to an attractive reproductive pheromone (Kermen et al. 2020b), which indicates that OB circuits might favor the detection of sensory cues relevant to immediate rather than delayed physiological needs. Interestingly, odorants with negative hedonic value, such as the predator odorants TMT and 2-phenylethylamine (a component of bobcat urine which is avoided by rats and mice (Ferrero et al. 2011)), systematically block or override mice's behavioral attraction to appetitive odorants, and result instead in neutral or avoidance responses (Saraiva et al. 2016). In addition, when predator odorants are presented as a target stimulus atop a continuous neutral hedonic odor background, the resulting OB and behavioral responses are highly correlated to those elicited by the predator odorants alone (Qiu et al. 2020b). This indicates that predator odorants are relatively well protected against suppression by other odorants and dominate behavioral outcome. Since OB representations of target odorants without ethological relevance can on some occasions be completely masked by the background (Vinograd et al. 2017), one hypothesis is that the animal's ability to detect an odorant within an olfactory background depends on the hedonic value of the target odorant. Whether the

representation of odorants with negative hedonic value is specifically preserved in a noisy olfactory context, or if it is rather a general feature of salient odorants important for survival (Vinograd et al. 2017), remains unknown and is an exciting area for future research.

Studying the organization of interglomerular inhibitory circuits (i.e., the periglomerular and short axon cell networks), which are thought to mediate suppressive interactions between odorants, could shed light on how the OB integrates hedonically complex odors. Recent anatomical and functional evidence shows that interglomerular inhibition, far from being randomly organized, is odor and/or glomerulus specific in zebrafish (Wanner et al. 2016) and mouse (Economo et al. 2016) OBs, which could favor inhibition between processing channels with different hedonic value. In particular, some murine glomeruli are suppressed by a large number of odorants, whereas other glomeruli are particularly resilient to suppression (Economo et al. 2016). Studies investigating the odor tuning of resilient and suppressed glomeruli with respect to ethological relevant stimuli would help to determine whether interglomerular suppression contributes to the representation of hedonically complex signals, as reported in the fruit-fly's antennal lobe (Berck et al. 2016; Mohamed et al. 2019).

Taken together, these studies show that nonlinear interactions within OB circuits between components of hedonically complex odor signals can support biased behavioral response to a specific component. Nonlinear interactions in the vertebrate OB may thus play an important and underexplored role in hedonic perception in natural conditions.

## Conclusion

Several representations of odor hedonics are present in the OB and seem to recruit different circuit mechanisms and/or topographical locations according to whether the odorant is ethologically relevant or not and whether the hedonic value was innate or learned. Overall, these studies critically demonstrate the crucial contribution of OB circuits in attributing hedonic value to odorants throughout life. To provide a better understanding of these differences, future studies should focus on the comparison between innate hedonic value of odorants with or without biological significance and learned odor hedonic value.

**Funding** This work was supported by the CNRS, INSERM and Lyon 1 University (N.M. and L.C.) and by NTNU and the Research Council of Norway (FRIPRO grant 262698 to F.K.).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Ethical approval** This article does not contain any studies with animals performed by any of the authors.

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## References

- Alaoui-Ismaïli O, Vernet-Maury E, Dittmar A, Delhomme G, Chanel J et al (1997) Odor hedonics: connection with emotional response estimated by autonomic parameters. *Chem Senses* 22(3):237–248
- Alonso M, Lepousez G, Sebastien W, Bardy C, Gabellec MM et al (2012) Activation of adult-born neurons facilitates learning and memory. *Nat Neurosci* 15(6):897–904
- Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J Comp Neurol* 137(4):433–57
- Atanasova B, El-Hage W, Chabanet C, Gaillard P, Belzung C, Camus V et al (2010) Olfactory anhedonia and negative olfactory alliesthesia in depressed patients. *Psychiatry Res* 176(2–3):190–196
- Auffarth B, Gutierrez-Galvez A, Marco S et al (2011) Continuous spatial representations in the olfactory bulb may reflect perceptual categories. *Front Syst Neurosci* 5:82
- Baier H, Korsching S (1994) Olfactory glomeruli in the zebrafish form an invariant pattern and are identifiable across animals. *J Neurosci Off J Soc Neurosci* 14(1):219–230
- Bensafi M, Rouby C, Farget V, Bertrand B, Vigouroux M, Holley A et al (2002) Autonomic nervous system responses to odours: the role of pleasantness and arousal. *Chem Senses* 27(8):703–709
- Bensafi M, Sobel N, Khan RM et al (2007) Hedonic-specific activity in piriform cortex during odor imagery mimics that during odor perception. *J Neurophysiol* 98(6):3254–3262
- Berck ME, Khandelwal A, Claus L, Hernandez-Nunez L, Si G et al (2016) The wiring diagram of a glomerular olfactory system. *Elife* 5:e14859
- Berglund B, Berglund U, Engen T, Ekman G et al (1973) Multidimensional analysis of twenty-one odors. *Scand J Psychol* 14(2):131–137
- Brauchli P, Rüegg PB, Etzweiler F, Zeier H et al (1995) Electrocutaneous and autonomic alteration by administration of a pleasant and an unpleasant odor. *Chem Senses* 20(5):505–515
- Breton-Provencher V, Bakhshetyan K, Hardy D, Bammann RR, Cavarretta F et al (2016) Principal cell activity induces spine relocation of adult-born interneurons in the olfactory bulb. *Nat Commun*. 7(1):12659
- Chae H, Kepple DR, Bast WG, Murthy VN, Koulakov AA, Albeanu DF et al (2019) Mosaic representations of odors in the input and output layers of the mouse olfactory bulb. *Nat Neurosci* 22(8):1306–1317

- Chia JSM, Wall ES, Wee CL, Rowland TAJ, Cheng RK et al (2019) Bacteria evoke alarm behaviour in zebrafish. *Nat Commun* 10(1):3831
- Cho JH, Prince JEA, Cutforth T, Cloutier JF et al (2011) The pattern of glomerular map formation defines responsiveness to aversive odorants in mice. *J Neurosci* 31(21):7920–7926
- Cleland TA, Sethupathy P (2006) Non-topographical contrast enhancement in the olfactory bulb. *BMC Neurosci* 7(1):7
- Coopersmith R, Lee S, Leon M et al (1986) Olfactory bulb responses after odor aversion learning by young rats. *Brain Res* 389(1–2):271–277
- Diaz-Verdugo C, Sun GJ, Fawcett CH, Zhu P, Fishman MC et al (2019) Mating suppresses alarm response in zebrafish. *Curr Biol* 29(15):2541–2546.e3
- Dieris M, Ahuja G, Krishna V, Korsching SI et al (2017) A single identified glomerulus in the zebrafish olfactory bulb carries the high-affinity response to death-associated odor cadaverine. *Sci Rep.* 7(1):1–11
- Doucette W, Gire DH, Whitesell J, Carmean V, Lucero MT, Restrepo D et al (2011) Associative cortex features in the first olfactory brain relay station. *Neuron* 69(6):1176–1187
- Economu MN, Hansen KR, Wachowiak M et al (2016) Control of mitral/tufted cell output by selective inhibition among olfactory bulb glomeruli. *Neuron* 91(2):397–411
- Ferdenzi C, Jossain P, Digard B, Luneau L, Djordjevic J, Bensafi M (2016) Individual differences in verbal and non-verbal affective responses to smells: influence of odor label across cultures. *Chem Senses* bjw098
- Ferrero DM, Lemon JK, Fluegge D, Pashkovski SL, Korzan WJ et al (2011) Detection and avoidance of a carnivore odor by prey. *Proc Natl Acad Sci U S A* 108(27):11235–11240
- Fletcher ML (2012) Olfactory aversive conditioning alters olfactory bulb mitral/tufted cell glomerular odor responses. *Front. Syst. Neurosci.* p 6
- Frank T, Mönig NR, Satou C, Higashijima S, Friedrich RW et al (2019) Associative conditioning remaps odor representations and modifies inhibition in a higher olfactory brain area. *Nat Neurosci* 22(11):1844–1856
- Friedrich RW, Korsching SI (1998) Chemotopic, combinatorial, and noncombinatorial odorant representations in the olfactory bulb revealed using a voltage-sensitive axon tracer. *J Neurosci* 18(23):9977
- Gadziola MA, Tylicki KA, Christian DL, Wesson DW et al (2015) The olfactory tubercle encodes odor valence in behaving mice. *J Neurosci* 35(11):4515–4527
- Grabenhorst F, Rolls ET, Margot C, da Silva MAAP, Velazco MI et al (2007) How pleasant and unpleasant stimuli combine in different brain regions: odor mixtures. *J Neurosci* 27(49):13532
- Grelat A, Benoit L, Wagner S, Moigneu C, Lledo PM, Alonso M et al (2018) Adult-born neurons boost odor–reward association. *Proc Natl Acad Sci* 115(10):2514–2519
- Haddad R, Weiss T, Khan R, Nadler B, Mandairon N et al (2010) Global features of neural activity in the olfactory system form a parallel code that predicts olfactory behavior and perception. *J Neurosci* 30(27):9017
- Hussain A, Saraiva LR, Ferrero DM, Ahuja G, Krishna VS et al (2013) High-affinity olfactory receptor for the death-associated odor cadaverine. *Proc Natl Acad Sci* 110(48):19579–19584
- Igarashi KM, Ieki N, An M, Yamaguchi Y, Nagayama S et al (2012) Parallel mitral and tufted cell pathways route distinct odor information to different targets in the olfactory cortex. *J Neurosci* 32(23):7970–7985
- Imamura F, Ayoub AE, Rakic P, Greer CA et al (2011) Timing of neurogenesis is a determinant of olfactory circuitry. *Nat Neurosci* 14(3):331–337
- Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T et al (2008) Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci* 11(10):1153–1161
- Jagetia S, Milton AJ, Stetzk LA, Liu S, Pai K et al (2018) Inter- and intra-mouse variability in odor preferences revealed in an olfactory multiple-choice test. *Behav Neurosci* 132(2):88–98
- Jossain P, Bessy M, Fournel A, Ferdenzi C, Rouby C et al (2015) Altered affective evaluations of smells in Alzheimer’s disease. *J Alzheimers Dis* 49(2):433–441
- Jossain P, Thevenet M, Rouby C, Bensafi M et al (2013) Effect of aging on hedonic appreciation of pleasant and unpleasant odors. *PLoS ONE* 8(4):e61376
- Kang N, Baum MJ, Cherry JA et al (2009) A direct main olfactory bulb projection to the ‘vomeronasal’ amygdala in female mice selectively responds to volatile pheromones from males. *Eur J Neurosci* 29(3):624–634
- Kass MD, McGann JP (2017) Persistent, generalized hypersensitivity of olfactory bulb interneurons after olfactory fear generalization. *Neurobiol Learn Mem* 146:47–57
- Katata K, Sakai N, Doi K, Kawamitsu H, Fujii M et al (2009) Functional MRI of regional brain responses to ‘pleasant’ and ‘unpleasant’ odors. *Acta Otolaryngol (Stockh)* 129(sup562):85–90
- Kay LM, Laurent G (1999) Odor- and context-dependent modulation of mitral cell activity in behaving rats. *Nat Neurosci* 2(11):1003–1009
- Kermen F, Darnet L, Wiest C, Palumbo F, Bechert J et al (2020) Stimulus-specific behavioral responses of zebrafish to a large range of odors exhibit individual variability. *BMC Biol.* 18(1):1–16
- Kermen F, Lal P, Faturos NG, Yaksi E et al (2020b) Interhemispheric connections between olfactory bulbs improve odor detection. *PLoS Biol* 18(4):e3000701
- Kermen F, Midroit M, Kuczewski N, Forest J, Thévenet M et al (2016) Topographical representation of odor hedonics in the olfactory bulb. *Nat Neurosci* 19(7):876–878
- Kermen F, Sultan S, Sacquet J, Mandairon N, Didier A et al (2010) Consolidation of an olfactory memory trace in the olfactory bulb is required for learning-induced survival of adult-born neurons and long-term memory. *PLoS ONE* 5(8):e12118
- Khan RM, Luk CH, Flinker A, Aggarwal A, Lapid H et al (2007) Predicting odor pleasantness from odorant structure: pleasantness as a reflection of the physical world. *J Neurosci* 27(37):10015–10023
- Knaden M, Strutz A, Ahsan J, Sachse S, Hansson BS et al (2012) Spatial representation of odorant valence in an insect brain. *Cell Rep* 1(4):392–399
- Kobayakawa K, Kobayakawa R, Matsumoto H, Oka Y, Imai T et al (2007) Innate versus learned odour processing in the mouse olfactory bulb. *Nature* 450(7169):503–508
- Koide T, Miyasaka N, Morimoto K, Asakawa K, Urasaki A et al (2009) Olfactory neural circuitry for attraction to amino acids revealed by transposon-mediated gene trap approach in zebrafish. *Proc Natl Acad Sci* 106(24):9884–9889
- Lapid H, Shushan S, Plotkin A, Voet H, Roth Y et al (2011) Neural activity at the human olfactory epithelium reflects olfactory perception. *Nat Neurosci* 14(11):1455–1461
- Linster C, Cleland TA (2004) Configurational and elemental odor mixture perception can arise from local inhibition. *J Comput Neurosci* 16(1):39–47
- Linster C, Devore S (2012) Noradrenergic and cholinergic modulation of olfactory bulb sensory processing. *Front Behav Neurosci* 6:52
- Linster C, Johnson BA, Yue E, Morse A, Xu Z et al (2001) Perceptual correlates of neural representations evoked by odorant enantiomers. *J Neurosci Off J Soc Neurosci* 21(24):9837–9843
- Lledo PM, Valley M (2016) Adult olfactory bulb neurogenesis. *Cold Spring Harb Perspect Biol* 8(8):a018945



- Lombion-Pouthier S, Vandel P, Nezelof S, Haffen E, Millot JL et al (2006) Odor perception in patients with mood disorders. *J Affect Disord* 90(2–3):187–191
- Mandairon N, Poncelet J, Bensafi M, Didier A et al (2009) Humans and mice express similar olfactory preferences. *PLoS ONE* 4(1):e4209
- Mandairon N, Sultan S, Nouvian M, Sacquet J, Didier A (2011) Involvement of newborn neurons in olfactory associative learning? The operant or non-operant component of the task makes all the difference. *J Neurosci Off J Soc Neurosci* 31(35):12455–12460
- Manoel D, Makhoulouf M, Scialdone A, Saraiva LR (2019) Interspecific variation of olfactory preferences in flies, mice, and humans. *Chem Senses* 44(1):7–9
- Mantel M, Ferdenzi C, Roy JM, Bensafi M (2019) Individual differences as a key factor to uncover the neural underpinnings of hedonic and social functions of human olfaction: current findings from PET and fMRI studies and future considerations. *Brain Topogr* 32(6):977–986
- Martel KL, Baum MJ (2007) Sexually dimorphic activation of the accessory, but not the main, olfactory bulb in mice by urinary volatiles: Sexually dimorphic activation of the olfactory bulb. *Eur J Neurosci* 26(2):463–475
- Martel KL, Keller M, Douhard Q, Bakker J, Baum MJ (2007) Comparison of urinary odor-induced glomerular activation in the main olfactory bulb of aromatase knock-out and wild type female mice. *Neurosci Lett* 421(2):101–105
- Mathuru AS, Kibat C, Cheong WF, Shui G, Wenk MR et al (2012) Chondroitin fragments are odorants that trigger fear behavior in fish. *Curr Biol* 22(6):538–544
- Midroit M, Chalencón L, Renier N, Milton A, Thevenet M et al (2020) Odorants as natural reward: behavioral evidence and underlying circuitry of odorant attraction from mice to humans. *SSRN Electron J*
- Miyamichi K, Amat F, Moussavi F, Wang C, Wickersham I et al (2011) Cortical representations of olfactory input by trans-synaptic tracing. *Nature* 472(7342):191–196
- Moberg PJ, Arnold SE, Doty RL, Kohler C, Kanes S et al (2003) Impairment of odor hedonics in men with schizophrenia. *Am J Psychiatry* 160(10):1784–1789
- Mohamed AAM, Retzke T, Das Chakraborty S, Fabian B, Hansson BS et al (2019) Odor mixtures of opposing valence unveil interglomerular crosstalk in the *Drosophila* antennal lobe. *Nat Commun* 10(1):1201
- Mori K, Sakano H (2011) How is the olfactory map formed and interpreted in the mammalian brain? *Annu Rev Neurosci* 34(1):467–499
- Mori K, Yoshihara Y (1995) Molecular recognition and olfactory processing in the mammalian olfactory system. *Prog Neurobiol* 45(6):585–619
- Moskowitz HR, Barbe CD (1977) Profiling of odor components and their mixtures. *Sens Processes* 1(3):212–226
- Mouret A, Lepousez G, Gras J, Gabellec MM, Lledo PM et al (2009) Turnover of newborn olfactory bulb neurons optimizes olfaction. *J Neurosci Off J Soc Neurosci* 29(39):12302–12314
- Mrochen A, Marxreiter F, Kohl Z, Schlachetzki J, Renner B et al (2016) From sweet to sweat: Hedonic olfactory range is impaired in Parkinson's disease. *Parkinsonism Relat Disord* 22:9–14
- Muthusamy N, Zhang X, Johnson CA, Yadav PN, Ghashghaei HT et al (2017) Developmentally defined forebrain circuits regulate appetitive and aversive olfactory learning. *Nat Neurosci* 20(1):20–23
- Nagayama S, Homma R, Imamura F et al (2014) Neuronal organization of olfactory bulb circuits. *Front, Neural Circuits*, p 8
- Naudin M, Carl T, Surguladze S, Guillen C, Gaillard P et al (2014) Perceptive biases in major depressive episode. *PLoS ONE* 9(2):e86832
- Pacifico R, Dewan A, Cawley D, Guo C, Bozza T et al (2012) An olfactory subsystem that mediates high-sensitivity detection of volatile amines. *Cell Rep* 2(1):76–88
- Padmanabhan K, Osakada F, Tarabrina A, Kizer E, Callaway EM et al (2019) Centrifugal inputs to the main olfactory bulb revealed through whole brain circuit-mapping. *Front Neuroanat* 12:115
- Qiu Q, Wu Y, Ma L, Ramalingam V, Yu CR (2020a) Acquisition of innate odor preference depends on spontaneous and experiential activities during critical period. *Neuroscience*
- Qiu Q, Wu Y, Ma L, Yu CR (2020b) Encoding innately recognized odors via a generalized population code. *Neuroscience*
- Richardson JTE, Zucco GM (1989) Cognition and olfaction: a review. *Psychol Bull* 105(3):352–360
- Root CM, Denny CA, Hen R, Axel R et al (2014) The participation of cortical amygdala in innate, odour-driven behaviour. *Nature* 515(7526):269–273
- Ross JM, Fletcher ML (2018) Learning-dependent and -independent enhancement of mitral/tufted cell glomerular odor responses following olfactory fear conditioning in awake mice. *J Neurosci* 38(20):4623
- Ross JM, Fletcher ML (2019) Aversive learning-induced plasticity throughout the adult mammalian olfactory system: insights across development. *J Bioenerg Biomembr* 51(1):15–27
- Rouby C, Pouliot S, Bensafi M et al (2009) Odor hedonics and their modulators. *Inst Paul Bocuse Spec Issue* 20(8):545–549
- Rubin BD, Katz LC (1999) Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* 23(3):499–511
- Saito H, Nishizumi H, Suzuki S, Matsumoto H, Ieki N et al (2017) Immobility responses are induced by photoactivation of single glomerular species responsive to fox odour TMT. *Nat Commun* 8(1):1–10
- Sakamoto M, Imayoshi I, Ohtsuka T, Yamaguchi M, Mori K, Kageyama R et al (2011) Continuous neurogenesis in the adult forebrain is required for innate olfactory responses. *Proc Natl Acad Sci* 108(20):8479–8484
- Saraiva LR, Kondoh K, Ye X, Yoon K, Hernandez M, Buck LB et al (2016) Combinatorial effects of odorants on mouse behavior. *Proc Natl Acad Sci* 113(23):E3300–3306
- Satou M (1990) Synaptic organization, local neuronal circuitry, and functional segregation of the teleost olfactory bulb. *Prog Neurobiol* 34(2):115–142
- Schaefer ML, Young DA, Restrepo D et al (2001) Olfactory fingerprints for major histocompatibility complex-determined body odors. *J Neurosci* 21(7):2481–2487
- Schiffman S, Robinson DE, Erickson RP et al (1977) Multidimensional scaling of odorants: examination of psychological and physicochemical dimensions. *Chem Senses* 2(3):375–390
- Seki Y, Dweck HKM, Rybak J, Wicher D, Sachse S, Hansson BS (2017) Olfactory coding from the periphery to higher brain centers in the *Drosophila* brain. *BMC Biol.* 15(1):1–20
- Sullivan RM, Leon M (1987) One-trial olfactory learning enhances olfactory bulb responses to an appetitive conditioned odor in 7-day-old rats. *Dev Brain Res* 35(2):307–311
- Sultan S, Mandairon N, Kermen F, Garcia S, Sacquet J, Didier A et al (2010) Learning-dependent neurogenesis in the olfactory bulb determines long-term olfactory memory. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* 24(7):2355–63
- Tabor R, Yaksi E, Weislogel JM, Friedrich RW et al (2004) Processing of odor mixtures in the zebrafish olfactory bulb. *J Neurosci* 24(29):6611
- Takahashi YK, Nagayama S, Mori K et al (2004) Detection and masking of spoiled food smells by odor maps in the olfactory bulb. *J Neurosci* 24(40):8690

- Tan J, Savigner A, Ma M, Luo M et al (2010) Odor information processing by the olfactory bulb analyzed in gene-targeted mice. *Neuron* 65(6):912–926
- Thomas-Danguin T, Sinding C, Romagny S, El Mountassir F, Atanasova B et al (2014) The perception of odor objects in everyday life: a review on the processing of odor mixtures. *Front Psychol* 5:504
- Tong MT, Peace ST, Cleland TA et al (2014) Properties and mechanisms of olfactory learning and memory. *Front Behav Neurosci* 8:238–238
- Valley MT, Mullen TR, Schultz LC, Sagdullaev BT, Firestein S et al (2009) Ablation of mouse adult neurogenesis alters olfactory bulb structure and olfactory fear conditioning. *Front Neurosci* 3:51
- Vinograd A, Livneh Y, Mizrahi A et al (2017) History-dependent odor processing in the mouse olfactory bulb. *J Neurosci Off J Soc Neurosci* 37(49):12018–12030
- Wakisaka N, Miyasaka N, Koide T, Masuda M, Hiraki-Kajiyama T, Yoshihara Y et al (2017) An adenosine receptor for olfaction in fish. *Curr Biol* 27(10):1437–1447.e4
- Walliczek-Dworschak U, Hummel T (2017) The human sense of olfaction. *Facial Plast Surg FPS* 33(4):396–404
- Walsh-Messinger J, Wong PS, Antonius D, McMahon K, Opler LA et al (2018) Sex differences in hedonic judgement of odors in schizophrenia cases and healthy controls. *Psychiatry Res* 269:345–353
- Wanner AA, Friedrich RW (2020) Whitening of odor representations by the wiring diagram of the olfactory bulb. *Nat Neurosci* 23(3):433–442
- Wanner AA, Genoud C, Masudi T, Siksou L, Friedrich RW et al (2016) Dense EM-based reconstruction of the interglomerular projectome in the zebrafish olfactory bulb. *Nat Neurosci* 19(6):816–825
- Wilson DA, Sullivan RM (1994) Neurobiology of associative learning in the neonate: early olfactory learning. *Behav Neural Biol* 61(1):1–18
- Winston JS, Gottfried JA, Kilner JM, Dolan RJ et al (2005) Integrated neural representations of odor intensity and affective valence in human amygdala. *J Neurosci* 25(39):8903
- Xu F, Schaefer M, Kida I, Schafer J, Liu N et al (2005) Simultaneous activation of mouse main and accessory olfactory bulbs by odors or pheromones. *J Comp Neurol* 489(4):491–500
- Yabuki Y, Koide T, Miyasaka N, Wakisaka N, Masuda M et al (2016) Olfactory receptor for prostaglandin F<sub>2</sub> $\alpha$  mediates male fish courtship behavior. *Nat Neurosci* 19(7):897–904
- Yaksi E, von Saint PF, Niessing J, Bundschuh ST, Friedrich RW et al (2009) Transformation of odor representations in target areas of the olfactory bulb. *Nat Neurosci* 12(4):474–482
- Yoshihara Y (2014) Zebrafish olfactory system. In: Mori K (ed) *The olfactory system: from odor molecules to motivational behaviors*. Springer Japan, Tokyo, pp 71–96
- Zarzo M (2008) Psychologic dimensions in the perception of everyday odors: pleasantness and edibility. *J Sens Stud* 23(3):354–376

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**ÉTUDE 1** : "Processus neuronaux de la valeur  
recompensante des odorants plaisants." (publiée)



# Current Biology

## Neural processing of the reward value of pleasant odors

### Highlights

- The posterior olfactory bulb is a site of intracranial self-stimulation
- Olfactory tubercle receives heavy projections from the posterior olfactory bulb
- Olfactory tubercle activity is related to odorant pleasantness in mice and humans
- Attractive odors are natural rewards in both mice and humans

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### In Brief

Midroit et al. reveal that the odors act as natural rewards. The olfactory tubercle gates olfactory information from the posterior olfactory bulb to the reward system to encode spontaneous odor-driven attraction.

Article

# Neural processing of the reward value of pleasant odorants

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<https://doi.org/10.1016/j.cub.2021.01.066>

## SUMMARY

Pleasant odorants are represented in the posterior olfactory bulb (pOB) in mice. How does this hedonic information generate odor-motivated behaviors? Using optogenetics, we report here that stimulating the representation of pleasant odorants in a sensory structure, the pOB, can be rewarding, self-motivating, and is accompanied by ventral tegmental area activation. To explore the underlying neural circuitry downstream of the olfactory bulb (OB), we use 3D high-resolution imaging and optogenetics and determine that the pOB preferentially projects to the olfactory tubercle, whose increased activity is related to odorant attraction. We further show that attractive odorants act as reinforcers in dopamine-dependent place preference learning. Finally, we extend those findings to humans, who exhibit place preference learning and an increase BOLD signal in the olfactory tubercle in response to attractive odorants. Thus, strong and persistent attraction induced by some odorants is due to a direct gateway from the pOB to the reward system.

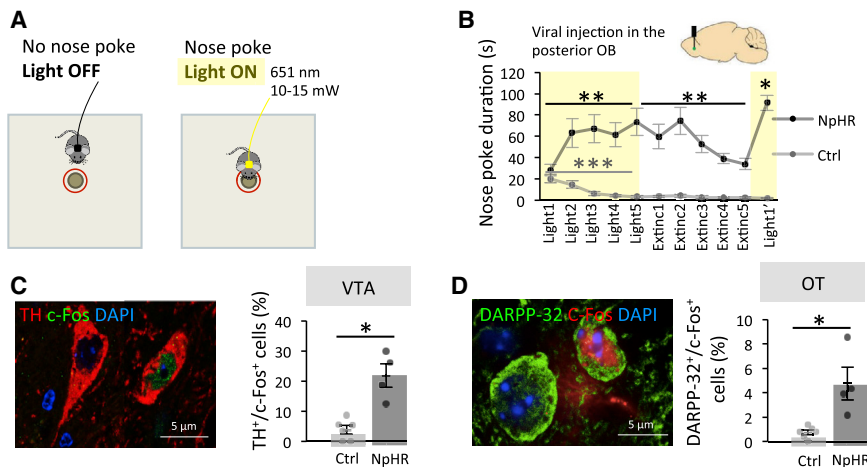
## INTRODUCTION

Since the dawn of time, we have flavored our dishes, perfumed our houses and ourselves. Our close relationship with smells exemplifies the strong attractive power odorants exert over us. Why do some odorants have such a strong power of attraction? Among the different dimensions of olfactory perception such as identity, intensity, or familiarity, the hedonic value (or pleasantness) dominates since it is the first criterion used by humans to describe and categorize odorants.<sup>1–3</sup> The hedonic value drives vital behaviors such as feeding, social relationships, and danger avoidance<sup>4</sup> and impacts a wide array of higher-order social functions in humans that contribute to our overall well-being.

The olfactory bulb (OB) is the first sensory structure for odor information processing.<sup>5</sup> We previously showed that in mice, pleasant odorants are represented in spatially restricted activity in the posterior olfactory bulb (pOB).<sup>6</sup> How does this hedonic information generate attraction to odor? Because motivated behaviors are known to rely on the reward/motivational system,<sup>7–9</sup> this question prompted us to explore the role of the reward system in odor-induced attraction.

Thus, using complementary approaches in mice and humans, we tested the hypothesis that pleasant odorants are attractive because they directly solicit the reward system.

First, we investigated whether activating the neural representation of pleasant odorants could be rewarding, and we found that optogenetic activation of pOB can serve as reinforcer using a self-stimulation paradigm, a widely used operant conditioning to assess the biological bases of motivation.<sup>10,11</sup> Second, we explored the underlying neural circuitry downstream of the OB and focused more particularly on the olfactory tubercle (OT), a direct target of the OB that is a component of the ventral striatum<sup>12,13</sup> and plays a role in motivated behaviors.<sup>14–16</sup> In this context, using 3D high-resolution imaging, electrophysiology, and cellular mapping and optogenetics, we deciphered the anatomical link between the pOB and the OT and revealed that stimulation of the pOB by spontaneously attractive odorants activates the OT and triggers motivated behaviors. Then, to further confirm the rewarding properties of odorants, we used a conditioned place preference test, a standard model used to evaluate the rewarding properties of drugs of abuse. This test showed that attractive odorants induced dopamine-dependent place preference learning. Finally, we extended



**Figure 1. The pOB is a site for optogenetic intracranial self-stimulation**

(A) Experimental setup.

(B) Top. Injection of NpHR-EYFP (NpHR  $n = 7$ ) or control virus (Ctrl  $n = 9$ ) in the posterior granule cell layer of the OB. Bottom. NpHR mice rapidly learned across days of learning to nose poke to trigger light-stimulation (Friedman test: trial effect  $F(4,35) = 13.60$   $p = 0.009$ , Two-Tailed Wilcoxon Light1 versus Light5  $W = 28.000$   $p = 0.016$ , Rank-Biserial Correlation = 1.000). This learning was subject to extinction when light was no more available (Friedman test: trial effect  $F(4,35) = 20.31$   $p = 0.001$ , Two-Tailed Wilcoxon Light5 versus Extinc5  $W = 27.000$   $p = 0.031$ , Rank-Biserial Correlation = 0.929). On the last trial, light was available again and nose poking reinstated (Two-Tailed Wilcoxon Extinc5 versus Light1'  $W = 28.000$   $p = 0.016$ , Rank-Biserial Correlation = 1.000). Nose pokes significantly decreased in Ctrl among the 5 first trials of light stimulation (Friedman trial effect  $F(4,35) = 21.36$   $p < 0.001$ , Two-

Tailed Wilcoxon Light1 versus Light5  $W = 36.000$   $p = 0.014$ , Rank-Biserial Correlation = 1.000). Values represent means  $\pm$  SEM.

(C) Left. Representative image of double labeled TH+/c-Fos+ cells in the VTA. Right. The percentage of TH+ cells expressing c-Fos was higher in NpHR mice ( $n = 4$ ) compared to Ctrl ( $n = 6$ ) in the VTA after self-conditioning (Two-Tailed Mann-Whitney,  $W = 24.000$   $p = 0.014$ , Rank-Biserial Correlation = 1.000). Bars represent means of individual data points  $\pm$  SEM.

(D) Left. Representative image of double labeled DARPP-32+/c-Fos+ cells in the OT. Right. The percentage of DARPP-32+ cells expressing c-Fos in OT was higher in NpHR ( $n = 4$ ) mice compared to Ctrl ( $n = 6$ ) after self-conditioning (Two-Tailed Mann-Whitney,  $W = 24.000$   $p = 0.014$ , Rank-Biserial Correlation = 1.000). Bars represent means of individual data points  $\pm$  SEM \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

See also Figure S1.

our findings to humans by identifying a similar recruitment of the reward system and more particularly of the olfactory tubercle by attractive odorants in humans, through both naturalistic living-lab experiments and functional brain imaging approaches.

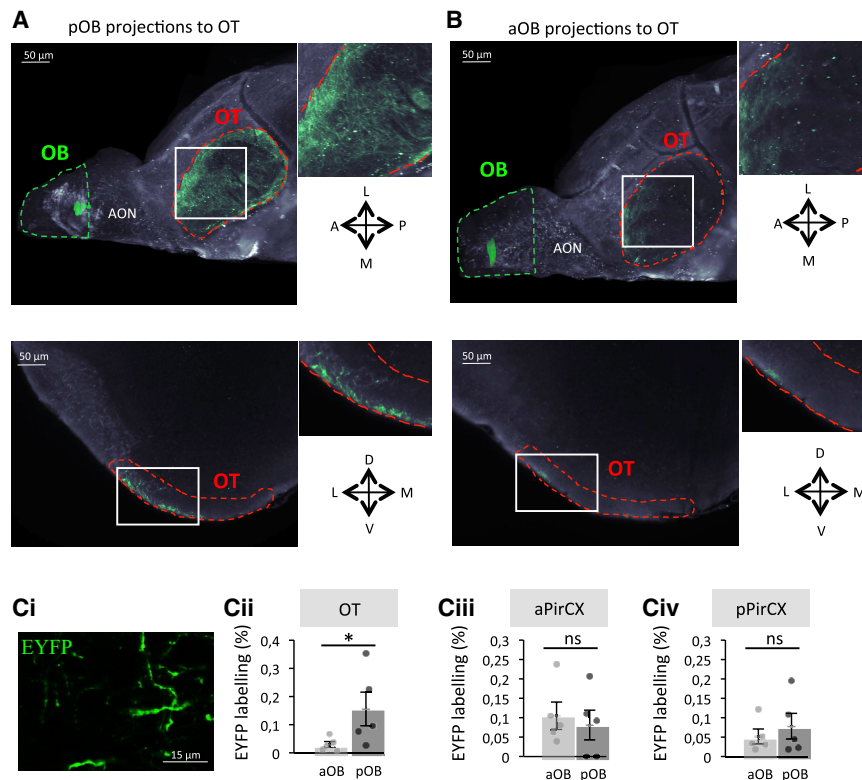
## RESULTS

### The posterior olfactory bulb as a site for rewarding intracranial self-stimulation

Since optogenetic stimulation of the pOB increases attraction toward odorants,<sup>6</sup> we assessed whether this stimulation would by itself be sufficient to serve as a reinforcer and thus induce operant conditioning using optogenetic intracranial self-stimulation. Two groups of mice were injected with a Lenti-hSyn-eNpHR3.0-EYFP virus (NpHR-EYFP virus, NpHR group) or a control Lenti-hSyn-EYFP virus (EYFP virus, Ctrl group) in the ventro-posterior granule cell layer of the OB with optical fibers implanted at the same location (Figures 1A and 1B; Figures S1A and S1B). Light stimulation in NpHR mice silenced inhibitory granule interneurons as shown by a lower percentage of EYFP+/c-Fos+ neurons in the targeted part of the OB, showing that light stimulation inhibits NpHR transduced neurons (Figure S1C). This was not observed in Ctrl mice, showing that light alone had no effect on granule cell activity (Figure S1C). This manipulation was chosen since it leads to disinhibition/activation of OB projection neurons (mitral/tufted cells) specifically in the pOB.<sup>6</sup>

During behavior, bilateral light stimulation was automatically triggered when mice nose poked within 1 cm around a non-odorized hole of a board apparatus (light-triggering zone) and lasted as long as the nose poke (Figure 1A). Across days of training, we found that NpHR mice readily learned to nose poke to receive optogenetic stimulation in their pOB.

More precisely, the nose poke duration increased over the five days of training in NpHR mice (nose poke duration Light1 =  $28.275 \pm 5.469$  s, Light5 =  $73.697 \pm 12.897$  s; Friedman test: trial effect  $F(4,35) = 13.60$   $p = 0.009$ , Two-Tailed Wilcoxon Light1 versus Light5  $W = 28.000$   $p = 0.016$  Rank-Biserial Correlation = 1.000; Figure 1B) indicating that light stimulation serve as reinforcer in the operant conditioning paradigm. We then proceeded to an extinction phase during which light stimulation was no longer delivered upon nose poke (Trial 6 to Trial 10). During this second phase, NpHR mice showed a decrease in nose poke duration (nose poke duration Light5 =  $73.697 \pm 12.897$  s, Extinc5 =  $34.163 \pm 5.286$  s; Friedman test: trial effect  $F(4,35) = 20.31$   $p = 0.001$ , Two-Tailed Wilcoxon Light5 versus Extinc5  $W = 27.000$   $p = 0.031$  Rank-Biserial Correlation = 0.929; Figure 1B). Finally, in a third phase, light stimulation was available again, and a reinstatement of self-stimulation behavior was observed (nose poke duration Extinc5 =  $34.163 \pm 5.286$  s, Light1' =  $91.434 \pm 7.114$  s; Two-Tailed Wilcoxon Extinc5 versus Light1'  $W = 28.000$   $p = 0.016$  Rank-Biserial Correlation = 1.000; Figure 1B). This learning/extinction/reinstatement sequence confirmed that self-stimulation behavior is dependent on optogenetic inhibition of granule cells in the pOB. This behavior was not observed in Ctrl mice (with no expression of NpHR in the granule cells), which instead showed a rapid and pronounced decrease in poke durations across trials regardless of light delivery (nose poke duration Light1 =  $20.274 \pm 4.074$  s, Light5 =  $3.222 \pm 1.169$  s; Friedman test: trial effect  $F(4,35) = 21.36$   $p < 0.001$ , Two-Tailed Wilcoxon Light1 versus Light5  $W = 36.000$   $p = 0.014$  Rank-Biserial Correlation = 1.000; Figure 1B). The difference between NpHR and Ctrl groups was observed at early as the second trial of light stimulation and remained over the five



**Figure 2. A privileged pathway exists between the pOB and the OT**

(A) Representative image of the pOB projecting onto the OT after iDISCO clarification. Top. Ventral projection. Bottom. Coronal optical section.

(B) Representative image of the aOB projecting onto the OT after iDISCO clarification. Top. Ventral projection. Bottom. Coronal optical section.

(C) Quantification of the percentage of EYFP labeling (Ci, EYFP labeling = EYFP+area/total area of the field\*100) after viral injections in the pOB (n = 5) and the aOB (n = 5) in the OT (Cii, Bonferroni corrected One-Tailed Mann-Whitney, W = 2.000 p = 0.048, Rank-Biserial Correlation = -0.840), aPirCX (Ciii, Bonferroni corrected One-Tailed Mann-Whitney, W = 14.000 p = 1, Rank-Biserial Correlation = 0.120) and pPirCX (Civ, Bonferroni corrected One-Tailed Mann-Whitney, W = 11.000 p = 1, Rank-Biserial Correlation = -0.120). Bars represent means of individual data points  $\pm$  SEM \*p < 0.05. A: Anterior; P: Posterior; L: Lateral; M: Medial; D: Dorsal; V: Ventral. See also [Figure S2](#).

injection and its efficiency by quantifying the activity of transduced cells after light stimulation ([Figures S1E and S1F](#)).

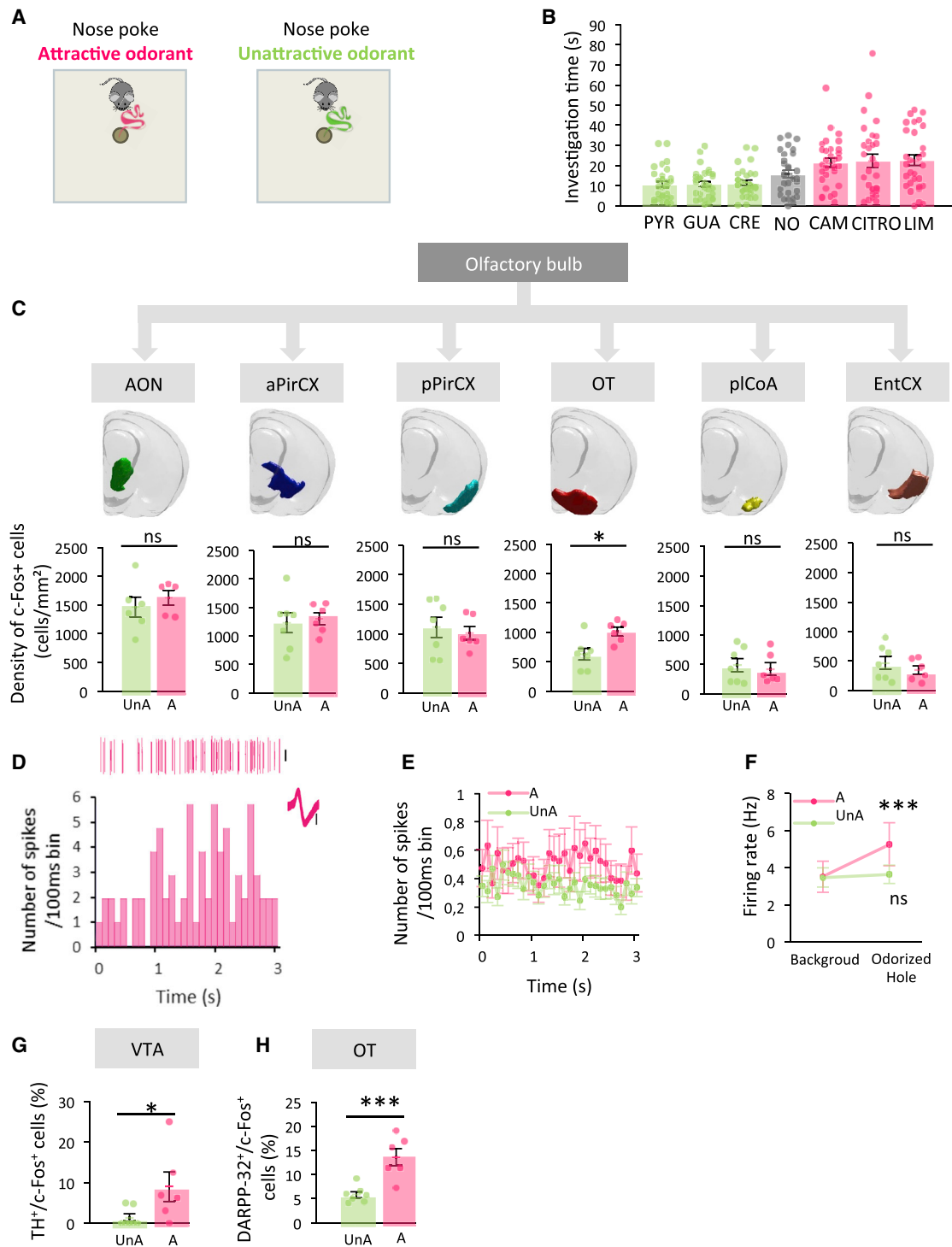
extinction trials revealing a rapid and persistent effect of the rewarding stimulation in NpHR mice (Bonferroni corrected One-Tailed Mann-Whitney, Light1 W = 43.000 p = 1 Rank-Biserial Correlation = 0.365, Light2 W = 61.000 p = 0.004 Rank-Biserial Correlation = 0.937, Light3 W = 63.000 p < 0.001 Rank-Biserial Correlation = 1.000, Light4 W = 63.000 p < 0.001 Rank-Biserial Correlation = 1.000, Light5 W = 63.000 p = 0.005 Rank-Biserial Correlation = 1.000, Extinc1 W = 63.000 p < 0.001 Rank-Biserial Correlation = 1.000, Extinc2 W = 63.000 p < 0.001 Rank-Biserial Correlation = 1.000, Extinc3 W = 63.000 p = 0.006 Rank-Biserial Correlation = 1.000, Extinc4 W = 63.000 p < 0.001 Rank-Biserial Correlation = 1.000, Extinc5 W = 63.000 p = 0.006 Rank-Biserial Correlation = 1.000, Light1' W = 63.000 p = 0.006 Rank-Biserial Correlation = 1.000). Moreover, we performed another set of experiments in mice which had NpHR-EYFP virus injected in their anterior OB (aOB) and were trained in the same behavioral protocol. NpHR mice injected in the aOB showed no increase in nose poking across trials (Light1-Light5) (nose poke duration Light1 = 7.835  $\pm$  1.791 s, Light5 = 6.458  $\pm$  2.466 s; Friedman test: trial effect  $F_{(4,35)} = 5.90$  p = 0.207; [Figure S1D](#)), indicating that self-stimulation behavior was specific to the stimulation of the pOB. In addition, on the first trial, NpHR aOB mice displayed less nose pokes than Ctrl mice (7.835  $\pm$  1.791 versus 20.274  $\pm$  4.074 s, when comparing Light1 trial in [Figure S1D](#) and Light1 trial Ctrl group in [Figure 1B](#); Two-Tailed Mann-Whitney, W = 11.000 p = 0.015 Rank-Biserial Correlation = -0.694). This suggests an aversive effect of aOB granule cell inhibition in line with previous data by our group.<sup>6</sup> We verified the anterior location of the virus

Next, to confirm that pOB self-stimulation recruits the reward system, we analyzed its impact on the activity of ventral tegmental area (VTA) dopaminergic neurons (TH<sup>+</sup> cells) using assessment of c-Fos expression. We found a higher percentage of TH<sup>+</sup>/c-Fos<sup>+</sup> double-labeled cells in light-stimulated NpHR mice injected in the pOB compared to the Ctrl group (TH<sup>+</sup>/c-Fos<sup>+</sup> cells NpHR = 21.834  $\pm$  3.888%, EYFP = 3.848  $\pm$  1.392%; Two-Tailed Mann-Whitney, W = 24.000 p = 0.014 Rank-Biserial Correlation = 1.000; [Figure 1C](#)). Because the OT is at the crossroad between the OB and VTA, we further analyzed the activation of OT projection neurons, the medium spiny neurons (MSNs), identified based on DARPP-32<sup>+</sup> expression.<sup>17</sup> We found a higher percentage of DARPP-32<sup>+</sup>/c-Fos<sup>+</sup> cells in NpHR mice injected in the pOB compared to the Ctrl group (DARPP-32<sup>+</sup>/c-Fos<sup>+</sup> cells NpHR = 4.758  $\pm$  1.356%, EYFP = 0.735  $\pm$  0.200%; Two-Tailed Mann-Whitney, W = 24.000 p = 0.014 Rank-Biserial Correlation = 1.000; [Figure 1D](#)), indicating the activation of a network including the OT and VTA after posterior bulbar self-stimulation.

Taken together, our results reveal that the pOB, but not the aOB, is a site of self-stimulation suggesting that the OT provides a direct gateway from the pOB to the reward system.

### A privileged pathway between the posterior olfactory bulb and the olfactory tubercle

We next hypothesized that the capacity of the pOB to support reinforcement and to recruit the VTA could be due to an enrichment of the axonal projection densities between pOB and the OT, relative to aOB projections. To test this hypothesis, mice were injected with a lentivirus expressing EYFP in the mitral cell layer of aOB or pOB. After 2 months, allowing expression of the EYFP within the entirety of the transduced neurons



**Figure 3. The OT, specifically, shows greater neural activity in response to attractive compared to unattractive odorants**

(A) Experimental setup.

(B) Level of attraction of unlearned odorants ( $n = 30$ , green: unattractive odorant (UnA), gray: no odor (NO), pink: attractive odorant [A]). The level of attraction varied among odorants (Friedman Odor effect,  $F_{(6,210)} = 27.86$   $p < 0.001$ , permutation test  $p < 0.001$ ). Bars represent means of individual data points  $\pm$  SEM.

(C) Top. ROI of secondary olfactory structures. Bottom. c-Fos+ cell density in response to UnA ( $n = 7$ ) versus A ( $n = 6$ ) odorants. No difference except in the OT (Bonferroni corrected One-Tailed Unpaired t-test, AON  $t = -0.704$   $p = 1$  Cohen's  $d = -0.426$ , aPirCX  $t = -0.330$   $p = 1$  Cohen's  $d = -0.184$ , pPirCX  $t = 0.477$   $p = 1$  Cohen's  $d = 0.265$ , OT  $t = -2.991$   $p = 0.037$  Cohen's  $d = -1.664$ , pLoA  $t = 0.380$   $p = 1$  Cohen's  $d = 0.211$ , EntCX  $t = 0.860$   $p = 1$  Cohen's  $d = 0.478$ ). Bars represent means of individual data points  $\pm$  SEM.

(legend continued on next page)



including their projecting axons,<sup>6</sup> EYFP was visualized using iDISCO.<sup>18</sup> The injection site was reconstructed with Imaris software<sup>18</sup> to validate the spread of the OB viral infection. In the brains showing localized injections either in the aOB or pOB, we observed that viral injections in the pOB (Figure 2A) led to a higher density of labeled fibers within the OT than viral injections in the aOB (Figure 2B).

To allow precise quantification of EYFP labeling, we performed additional groups of mice (aOB,  $n = 5$ ; pOB,  $n = 5$ ; Figure 2Ci) and confirmed a higher percentage of EYFP labeling in the OT's ventral surface (known to receive input from the OB) of mice injected in the pOB compared to mice injected in the aOB (EYFP labeling pOB =  $0.155 \pm 0.059\%$ , aOB =  $0.030 \pm 0.010\%$ ; Bonferroni corrected One-Tailed Mann-Whitney,  $W = 2.000$   $p = 0.048$  Rank-Biserial Correlation =  $-0.840$ ; Figure 2Cii). Differences in the injection sites cannot account for this finding, since a similar percentage of transduced EYFP<sup>+</sup> mitral cells (Tbx21+ neurons) was assessed in the OB of mice injected in the aOB or the pOB (Figure S2A-B).

In contrast to OT innervation, quantification of OB projections into the anterior (aPirCX) and posterior piriform cortex (pPirCX) revealed no difference of labeling based on the anterior or posterior OB injection sites (EYFP labeling in aPirCX pOB =  $0.081 \pm 0.039\%$ , aOB =  $0.105 \pm 0.035\%$ ; EYFP labeling in pPirCX pOB =  $0.078 \pm 0.033\%$ , aOB =  $0.052 \pm 0.019\%$ ; Bonferroni corrected One-Tailed Mann-Whitney, aPirCX  $W = 14.000$   $p = 1$  Rank-Biserial Correlation =  $0.120$ , pPirCX  $W = 11.000$   $p = 1$  Rank-Biserial Correlation =  $-0.120$ ; Figure 2Ciii and Civ). Altogether, these results highlight the existence of a privileged connection between the pOB and the OT that could support odorant attraction.

### The olfactory tubercle, a key structure mediating odorant attraction

To now investigate the functional role of the OT in spontaneous attraction toward unlearned odorants, we used the one hole-board apparatus (Figure 3A) to quantify the duration of odor investigation as a measure for odor-driven attraction (Table S1).<sup>6,19-21</sup> In line with previous work, different odorants, chosen to be unfamiliar and with no particular biological significance (pyridine: PYR, guaiacol: GUA, p-cresol: CRE, control no odor: NO, camphor: CAM, citronellol: CITRO, +limonene: LIM) elicited diverse investigation times (Friedman test: Odor effect,  $F_{(6,210)} = 27.86$   $p < 0.001$ , permutation test  $p < 0.001$ ; Figure 3B).<sup>6</sup> Following behavioral testing, we used c-Fos labeling to map neural activity in response to the spontaneously investigated odorants. C-Fos<sup>+</sup> cells were automatically detected on serial sections

of the anterior brain, and precisely allocated to specific brain regions, i.e., the direct synaptic targets of the OB (anterior olfactory nucleus: AON, aPirCX, pPirCX, OT, postero-lateral amygdala: pLCoA and entorhinal cortex: EntCX) (Figure 3C; Figure S3A and Table S2).<sup>22</sup>

To uncover the spatial representation of activity underlying the attraction toward odorants independently of their identity, we averaged the activity evoked by each of the three spontaneously attractive (Attractive group, A) and the three spontaneously unattractive odorants (Unattractive group, UnA) used in the experiment (investigation time  $A = 22.040 \pm 1.929$  s,  $UnA = 10.950 \pm 0.912$  s; Two-Tailed Wilcoxon,  $W = 35.000$   $p < 0.001$  Rank-Biserial Correlation =  $-0.849$ ). Remarkably, we found no difference of c-Fos<sup>+</sup> cell density in any of the secondary olfactory areas between odorant groups except for the OT (c-Fos<sup>+</sup> cell in OT  $A = 1014.090 \pm 73.140$  cells/mm<sup>2</sup>,  $UnA = 631.194 \pm 100.211$  cells/mm<sup>2</sup>; Bonferroni corrected One-Tailed Unpaired t-test, AON  $t = -0.704$   $p = 1$  Cohen's  $d = -0.426$ , aPirCX  $t = -0.330$   $p = 1$  Cohen's  $d = -0.184$ , pPirCX  $t = 0.477$   $p = 1$  Cohen's  $d = 0.265$ , OT  $t = -2.991$   $p = 0.037$  Cohen's  $d = -1.664$ , pLCoA  $t = 0.380$   $p = 1$  Cohen's  $d = 0.211$ , EntCX  $t = 0.860$   $p = 1$  Cohen's  $d = 0.478$ ; Figure 3C). Indeed, the OT showed a higher density of c-Fos<sup>+</sup> cell density in response to attractive compared to unattractive odorants (Figure 3C). This difference arose principally from its medial domain (mOT) compared to the lateral OT (lOT) (Figure S3C).

To confirm that the increase of c-Fos expression observed in the OT reflects an increase in neural activity during odor sampling itself, we directly probed the activity of the mOT in freely moving mice, using an eight-channel electrode array implanted in the mOT (Figure S3D). Extracellular single-unit recordings of mOT activity were performed during 120 s trials in which mice were allowed to explore the hole-board. Each mouse was tested in different trials with the three attractive (LIM, CITRO, CAM) and the three unattractive odorants (PYR, CRE, GUA). We first confirmed that the distribution of attraction to odorants is similar to that shown on Figure 3B (Kolmogorov-Smirnov  $p = 0.100$ ), thus replicating the previous behavioral data. Although attractive and unattractive odorants led to similar firing rate throughout the entire 120 s of the trial (background firing rate  $A = 3.512 \pm 0.846$  Hz,  $UnA = 3.456 \pm 0.512$  Hz; One-Tailed Mann-Whitney  $W = 2610.000$   $p = 0.237$  Rank-Biserial Correlation =  $-0.070$ ), approach (defined by the 3 s phase preceding the nose poke) toward attractive odorants resulted in a significant increase in firing frequency compared to background firing (firing rate for A Odorized Hole =  $5.248 \pm 1.155$  Hz, firing rate for UnA Odorized Hole =  $3.637 \pm 0.496$  Hz; Bonferroni corrected One-Tailed Wilcoxon for

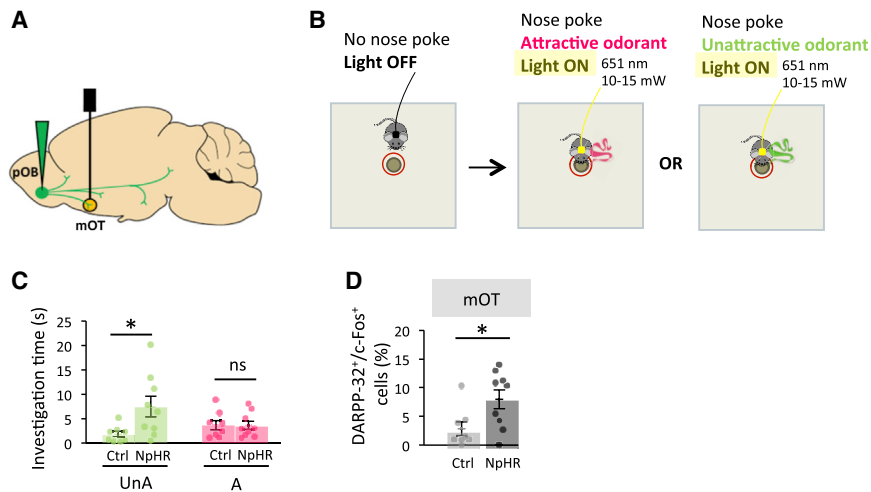
(D) Activity of a single OT neuron during the 3 s period preceding the first hole investigation in response to the A odorant CITRO. Data include example spike events (top), firing histogram (bottom) and overdrawn waveform (inset). Scale bars represent 0.1 mV.

(E) Population average values for all recorded neurons in the OT during the 3 s period preceding the first hole investigation by mice ( $n = 10$ ) in response to A ( $n = 55$  units) or UnA odorants ( $n = 102$  units). Values represent means  $\pm$  SEM.

(F) Firing rate across the entire trial (background) and during the 3 s period preceding the first hole investigation (Odorized Hole). Firing rate increased when animals ( $n = 10$ ) approached A ( $n = 55$  units) but not UnA odorants ( $n = 102$  units) compared to background (Bonferroni corrected One-Tailed Wilcoxon, A  $W = 354.000$   $p < 0.001$  Rank-Biserial Correlation =  $-0.523$ , UnA  $W = 2185.000$   $p = 0.136$  Rank-Biserial Correlation =  $-0.168$ ). Values represent means  $\pm$  SEM. (G) A higher percentage of TH+ cells expressing c-Fos in the VTA is observed in A ( $n = 6$ ) compared to UnA group ( $n = 5$ ) (One-Tailed Unpaired t-test,  $t = -2.176$   $p = 0.026$  Cohen's  $d = -1.211$ ). Bars represent means of individual data points  $\pm$  SEM.

(H) A higher percentage of DARPP-32+ cells expressing c-Fos in the OT is observed in A ( $n = 6$ ) compared to UnA group ( $n = 5$ ) (One-Tailed Unpaired t-test,  $t = -4.369$   $p < 0.001$  Cohen's  $d = -2.431$ ). Bars represent means of individual data points  $\pm$  SEM \* $p < 0.05$ , \*\*\* $p < 0.001$ .

See also Tables S1, S2, and Figure S3.



**Figure 4. Impact of pOB-mOT pathway activity on odor-driven attraction**

(A) Injection of NpHR-EYFP (NpHR;  $n = 9$ ) or control virus (Ctrl;  $n = 8$ ) in the mitral cells layer of the pOB and optical fiber implantation in mOT.

(B) The bilateral continuous light stimulation was automatically triggered by mice nose poking within 1 cm zone around the odorized hole (attractive or unattractive odors) and stopped automatically when nose poke stopped.

(C) Inhibition of pOB-mOT pathway activity increased mice attraction toward unattractive odors (UnA; Bonferroni corrected Two-Tailed Mann-Whitney,  $W = 12.000$   $p = 0.047$  Rank-Biserial Correlation =  $-0.667$ ) but not toward attractive ones (A; Bonferroni corrected Two-Tailed Mann-Whitney,  $W = 36.000$   $p = 1$  Rank-Biserial Correlation =  $0.000$ ). Bars represent means of individual data points  $\pm$  SEM.

(D) The percentage of DARPP-32+ cells expressing

c-Fos was higher in NpHR mice compared to Ctrl in mOT (Bonferroni corrected One-Tailed Mann-Whitney,  $W = 13.500$   $p = 0.034$  Rank-Biserial Correlation =  $-0.625$ ). Points represent individual data  $\pm$  SEM \* $p < 0.05$ .

See also Table S1 and Figure S4.

comparison to background,  $A$   $W = 354.000$   $p < 0.001$  Rank-Biserial Correlation =  $-0.523$ , UnA  $W = 2185.000$   $p = 0.136$  Rank-Biserial Correlation =  $-0.168$ ; Figures 3D–3F). Thus, during this time window, the mOT activity is modulated by the hedonic value of the odorant and the animal can use the odor cue emanating from the hole to initiate its approach to the odor source. These results support that the mOT encodes attraction to odors.

To further explore the link between OT activation during attraction to odor and the triggering of the motivated behavior, we next studied the recruitment of the OT-VTA network. We assessed activity of VTA dopaminergic neurons ( $TH^+/c-Fos^+$ ) and OT medium spiny neurons ( $DARPP-32^+/c-Fos^+$ ) in response to odors. We found a higher percentage of double-labeled cells in both structures in response to attractive versus unattractive odors ( $TH^+/c-Fos^+$  cells  $A = 8.962 \pm 3.632\%$  UnA =  $1.395 \pm 0.901\%$ ,  $DARPP-32^+/c-Fos^+$  cells  $A = 13.604 \pm 1.779\%$  UnA =  $5.830 \pm 0.647\%$ ; One-Tailed Unpaired  $t$ -test, VTA  $t = -2.176$   $p = 0.026$  Cohen's  $d = -1.211$ , OT  $t = -4.369$   $p < 0.001$  Cohen's  $d = -2.431$ ; Figures 3G and 3H). Altogether, these data highlight a key role of the OT in encoding odor-driven attraction.

### The pathway between the posterior olfactory bulb and the medial olfactory tubercle drives odorant attraction

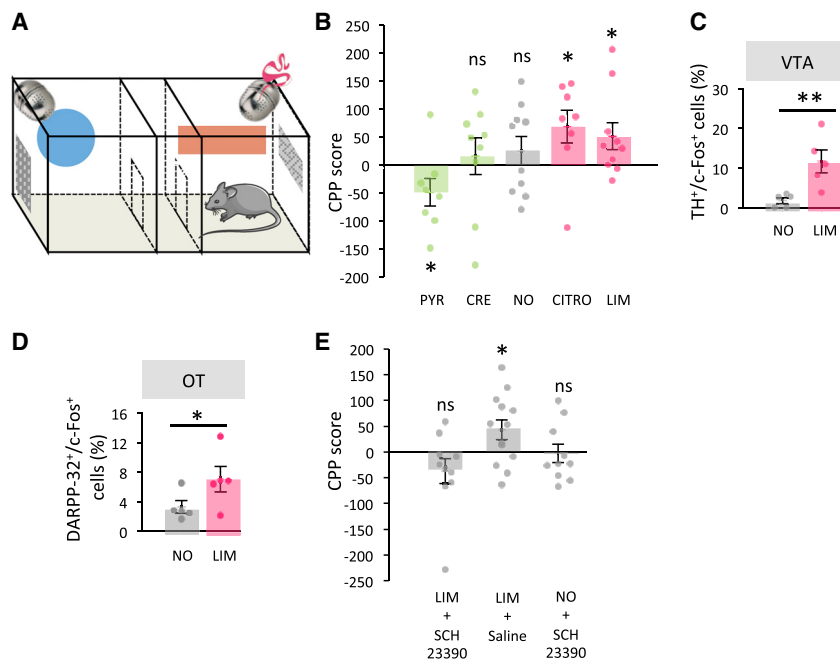
To uncover the functional involvement of the pOB-mOT pathway in driving odorant attraction, we modulated pOB-mOT activity during mice approach toward odors. Two groups of mice were injected with a Lenti-hSyn-eNpHR3.0-EYFP virus (NpHR group) or a control Lenti-hSyn-EYFP virus (Ctrl group) in the ventro-posterior mitral cell layer of the OB with optical fibers implanted in mOT (Figure 4A; Figures S4A and S4B). Mice were placed on the odorized hole board and bilateral light stimulation was automatically triggered when mice nose poked within 1 cm around the hole (light-triggering zone) and lasted as long as the nose poke (Figure 4B). The hole contained attractive or unattractive odors and each mouse was tested in the two conditions. First, Ctrl mice spent more time investigating attractive odors than unattractive ones (investigation time  $A = 3.683 \pm 0.962$  s UnA =  $1.793 \pm 0.602$  s; One-Tailed Wilcoxon  $W = 31.000$   $p =$

$0.034$  Rank-Biserial Correlation =  $0.722$ ), indicating no effect of light per se on odor-driven attraction. We then found that optogenetic inhibition of the pOB-mOT path increased investigation time of unattractive odors (NpHR =  $7.451 \pm 2.135$  s versus Ctrl =  $1.793 \pm 0.602$  s; Bonferroni corrected Two-Tailed Mann-Whitney,  $W = 12.000$   $p = 0.047$  Rank-Biserial Correlation =  $-0.667$ ). The investigation time of attractive odors was not different between NpHR and Ctrl mice (NpHR =  $3.600 \pm 0.849$  s; Ctrl =  $3.683 \pm 0.962$  s; Bonferroni corrected Two-Tailed Mann-Whitney,  $W = 36.000$   $p = 1$  Rank-Biserial Correlation =  $0.000$ ; Figure 4C).

We then assessed the effect of the optogenetic manipulation on mOT neuronal activity and found a higher percentage of medium spiny neurons expressing c-Fos ( $DARPP-32^+/c-Fos^+$ ) in NpHR than in Ctrl mice ( $DARPP-32^+/c-Fos^+$  cells, NpHR =  $7.969 \pm 1.652\%$  EYFP =  $2.863 \pm 1.192\%$ ; Bonferroni corrected One-Tailed Mann-Whitney,  $W = 13.500$   $p = 0.002$  Rank-Biserial Correlation =  $-0.625$ ; Figure 4D). No effect of optogenetic manipulation on neuronal activity was observed in IOT confirming the main effect of optogenetic stimulation in the mOT (Figure S4C). Altogether, these results demonstrated that the pOB modulated activity of mOT and enabled increasing attraction to unpleasant odors.

### Attractive odors induce conditioned place preference mediated by dopamine signaling

A classic way of testing the functional recruitment of the reward system by a given stimulus is to investigate whether this stimulus is able to serve as a reinforcer.<sup>23–25</sup> Conditioned place preference (CPP) is a well-established test to measure the reinforcing effects of a stimulus by evaluating if animals develop a preference for the location where they received the stimulus. To further confirm the rewarding property of attractive odors, we assessed whether mice can be conditioned in an odorant place preference paradigm. Following a period of habituation, we conditioned mice by alternatively confining them in two distinct compartments, one of which contained the odorant of interest (4 sessions of 15 min/day across 5 days) (Figure 5A) and the other compartment contained no odorant. Before (pre-conditioning) and after (post-conditioning) the conditioning phase, mice were allowed to freely



**Figure 5. Spontaneously attractive odorants induce conditioned place preference**  
(A) Schema of conditioned place preference (CPP) apparatus.

(B) CPP score (time in s spent in the odor paired compartment during post-conditioning – pre-conditioning test) is different between experimental groups. Only attractive odorants (CITRO  $n = 8$ , LIM  $n = 10$ ) induced CPP (One-Tailed One-Sample  $t$ -test, CITRO  $t = 2.331$   $p = 0.026$  Cohen's  $d = 0.824$ , LIM  $t = 2.123$   $p = 0.031$  Cohen's  $d = 0.671$ ). Unattractive ones (CRE  $n = 9$ , PYR  $n = 8$ ) induced no CPP (One-Tailed One-Sample  $t$ -test, CRE  $t = 0.473$   $p = 0.324$ , Cohen's  $d = 0.158$ ) or aversion (One-Tailed One-Sample  $t$ -test, PYR  $t = -1.974$   $p = 0.045$ , Cohen's  $d = -0.698$ ). Control group (NO,  $n = 10$ ) showed no CPP (One-Tailed One-Sample  $t$ -test, NO  $t = 1.005$   $p = 0.171$  Cohen's  $d = 0.318$ ). Bars represent means of individual data points  $\pm$  SEM.

(C) The percentage of TH<sup>+</sup> cells expressing c-Fos in VTA was higher in mice conditioned with LIM ( $n = 5$ ) compared to NO mice ( $n = 5$ ) (One-Tailed Unpaired  $t$ -test,  $t = -3.302$   $p = 0.005$ , Cohen's  $d = -2.088$ ). Bars represent means of individual data points  $\pm$  SEM.

(D) The percentage of DARPP-32<sup>+</sup> cells expressing c-Fos in OT was higher in LIM conditioned ( $n = 5$ )

compared to control ( $n = 5$ ) mice (One-Tailed Unpaired  $t$ -test,  $t = -1.968$   $p = 0.042$ , Cohen's  $d = -1.245$ ). Bars represent means of individual data points  $\pm$  SEM. (E) CPP to LIM was suppressed by D1 receptor antagonist SCH23390 (LIM+SCH23390,  $n = 10$ ) (Two-Tailed One-Sample  $t$ -test,  $t = -1.509$   $p = 0.166$ , Cohen's  $d = -0.477$ ). This was not observed after Saline infusion (LIM+Saline,  $n = 13$ ) (Two-Tailed One-Sample  $t$ -test,  $t = 2.258$   $p = 0.043$ , Cohen's  $d = 0.626$ ) and no conditioned aversion was observed in control group injected with SCH23390 (NO+SCH23390,  $n = 10$ ) (Two-Tailed One-Sample  $t$ -test,  $t = -0.180$   $p = 0.861$ , Cohen's  $d = -0.057$ ) indicating that drug alone did not induce avoidance. Bars represent means of individual data points  $\pm$  SEM \* $p < 0.05$ , \*\* $p < 0.01$ .

See also [Table S1](#) and [Figure S5](#).

explore both compartments in the absence of reinforcer (odorant), and the time spent in each of them was recorded. Changes in the time spent in the stimulus-paired compartment between pre-conditioning and post-conditioning is calculated as an index of the preference for the reinforced compartment (conditioned place preference score) and thus of the reinforcing effect of the stimulus.<sup>24</sup> In a first series of experiments, five groups of mice were tested, two groups were trained with spontaneously attractive odorants (CITRO or LIM,  $n = 8$  and  $10$ , respectively), two with spontaneously unattractive odorants (PYR or CRE,  $n = 8$  and  $9$ , respectively), and a control group of mice was submitted to the same sequence of behavior but in the absence of odorant (NO,  $n = 10$ ) ([Table S1](#)).

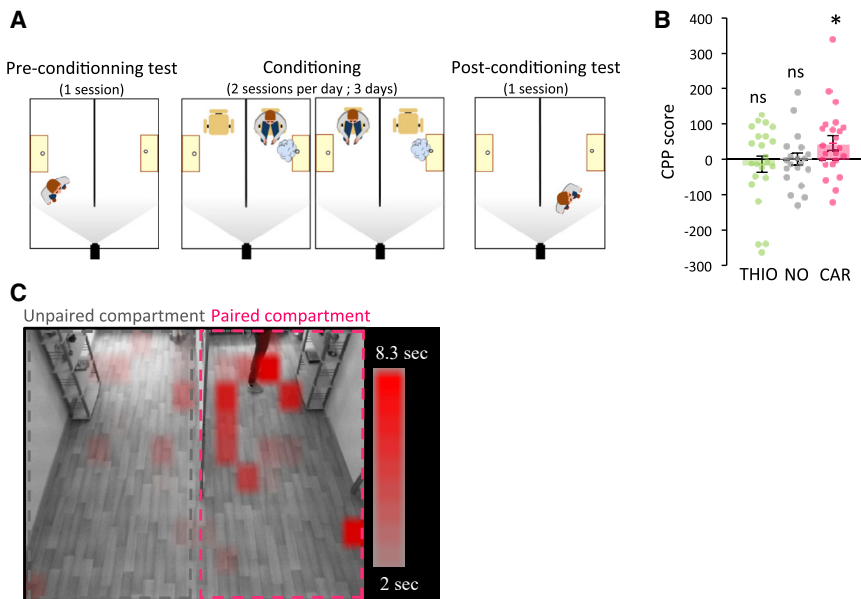
We found that mice spent more time in the compartment previously paired with a spontaneously attractive odorant indicating a place preference conditioning induced by attractive odorant and thus a rewarding effect of the odorants (CPP score CITRO =  $68.705 \pm 29.480$  s LIM =  $50.869 \pm 23.963$  s; One-Tailed One-Sample  $t$ -test, CITRO  $t = 2.331$   $p = 0.026$  Cohen's  $d = 0.824$  LIM  $t = 2.123$   $p = 0.031$  Cohen's  $d = 0.671$ ; [Figure 5B](#)). Second, mice spent less time in the compartment previously paired with one of the two unattractive odorants (PYR) (CPP score PYR =  $-48.900 \pm 24.000$  s; One-Tailed One-Sample  $t$ -test,  $t = -1.974$   $p = 0.045$  Cohen's  $d = -0.698$ ; [Figure 5B](#)) indicative of a conditioned place aversion. CRE group and control animals (NO) showed neither place preference nor aversion (CPP score CRE =  $15.678 \pm 33.127$  s NO =  $25.405 \pm 25.271$  s; One-Tailed One-Sample  $t$ -test, CRE  $t = 0.473$   $p = 0.324$  Cohen's  $d = 0.158$  NO  $t = 1.005$   $p = 0.171$  Cohen's  $d = 0.318$ ; [Figure 5B](#)). These

results showed that attractive odorants possess positive reinforcing properties. Further, these results demonstrated that at least one unattractive odorant can have an opposite effect and induce a conditioned place aversion showing negative reinforcing properties.

To go further, we analyzed the neural activity within both the OT and the VTA using assessment of c-Fos expression, in medium spiny neurons (DARPP-32<sup>+</sup> cells) and dopaminergic cells (TH<sup>+</sup> cells), respectively, following place preference in response to an odorant (LIM-CPP versus control NO groups). We found a higher percentage of double-labeled cells in both neuronal populations in LIM-CPP compared to control NO group (TH<sup>+</sup>/c-Fos<sup>+</sup> cells LIM =  $11.726 \pm 2.894\%$  NO =  $1.841 \pm 0.764\%$ , DARPP-32<sup>+</sup>/c-Fos<sup>+</sup> cells LIM =  $7.055 \pm 1.706\%$  NO =  $3.301 \pm 0.853\%$ ; One-Tailed Unpaired  $t$ -test,  $t = -3.302$  VTA  $p = 0.005$  Cohen's  $d = -2.088$  and OT  $t = -1.968$   $p = 0.042$  Cohen's  $d = -1.245$ ; [Figures 5C](#) and [5D](#)), indicating an activation of both OT and VTA by CPP.

Since the release of dopamine (DA) within the reward system is a common substrate of rewarding stimuli,<sup>12</sup> we performed a second series of experiments aimed at altering dopaminergic transmission during odor CPP to more directly assess the recruitment of the reward system by spontaneously attractive odorants. In a first group of animals, the DAergic D1 receptor antagonist SCH23390<sup>26,27</sup> was systemically injected 15 min before confinement in the LIM-paired compartment while saline was injected 15 min before confinement in the unpaired compartment (LIM+SCH23390 group). Remarkably, D1 antagonism suppressed LIM-induced CPP in the LIM+SCH23390 group while mice of





**Figure 6. Attractive odorant induced conditioned place preference in humans**

(A) Schema of conditioned place preference paradigm (CPP).

(B) CPP score in odor paired compartment was calculated as follows, time spent in the conditioned compartment during post-conditioning – pre-conditioning. CAR (n = 23) (One-Tailed One-sample t-test,  $t = 2.219$   $p = 0.019$ , Cohen's  $d = 0.463$ ) and not control (NO, n = 21) (One-Tailed One-sample t-test,  $t = 0.043$   $p = 0.483$ , Cohen's  $d = 0.009$ ) or THIO (n = 23) (One-Tailed One-sample t-test,  $t = -0.592$   $p = 0.280$ , Cohen's  $d = -0.123$ ) induce CPP. Bars represent means of individual data points  $\pm$  SEM.

(C). CAR-CPP score representation in discretized space of CPP apparatus. \* $p < 0.05$ .

See also [Table S1](#) and [Figure S6](#).

second group injected with saline (LIM+Saline group) continued expressing CPP (CPP score LIM+SCH23390 =  $-37.033 \pm 24.547$  s; LIM+Saline =  $42.705 \pm 18.916$  s; Two-Tailed One-Sample t-test, LIM+SCH23390  $t = -1.509$   $p = 0.166$  Cohen's  $d = -0.477$ , LIM+Saline  $t = 2.258$   $p = 0.043$  Cohen's  $d = 0.626$ ; [Figure 5E](#)), similarly to non-injected mice ([Figure 5B](#)). Importantly, because the OB contains DAergic periglomerular interneurons involved in odor processing, we verified that SCH23390 did not alter LIM detection ([Figure S5A](#)). In addition, to exclude any adverse effect of SCH23390, we performed testing in an additional control group in which SCH23390 injection was paired to one of the two compartments without any odorant (NO+SCH23390 group) to confirm that the drug alone did not induce conditioned place aversion (CPP score NO+SCH23390 =  $-3.210 \pm 17.833$  s; Two-Tailed One-Sample t-test, NO+SCH23390  $t = -0.180$   $p = 0.861$  Cohen's  $d = -0.057$ ; [Figure 5E](#)). Notably, we observed a reduction in the average locomotion speed of mice during confinement ([Figure S5B](#)) following SCH23390, in line with alteration of locomotion associated with dopaminergic antagonists.<sup>28</sup> However, because SCH23390 was injected during the conditioning phase only (confinement) and not during pre-conditioning or post-conditioning tests, and since a 24 h wash-out was allowed between SCH23390 injection and testing, we reason that the speed reduction is not a major influencer on the CPP score. Together, these results show that dopamine signaling bestows odorants with rewarding properties thereby driving their spontaneous attraction.

### Attractive odorants induce conditioned place preference in humans

To analyze whether our observations can be extended to humans, 67 participants (34 women, 35 men) between the ages of 18 and 30 years (mean  $\pm$  SD,  $21.5 \pm 2.6$  years) were recruited to take part in a similar CPP test. The CPP that is classically used in the animal model to evaluate the rewarding power of a stimulus (such as drugs or food), has also occasionally been applied

to humans.<sup>29,30</sup> In our study, the CPP facility consisted of an experimental room separated into two compartments of the same size, shape, and furnishing but clearly distinguishable based on decorative elements ([Figure 6A](#)). As in mice, before and after a conditioning phase (2 sessions of 10 min/day across 3 days) in which participants were alternatively confined in two distinct compartments, one of which contained an attractive odorant (L-carvone: CAR; n = 23) and the other one no odorant, participants were allowed to freely explore both compartments in the absence of odorant and the time spent in each of them was compared between pre- and post-conditioning test. The odorant CAR was selected since this odorant is rated as strongly attractive in humans.<sup>20,31,32</sup> When comparing the time spent in each compartment in the post-conditioning versus the pre-conditioning phases, we found that participants spent more time in the room previously paired with CAR (CPP score CAR =  $45.877 \pm 20.678$  s; One-Tailed One-sample t-test  $t = 2.219$   $p = 0.019$  Cohen's  $d = 0.463$ ; [Figures 6B](#) and [6C](#)), indicating an odor-driven CPP in humans.

To validate this result, two additional experimental groups were tested. First, a control no odor group (NO; n = 21) was found to display no place preference following being subjected to the same behavioral paradigm but with no odor associated to any compartment (CPP score NO =  $0.738 \pm 17.091$  s; One-Tailed One-sample t-test  $t = 0.043$   $p = 0.483$  Cohen's  $d = 0.009$ ; [Figure 6B](#)). Second, we also tested the effect of CPP training with an unattractive odorant (thioglycolic acid: THIO; n = 23) and found no place preference (CPP score THIO =  $-13.801 \pm 23.330$  s; One-Tailed One-sample t-test  $t = -0.592$   $p = 0.280$  Cohen's  $d = -0.123$ ; [Figure 6B](#)). We verified that the CPP scores cannot be explained by a spontaneous preference for one specific compartment ([Figure S6A](#)) or by a difference in intensity perception between the two odorants ([Figure S6B](#)). We also confirmed that odorant smelled during the conditioning phase by the CAR group was rated as more attractive than by the THIO group ([Figure S6C](#)). Taken together, these results show that spontaneously attractive odorants are rewarding in humans as they are in mice when assessed by CPP.

### The activity of the olfactory tubercle reflects the odorants attraction in humans

We finally confirmed the recruitment of the human OT by attractive odorants. 30 volunteers were asked to rate, during an fMRI session, the attraction of seven odorants (butanoic acid: BUT, 3-hydroxy-3-methylhexanoic acid: HMHA, 3-methyl-3-sulfanyhexan-1-ol: MSH, cis-3-hexenol: CIS3, terpinen-4-ol: TER, +limonene: LIM and isoamyl acetate: ISO) from 1 (weakly/unattractive) to 5 (highly attractive) during a fMRI recording session (Table S1). For each participant, odorants rated from 3 to 5 were classified in the “attractive group,” whereas odorants rated 1 or 2 were classified in the “unattractive group.” First, as expected, we found activation in olfactory areas including the PirCX and area bordering the OT in response to odorants (Figure 7A). Sniffing was digitally recorded during the experiment and was used as a covariate in the fMRI contrast estimation. The fMRI images were then averaged within the two attraction conditions based on the individual odorant rating (attractive ratings, unattractive ratings). Then, mean activity levels were measured in the OT, aPirCX, and pPirCX using ROIs. Mean activity of the OT, aPirCX, and pPirCX were averaged between hemispheres (no hemisphere\*attraction interaction, Repeated-measure ANOVA, OT  $F_{(3,120)} = 0.12$   $p = 0.914$ , aPirCX  $F_{(3,120)} = 0.94$   $p = 0.336$ , pPirCX  $F_{(3,120)} = 0.07$   $p = 0.797$ ). These results uncovered higher OT activation in response to the attractive odorants compared to those unattractive (Mean  $\beta$ value A =  $2.109 \pm 0.821$  UnA =  $0.708 \pm 0.706$ ; Bonferroni corrected One-Tailed Paired t-test, OT  $t = 2.572$   $p = 0.023$  Cohen's  $d = 0.470$ ; Figures 7B–7D), whereas no difference was found in either the aPirCX or pPirCX (Mean  $\beta$ value in aPirCX A =  $2.342 \pm 0.609$  UnA =  $1.823 \pm 0.507$ , Mean  $\beta$ value in pPirCX A =  $3.148 \pm 0.799$  UnA =  $2.515 \pm 0.738$ ; Bonferroni corrected One-Tailed Paired t-test, aPirCX  $t = 1.320$   $p = 0.296$  Cohen's  $d = 0.241$ , pPirCX  $t = 1.081$   $p = 0.432$  Cohen's  $d = 0.197$ ; Figures 7E–7J). This confirms the selective recruitment of the OT by attractive odorants.

### DISCUSSION

Our close relationship with smells exemplifies the strong attractive power they exert over us. Why do some odorants possess such strong effects? This study provides behavioral, anatomical, and functional evidence that odorants can act as natural rewards in the absence of associated food or social cues.

We first discovered that the pOB supports self-stimulation conditioning, suggesting that this sensory structure strongly interfaces with the reward system. Multiple brain areas support self-stimulation operant conditioning, especially those in the reward system.<sup>10,11</sup> These areas include the VTA itself, as well as its direct synaptic targets such as the nucleus accumbens, the prefrontal cortex, or the septum. Self-stimulation conditioning can also be obtained from brain areas projecting to the VTA such as the OT or the hypothalamus.<sup>33,34</sup> Our data reveal that the pOB, not receiving from nor sending direct synaptic contacts to the VTA, supports self-stimulation conditioning, in line with an isolated report from the early 1970s suggesting electrical self-stimulation in the rat OB.<sup>35</sup> Thus, our results highlight an unexpectedly new region-dependent role of the OB, and therefore the rewarding power of odorants. We found that attractive odorants induced DA-

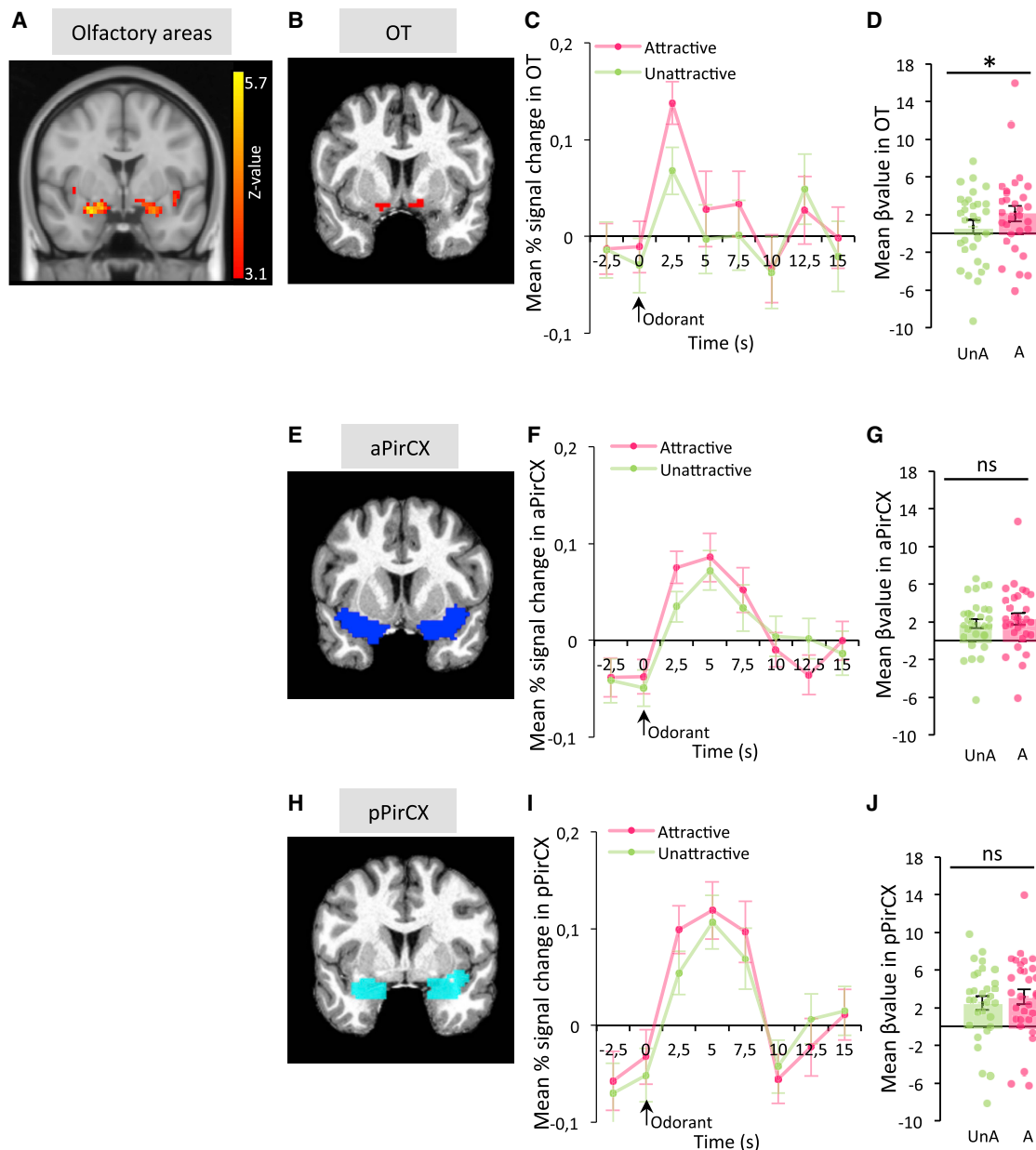
dependent CPP, indicating that they have reinforcing properties as other well-known natural reinforcers, such as food or mating.<sup>36,37</sup> Importantly, we were able to show that pleasant odorants can drive CPP in humans, suggesting that the reinforcing properties of odorants are not limited to macroscopic animals but perpetuate in humans. Further, our finding that the pOB but not the aOB is a site for self-stimulation has two major implications. First, this strongly suggests that hedonic representations of odorants are incentive information relayed to the reward system to induce attraction. Second, it confirms that the olfactory system's representation of hedonics is functionally partitioned along its antero-posterior axis.<sup>6</sup> Consistent with a dual processing of olfactory hedonics, we also found that at least some unattractive odorants can induce conditioned place aversion.

The next question we addressed relates to the brain circuit accounting for the behavioral reinforcing properties of odorants. pOB self-stimulation activated the DAergic neurons of the VTA and the relay neurons of the OT indicating actual recruitment of the reward system that could form the neural basis of the incentive value of attractive odorants. Furthermore, both unit recordings and c-Fos cell mapping indicated that the OT responded selectively to the exposure of attractive odorants. Remarkably, c-Fos cell mapping data further revealed that among all the direct synaptic targets of the OB, only the OT exhibits a selective response with regard to odor hedonic tone. Consistently, increasing MSNs activity through optogenetic manipulation of the pOB-mOT pathway made unattractive odorant more attractive. This effect was obtained by local optogenetic inhibition of mitral cells which project to the GABAergic interneurons of the OT layer1, possibly leading to MSNs disinhibition.<sup>38</sup>

It is tempting to relate the hedonic-specific responses of the OT to its location at the cross road between the olfactory and reward systems and more specifically the VTA to which the OT is connected.<sup>12,13,38</sup> In animal models, the role of the OT in processing attractive or aversive odorants with strong ethological significance such as food, predator or opposite-sex odors<sup>15,39</sup> or learned odorants that predict a reward<sup>13,16,17,40</sup> is well documented. Here, we provide evidence for a new role of the OT—that being in processing attraction induced by unlearned odorants. Moreover, the direct stimulation of OT<sup>39</sup> or the modulation of the VTA-mOT pathway<sup>40</sup> are known to modify olfactory preferences; here, we revealed that afferent projections from pOB to mOT are also implicated in the build-up of odor-driven attraction.

We rigorously identified that the pOB sends denser projections to the OT than the aOB does. This distinct pattern of connectivity from the aOB or the pOB to the OT may relate to the timing of mitral cell development<sup>41</sup> and adds a layer to the functional significance of the distribution of hedonic information along the antero-posterior axis of the OB. Finally, the privileged connection between the pOB and the OT highlighted the pOB-OT-VTA axis as a key pathway for processing odor information and converting it into motivated behavior. A question of interest for future investigation relates to how the activity of such hard-wired pathway is modified or overcome as the initial odorant value is changed by learning or experience.

The role of the OT in coding odor hedonics in humans remained controversial.<sup>42,43</sup> Using ROI analysis to take into account the anatomical variabilities between subjects, our findings bring new evidence for selective activation of the OT, but



**Figure 7. Attractive odorants specifically activate the OT in humans**

(A) Brain activity in response to odorants ( $n = 30$  subjects). Brain activity was present in the olfactory areas including the PirCX and bordering the OT.

(B) Example of OT ROI for one participant.

(C) OT activity in response to attractive (A) or unattractive (UnA) odorants. Values represent means  $\pm$  SEM.

(D) The OT showed higher level of activity in response to A compared to UnA odorants (Bonferroni corrected One-Tailed Paired  $t$ -test,  $t = 2.572$   $p = 0.023$ , Cohen's  $d = 0.470$ ). Bars represent means of individual data points  $\pm$  SEM.

(E) Example of aPirCX ROI for one participant.

(F) aPirCX activity in response to A or UnA odorants. Values represent means  $\pm$  SEM.

(G) There is no difference of activity in the aPirCX between A and UnA odorants (Bonferroni corrected One-Tailed Paired  $t$ -test,  $t = 1.320$   $p = 0.296$ , Cohen's  $d = 0.241$ ). Bars represent means of individual data points  $\pm$  SEM.

(H) Example of pPirCX ROI for one participant.

(I) pPirCX activity in response to A or UnA odorants. Values represent means  $\pm$  SEM. The activity in pPirCX was similar between response to A and UnA odorants (Bonferroni corrected One-Tailed Paired  $t$ -test,  $t = 1.081$   $p = 0.432$ , Cohen's  $d = 0.197$ ). Bars represent means of individual data points  $\pm$  SEM \* $p < 0.05$ .

See also [Table S1](#).

not of the PirCX, in response to attractive odorants. Thus, our data obtained in mice were confirmed in humans, suggesting a highly conserved role of the OT between mice and humans, despite anatomical differences between the two species. In rodents, the OT is visible as a round bulge that covers a wide area of the basal forebrain and is located between the lateral olfactory tract and the optic chiasm.<sup>13</sup> Data from human brain imaging identified it as a small structure ventrally located to the anterior olfactory cortex, between the uncus and the medial forebrain bundles.<sup>44,45</sup> Despite these regional differences, the OT is targeted by the OB output, making it a structure belonging to the primary olfactory cortex both in humans<sup>46</sup> and rodents.<sup>13</sup> Interestingly, the PirCX showed no change in activity according to odor hedonics. The PirCX contributes to odor identity coding, habituation mechanisms, olfactory learning, and memory.<sup>47–54</sup> Several studies carried out in humans and animals also argued in favor of the involvement of PirCX in the representation of odor hedonics.<sup>53,55–61</sup> This apparent discrepancy with our data may arise from the use of odorants in a learning context or with strong biological meaning. The odorant cerebral representation may thus reflect associative and hedonic characteristics related to smell. Indeed, the role of the PirCX in encoding associative olfactory information is well documented.<sup>62–66</sup> Finally, the different functional roles of the OT and PirCX can be explained by their difference of brain connectivity. fMRI whole-brain functional connectivity cluster analysis yields different clusters corresponding respectively to OT and PirCX,<sup>46</sup> making the OT a specific pathway for spontaneous odorant-driven attraction.

Altogether, these data demonstrate that unlearned attractive odorants are rewarding in mice and humans thanks to strong functional and anatomical connections between the OB and the OT thereby allowing a shortcut entrance of olfactory information in the reward system. Several stimuli are known to be rewarding in humans, including sweet tastes, money, smiling faces, some artwork, and some music.<sup>67–70</sup> Preferred music induces a release of dopamine in striatal regions<sup>68</sup> and administration of dopamine precursor or antagonist respectively enhanced or decreased music motivated responses.<sup>69</sup> It is worth noting that not all sensory stimuli are rewarding. For instance, only preferred music (and not random music) recruits the reward system.<sup>68</sup> In line with this, we found that not all odorants are rewarding, but only attractive ones. These data suggest that the involvement of the reward system is based on intrinsic hedonic properties of sensory stimuli and not a global rewarding effect of sensory arousal.<sup>71</sup>

In sum, our data propose a direct gateway from the olfactory bulb to the reward system as the neural basis of the strong and persistent attraction of some odors.

## STAR★METHODS

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## SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2021.01.066>.

## ACKNOWLEDGMENTS

This work was supported by the CNRS, Inserm, Lyon 1 University, Université de Lyon PALSE program (travel fellowship to M.M.), Roudnitska Foundation (PhD fellowship to M.M.), NIH NIDCD grants R01DC014443, R01DC016519, R01DA049545, and R01DA049449 to D.W. and by a grant from the ANR/DFG SHS FRAL program (MEROD Project, ANR-15-FRAL-0002) to M.B. We would like to thank K. Deisseroth for the gift of the halorhodopsin construct, C. Benetollo from the Neurogenetic and Optogenetic Platform of the CRNL for lentiviral production and G. Froment, D. Nègre, and C. Costa from the lentivector production facility /SFR BioSciences de Lyon (UMS3444/US8).

## AUTHOR CONTRIBUTIONS

M.M., L.C., N.R., M.T., N.N., C.F., A.F., D.W.W., M.B., A.D., and N.M., designed the experiments. M.M., L.C., N.R., A.M., M.T., J.S., M.B., J.F., C.F., A.F., and N.M. conducted the experiments. M.M., L.C., N.R., M.R., O.R., M.B., A.D., and N.M. wrote the paper.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: September 30, 2020

Revised: January 7, 2021

Accepted: January 19, 2021

Published: February 17, 2021

## REFERENCES

1. Schiffman, S., Robinson, D.E., and Erickson, R.P. (1977). Multidimensional scaling of odorants: examination of psychological and physicochemical dimensions. *Chem. Senses* 2, 375–390.
2. Richardson, J.T., and Zucco, G.M. (1989). Cognition and olfaction: a review. *Psychol. Bull.* 105, 352–360.
3. Yeshurun, Y., and Sobel, N. (2010). An odor is not worth a thousand words: from multidimensional odors to unidimensional odor objects. *Annu. Rev. Psychol.* 61, 219–241.
4. Walliczek-Dworschak, U., and Hummel, T. (2017). The Human Sense of Olfaction. *Facial Plast. Surg.* 33, 396–404.



5. Kosaka, T., and Kosaka, K. (2009). Olfactory Bulb Anatomy. In *Encyclopedia of Neuroscience*, L.R. Squire, ed. (Academic Press), pp. 59–69.
6. Kermen, F., Midroit, M., Kuczewski, N., Forest, J., Thévenet, M., Sacquet, J., Benetollo, C., Richard, M., Didier, A., and Mandairon, N. (2016). Topographical representation of odor hedonics in the olfactory bulb. *Nat. Neurosci.* *19*, 876–878.
7. Schultz, W. (1998). Predictive reward signal of dopamine neurons. *J. Neurophysiol.* *80*, 1–27.
8. Ikemoto, S., and Wise, R.A. (2004). Mapping of chemical trigger zones for reward. *Neuropharmacology* *47* (Suppl 1), 190–201.
9. Ikemoto, S. (2010). Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. *Neurosci. Biobehav. Rev.* *35*, 129–150.
10. Olds, J., and Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J. Comp. Physiol. Psychol.* *47*, 419–427.
11. Carlezon, W.A., Jr., and Chartoff, E.H. (2007). Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nat. Protoc.* *2*, 2987–2995.
12. Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res. Brain Res. Rev.* *56*, 27–78.
13. Wesson, D.W., and Wilson, D.A. (2011). Sniffing out the contributions of the olfactory tubercle to the sense of smell: hedonics, sensory integration, and more? *Neurosci. Biobehav. Rev.* *35*, 655–668.
14. Agustín-Pavón, C., Martínez-García, F., and Lanuza, E. (2014). Focal lesions within the ventral striato-pallidum abolish attraction for male chemosignals in female mice. *Behav. Brain Res.* *259*, 292–296.
15. DiBenedictis, B.T., Olugbemi, A.O., Baum, M.J., and Cherry, J.A. (2015). DREADD-Induced Silencing of the Medial Olfactory Tubercle Disrupts the Preference of Female Mice for Opposite-Sex Chemosignals(1,2,3). *eNeuro* *2*, <https://doi.org/10.1523/ENEURO.0078-15.2015>.
16. Gadziola, M.A., Tylicki, K.A., Christian, D.L., and Wesson, D.W. (2015). The olfactory tubercle encodes odor valence in behaving mice. *J. Neurosci.* *35*, 4515–4527.
17. Murata, K., Kanno, M., Ieki, N., Mori, K., and Yamaguchi, M. (2015). Mapping of Learned Odor-Induced Motivated Behaviors in the Mouse Olfactory Tubercle. *J. Neurosci.* *35*, 10581–10599.
18. Renier, N., Wu, Z., Simon, D.J., Yang, J., Ariel, P., and Tessier-Lavigne, M. (2014). iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. *Cell* *159*, 896–910.
19. Kobayakawa, K., Kobayakawa, R., Matsumoto, H., Oka, Y., Imai, T., Ikawa, M., Okabe, M., Ikeda, T., Itoharu, S., Kikusui, T., et al. (2007). Innate versus learned odour processing in the mouse olfactory bulb. *Nature* *450*, 503–508.
20. Mandairon, N., Poncelet, J., Bensafi, M., and Didier, A. (2009). Humans and mice express similar olfactory preferences. *PLoS ONE* *4*, e4209.
21. Mandairon, N., Sultan, S., Rey, N., Kermen, F., Moreno, M., Busto, G., Farget, V., Messaoudi, B., Thevenet, M., and Didier, A. (2009). A computer-assisted odorized hole-board for testing olfactory perception in mice. *J. Neurosci. Methods* *180*, 296–303.
22. Midroit, M., Thevenet, M., Fournel, A., Sacquet, J., Bensafi, M., Breton, M., Chalençon, L., Cavelius, M., Didier, A., and Mandairon, N. (2018). Non-imaged based method for matching brains in a common anatomical space for cellular imagery. *J. Neurosci. Methods* *304*, 136–145.
23. Tahsili-Fahadan, P., Yahyavi-Firouz-Abadi, N., Khoshnoodi, M.A., Motiei-Langroudi, R., Tahaei, S.A., Ghahremani, M.H., and Dehpour, A.R. (2006). Agmatine potentiates morphine-induced conditioned place preference in mice: modulation by alpha2-adrenoceptors. *Neuropsychopharmacology* *31*, 1722–1732.
24. Huston, J.P., Silva, M.A. de S., Topic, B., and Müller, C.P. (2013). What's conditioned in conditioned place preference? *Trends Pharmacol. Sci.* *34*, 162–166.
25. McReynolds, J.R., Taylor, A., Vranjkovic, O., Ambrosius, T., Derricks, O., Nino, B., Kurtoglu, B., Wheeler, R.A., Baker, D.A., Gasser, P.J., and Mantsch, J.R. (2017). Corticosterone Potentiation of Cocaine-Induced Reinstatement of Conditioned Place Preference in Mice Is Mediated by Blockade of the Organic Cation Transporter 3. *Neuropsychopharmacology* *42*, 757–765.
26. Nazarian, A., Russo, S.J., Festa, E.D., Kraish, M., and Quinones-Jenab, V. (2004). The role of D1 and D2 receptors in the cocaine conditioned place preference of male and female rats. *Brain Res. Bull.* *63*, 295–299.
27. Pina, M.M., and Cunningham, C.L. (2014). Effects of dopamine receptor antagonists on the acquisition of ethanol-induced conditioned place preference in mice. *Psychopharmacology (Berl.)* *231*, 459–468.
28. Schindler, C.W., and Carmona, G.N. (2002). Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. *Pharmacol. Biochem. Behav.* *72*, 857–863.
29. Astur, R.S., Carew, A.W., and Deaton, B.E. (2014). Conditioned place preferences in humans using virtual reality. *Behav. Brain Res.* *267*, 173–177.
30. Childs, E., and de Wit, H. (2009). Amphetamine-induced place preference in humans. *Biol. Psychiatry* *65*, 900–904.
31. Licon, C.C., Manesse, C., Dantec, M., Fournel, A., and Bensafi, M. (2018). Pleasantness and trigeminal sensations as salient dimensions in organizing the semantic and physiological spaces of odors. *Sci. Rep.* *8*, 8444.
32. Jousain, P., Thevenet, M., Rouby, C., and Bensafi, M. (2013). Effect of aging on hedonic appreciation of pleasant and unpleasant odors. *PLoS ONE* *8*, e61376.
33. Swanson, L.W. (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res. Bull.* *9*, 321–353.
34. Morales, M., and Margolis, E.B. (2017). Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. *Nat. Rev. Neurosci.* *18*, 73–85.
35. Phillips, A.G., and Mogenson, G.J. (1969). Self-Stimulation of the Olfactory Bulb. *Physiol. Behav.* *4*, 195–197.
36. Imaizumi, M., Takeda, M., and Fushiki, T. (2000). Effects of oil intake in the conditioned place preference test in mice. *Brain Res.* *870*, 150–156.
37. Domínguez-Salazar, E., Naser, H.F., and Velázquez-Moctezuma, J. (2014). D1-like antagonist blocks conditioned place preference induced by ejaculation in male rats. *Behav. Brain Res.* *269*, 15–19.
38. Xiong, A., and Wesson, D.W. (2016). Illustrated Review of the Ventral Striatum's Olfactory Tubercle. *Chem. Senses* *41*, 549–555.
39. Fitzgerald, B.J., Richardson, K., and Wesson, D.W. (2014). Olfactory tubercle stimulation alters odor preference behavior and recruits forebrain reward and motivational centers. *Front. Behav. Neurosci.* *8*, 81.
40. Zhang, Z., Zhang, H., Wen, P., Zhu, X., Wang, L., Liu, Q., Wang, J., He, X., Wang, H., and Xu, F. (2017). Whole-Brain Mapping of the Inputs and Outputs of the Medial Part of the Olfactory Tubercle. *Front. Neural Circuits* *11*, 52.
41. Imamura, F., Ayoub, A.E., Rakic, P., and Greer, C.A. (2011). Timing of neurogenesis is a determinant of olfactory circuitry. *Nat. Neurosci.* *14*, 331–337.
42. Zelano, C., Montag, J., Johnson, B., Khan, R., and Sobel, N. (2007). Dissociated representations of irritation and valence in human primary olfactory cortex. *J. Neurophysiol.* *97*, 1969–1976.
43. de Araujo, I.E., Rolls, E.T., Velazco, M.I., Margot, C., and Cayeux, I. (2005). Cognitive modulation of olfactory processing. *Neuron* *46*, 671–679.
44. Sobel, N., Prabhakaran, V., Zhao, Z., Desmond, J.E., Glover, G.H., Sullivan, E.V., and Gabrieli, J.D. (2000). Time course of odorant-induced activation in the human primary olfactory cortex. *J. Neurophysiol.* *83*, 537–551.
45. Weismann, M., Yousry, I., Heuberger, E., Nolte, A., Ilmberger, J., Kobal, G., Yousry, T.A., Kettenmann, B., and Naidich, T.P. (2001). Functional

- magnetic resonance imaging of human olfaction. *Neuroimaging Clin. N. Am.* *11*, 237–250, viii.
46. Zhou, G., Lane, G., Cooper, S.L., Kahnt, T., and Zelano, C. (2019). Characterizing functional pathways of the human olfactory system. *eLife* *8*, <https://doi.org/10.7554/eLife.47177>.
  47. Hasselmo, M.E., Wilson, M.A., Anderson, B.P., and Bower, J.M. (1990). Associative memory function in piriform (olfactory) cortex: computational modeling and neuropharmacology. *Cold Spring Harb. Symp. Quant. Biol.* *55*, 599–610.
  48. Wilson, D.A. (1998). Habituation of odor responses in the rat anterior piriform cortex. *J. Neurophysiol.* *79*, 1425–1440.
  49. Barkai, E., and Saar, D. (2001). Cellular correlates of olfactory learning in the rat piriform cortex. *Rev. Neurosci.* *12*, 111–120.
  50. Illig, K.R., and Haberly, L.B. (2003). Odor-evoked activity is spatially distributed in piriform cortex. *J. Comp. Neurol.* *457*, 361–373.
  51. Kadohisa, M., and Wilson, D.A. (2006). Separate encoding of identity and similarity of complex familiar odors in piriform cortex. *Proc. Natl. Acad. Sci. USA* *103*, 15206–15211.
  52. Barnes, D.C., Hofacer, R.D., Zaman, A.R., Rennaker, R.L., and Wilson, D.A. (2008). Olfactory perceptual stability and discrimination. *Nat. Neurosci.* *11*, 1378–1380.
  53. Li, W., Howard, J.D., Parrish, T.B., and Gottfried, J.A. (2008). Aversive learning enhances perceptual and cortical discrimination of indiscriminable odor cues. *Science* *319*, 1842–1845.
  54. Howard, J.D., Plailly, J., Grueschow, M., Haynes, J.-D., and Gottfried, J.A. (2009). Odor quality coding and categorization in human posterior piriform cortex. *Nat. Neurosci.* *12*, 932–938.
  55. Gottfried, J.A., Deichmann, R., Winston, J.S., and Dolan, R.J. (2002). Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. *J. Neurosci.* *22*, 10819–10828.
  56. Sevelinges, Y., Gervais, R., Messaoudi, B., Granjon, L., and Mouly, A.-M. (2004). Olfactory fear conditioning induces field potential potentiation in rat olfactory cortex and amygdala. *Learn. Mem.* *11*, 761–769.
  57. Li, W., Luxenberg, E., Parrish, T., and Gottfried, J.A. (2006). Learning to smell the roses: experience-dependent neural plasticity in human piriform and orbitofrontal cortices. *Neuron* *52*, 1097–1108.
  58. Bensafi, M., Sobel, N., and Khan, R.M. (2007). Hedonic-specific activity in piriform cortex during odor imagery mimics that during odor perception. *J. Neurophysiol.* *98*, 3254–3262.
  59. Sevelinges, Y., Sullivan, R.M., Messaoudi, B., and Mouly, A.-M. (2008). Neonatal odor-shock conditioning alters the neural network involved in odor fear learning at adulthood. *Learn. Mem.* *15*, 649–656.
  60. Barnes, D.C., Chapuis, J., Chaudhury, D., and Wilson, D.A. (2011). Odor fear conditioning modifies piriform cortex local field potentials both during conditioning and during post-conditioning sleep. *PLoS ONE* *6*, e18130.
  61. Pantazopoulos, H., Dolatshad, H., and Davis, F.C. (2011). A fear-inducing odor alters PER2 and c-Fos expression in brain regions involved in fear memory. *PLoS ONE* *6*, e20658.
  62. Schoenbaum, G., and Eichenbaum, H. (1995). Information coding in the rodent prefrontal cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform cortex. *J. Neurophysiol.* *74*, 733–750.
  63. Litaudon, P., Mouly, A.M., Sullivan, R., Gervais, R., and Cattarelli, M. (1997). Learning-induced changes in rat piriform cortex activity mapped using multisite recording with voltage sensitive dye. *Eur. J. Neurosci.* *9*, 1593–1602.
  64. Cohen, Y., Reuveni, I., Barkai, E., and Maroun, M. (2008). Olfactory learning-induced long-lasting enhancement of descending and ascending synaptic transmission to the piriform cortex. *J. Neurosci.* *28*, 6664–6669.
  65. Gottfried, J.A. (2010). Central mechanisms of odour object perception. *Nat. Rev. Neurosci.* *11*, 628–641.
  66. Wilson, D.A., and Sullivan, R.M. (2011). Cortical processing of odor objects. *Neuron* *72*, 506–519.
  67. Berridge, K.C., Robinson, T.E., and Aldridge, J.W. (2009). Dissecting components of reward: ‘liking’, ‘wanting’, and learning. *Curr. Opin. Pharmacol.* *9*, 65–73.
  68. Salimpoor, V.N., Benovoy, M., Larcher, K., Dagher, A., and Zatorre, R.J. (2011). Anatomically distinct dopamine release during anticipation and experience of peak emotion to music. *Nat. Neurosci.* *14*, 257–262.
  69. Ferreri, L., Mas-Herrero, E., Zatorre, R.J., Ripollés, P., Gomez-Andres, A., Alicart, H., Olivé, G., Marco-Pallarés, J., Antonijoan, R.M., Valle, M., et al. (2019). Dopamine modulates the reward experiences elicited by music. *Proc. Natl. Acad. Sci. USA* *116*, 3793–3798.
  70. Lacey, S., Hagtvedt, H., Patrick, V.M., Anderson, A., Stilla, R., Deshpande, G., Hu, X., Sato, J.R., Reddy, S., and Sathian, K. (2011). Art for reward’s sake: visual art recruits the ventral striatum. *Neuroimage* *55*, 420–433.
  71. Yu, X., Li, W., Ma, Y., Tossell, K., Harris, J.J., Harding, E.C., Ba, W., Miracca, G., Wang, D., Li, L., et al. (2019). GABA and glutamate neurons in the VTA regulate sleep and wakefulness. *Nat. Neurosci.* *22*, 106–119.
  72. Yoshihara, S., Omichi, K., Yanazawa, M., Kitamura, K., and Yoshihara, Y. (2005). *Arx* homeobox gene is essential for development of mouse olfactory system. *Development* *132*, 751–762.
  73. Abraham, A., Pedregosa, F., Eickenberg, M., Gervais, P., Mueller, A., Kossaifi, J., Gramfort, A., Thirion, B., and Varoquaux, G. (2014). Machine learning for neuroimaging with scikit-learn. *Front. Neuroinformatics* *8*, <https://doi.org/10.3389/fninf.2014.00014>.
  74. Esteban, O., Markiewicz, C.J., Blair, R.W., Moodie, C.A., Isik, A.I., Erramuzpe, A., Kent, J.D., Goncalves, M., DuPre, E., Snyder, M., et al. (2019). fMRIPrep: a robust preprocessing pipeline for functional MRI. *Nat. Methods* *16*, 111–116.
  75. Gorgolewski, K., Burns, C.D., Madison, C., Clark, D., Halchenko, Y.O., Waskom, M.L., and Ghosh, S.S. (2011). Nipype: a flexible, lightweight and extensible neuroimaging data processing framework in python. *Front. Neuroinform.* *5*, 13.
  76. Avants, B.B., Epstein, C.L., Grossman, M., and Gee, J.C. (2008). Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. *Med. Image Anal.* *12*, 26–41.
  77. Tustison, N.J., Avants, B.B., Cook, P.A., Zheng, Y., Egan, A., Yushkevich, P.A., and Gee, J.C. (2010). N4ITK: improved N3 bias correction. *IEEE Trans. Med. Imaging* *29*, 1310–1320.
  78. Dielenberg, R.A., Hunt, G.E., and McGregor, I.S. (2001). “When a rat smells a cat”: the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. *Neuroscience* *104*, 1085–1097.
  79. Kovács, K.J. (2008). Measurement of immediate-early gene activation-c-fos and beyond. *J. Neuroendocrinol.* *20*, 665–672.
  80. Cruz, F.C., Koya, E., Guez-Barber, D.H., Bossert, J.M., Lupica, C.R., Shaham, Y., and Hope, B.T. (2013). New technologies for examining the role of neuronal ensembles in drug addiction and fear. *Nat. Rev. Neurosci.* *14*, 743–754.
  81. Maviel, T., Durkin, T.P., Menzaghi, F., and Bontempi, B. (2004). Sites of neocortical reorganization critical for remote spatial memory. *Science* *305*, 96–99.
  82. Frankland, P.W., Bontempi, B., Talton, L.E., Kaczmarek, L., and Silva, A.J. (2004). The involvement of the anterior cingulate cortex in remote contextual fear memory. *Science* *304*, 881–883.
  83. Okuyama, T., Kitamura, T., Roy, D.S., Itoharu, S., and Tonegawa, S. (2016). Ventral CA1 neurons store social memory. *Science* *353*, 1536–1541.
  84. Gradinaru, V., Zhang, F., Ramakrishnan, C., Mattis, J., Prakash, R., Diester, I., Goshen, I., Thompson, K.R., and Deisseroth, K. (2010). Molecular and cellular approaches for diversifying and extending optogenetics. *Cell* *141*, 154–165.

85. Johnson, K.A., Voyvodic, L., Loewinger, G.C., Mateo, Y., and Lovinger, D.M. (2020). Operant self-stimulation of thalamic terminals in the dorso-medial striatum is constrained by metabotropic glutamate receptor 2. *Neuropsychopharmacology* *45*, 1454–1462.
86. Cleland, T.A., Morse, A., Yue, E.L., and Linster, C. (2002). Behavioral models of odor similarity. *Behav. Neurosci.* *116*, 222–231.
87. Kermen, F., Chakirian, A., Sezille, C., Joussain, P., Le Goff, G., Ziesel, A., Chastrette, M., Mandaïron, N., Didier, A., Rouby, C., and Bensafi, M. (2011). Molecular complexity determines the number of olfactory notes and the pleasantness of smells. *Sci. Rep.* *1*, <https://doi.org/10.1038/srep00206>.
88. Gadziola, M.A., and Wesson, D.W. (2016). The Neural Representation of Goal-Directed Actions and Outcomes in the Ventral Striatum's Olfactory Tubercle. *J. Neurosci.* *36*, 548–560.
89. Mandaïron, N., Kuczewski, N., Kermen, F., Forest, J., Midroit, M., Richard, M., Thévenet, M., Sacquet, J., Linster, C., and Didier, A. (2018). Opposite regulation of inhibition by adult-born granule cells during implicit versus explicit olfactory learning. *eLife* *7*, <https://doi.org/10.7554/eLife.34976>.
90. Hietala, J., Seppälä, T., Lappalainen, J., and Syvälahti, E. (1992). Quantification of SCH 39166, a novel selective D1 dopamine receptor antagonist, in rat brain and blood. *Psychopharmacology (Berl.)* *106*, 455–458.
91. Hegoburu, C., Sevelinges, Y., Thévenet, M., Gervais, R., Parrot, S., and Mouly, A.-M. (2009). Differential dynamics of amino acid release in the amygdala and olfactory cortex during odor fear acquisition as revealed with simultaneous high temporal resolution microdialysis. *Learn. Mem.* *16*, 687–697.
92. Richard, S., Aguilera, N., Thévenet, M., Dkhissi-Benyahya, O., and Flamant, F. (2017). Neuronal expression of a thyroid hormone receptor  $\alpha$  mutation alters mouse behaviour. *Behav. Brain Res.* *321*, 18–27.
93. Sezille, C., Messaoudi, B., Bertrand, A., Joussain, P., Thévenet, M., and Bensafi, M. (2013). A portable experimental apparatus for human olfactory fMRI experiments. *J. Neurosci. Methods* *218*, 29–38.
94. Fonov, V., Evans, A., McKinstry, R., Almlí, C., and Collins, D. (2009). Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *Neuroimage* *47*, S102.
95. Zhang, Y., Brady, M., and Smith, S. (2001). Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans. Med. Imaging* *20*, 45–57.
96. Cox, R.W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput. Biomed. Res.* *29*, 162–173.
97. Jenkinson, M. (2003). Fast, automated, N-dimensional phase-unwrapping algorithm. *Magn. Reson. Med.* *49*, 193–197.
98. Greve, D.N., and Fischl, B. (2009). Accurate and robust brain image alignment using boundary-based registration. *Neuroimage* *48*, 63–72.
99. Behzadi, Y., Restom, K., Liu, J., and Liu, T.T. (2007). A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *Neuroimage* *37*, 90–101.
100. Power, J.D., Mitra, A., Laumann, T.O., Snyder, A.Z., Schlaggar, B.L., and Petersen, S.E. (2014). Methods to detect, characterize, and remove motion artifact in resting state fMRI. *Neuroimage* *84*, 320–341.
101. Zelano, C., Bensafi, M., Porter, J., Mainland, J., Johnson, B., Bremner, E., Telles, C., Khan, R., and Sobel, N. (2005). Attentional modulation in human primary olfactory cortex. *Nat. Neurosci.* *8*, 114–120.
102. Moreno, M.M., Linster, C., Escanilla, O., Sacquet, J., Didier, A., and Mandaïron, N. (2009). Olfactory perceptual learning requires adult neurogenesis. *Proc. Natl. Acad. Sci. USA* *106*, 17980–17985.
103. Mandaïron, N., Sultan, S., Nouvian, M., Sacquet, J., and Didier, A. (2011). Involvement of newborn neurons in olfactory associative learning? The operant or non-operant component of the task makes all the difference. *J. Neurosci.* *31*, 12455–12460.
104. Sultan, S., Rey, N., Sacquet, J., Mandaïron, N., and Didier, A. (2011). Newborn neurons in the olfactory bulb selected for long-term survival through olfactory learning are prematurely suppressed when the olfactory memory is erased. *J. Neurosci.* *31*, 14893–14898.
105. Moreno, M.M., Bath, K., Kuczewski, N., Sacquet, J., Didier, A., and Mandaïron, N. (2012). Action of the noradrenergic system on adult-born cells is required for olfactory learning in mice. *J. Neurosci.* *32*, 3748–3758.

**STAR★METHODS**

**KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
anti-c-Fos	Santa Cruz Biotechnology	Cat# sc-189; RRID:AB_2231020
chicken anti-EYFP	Anaspec TEBU	Cat# 55423; RRID:AB_1657428
chicken anti-TH	Abcam	Cat# ab76442; RRID:AB_1524535
rabbit anti-c-Fos	Proteintech	Cat# 26192; N/A
anti-DARPP-32	Abcam	Cat# ab40801; RRID:AB_731843
mouse anti-c-Fos	Proteintech	Cat# 66590-1-Ig; RRID:AB_2881950
guinea pig anti-Tbx21	<sup>72</sup>	N/A
rabbit anti-EYFP	Millipore	Cat# AB3080P; RRID:AB_2630379
<b>Bacterial and Virus Strains</b>		
Lenti-hSyn-eNpHR3.0-EYFP lentivirus	Addgene	RRID:Addgene_26775
Control Lenti-hSyn-EYFP lentivirus	<sup>6</sup>	N/A
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
R(+)-SCH-23390 hydrochloride	Addgene	RRID:AB_11159773
<b>Deposited Data</b>		
Analyzed data	This paper	<a href="https://github.com/Neuropop/Reward2021">https://github.com/Neuropop/Reward2021</a>
<b>Experimental Models: Organisms/Strains</b>		
C57Bl6J	Charles River Laboratories	Cat# JAX:000664, RRID:IMSR_JAX:000664
<b>Software and Algorithms</b>		
Statistica	Statistica	<a href="https://www.statistica.com">https://www.statistica.com</a>
Volcan	<sup>22</sup>	N/A
NiLearn	NiLearn <sup>73</sup>	<a href="http://nilearn.github.io">http://nilearn.github.io</a>
SciPy	Python Library	<a href="http://www.scipy.org/">http://www.scipy.org/</a> ; RRID:SCR_008058
MRICron	MRICron	<a href="http://www.mricron.com">http://www.mricron.com</a>
fMRIPrep	<sup>74</sup>	<a href="https://fmripred.org">https://fmripred.org</a>
Nipype	<sup>75</sup>	<a href="https://nipype.readthedocs.io">https://nipype.readthedocs.io</a>
SPM	MATLAB Toolbox	<a href="https://www.fil.ion.ucl.ac.uk/spm/">https://www.fil.ion.ucl.ac.uk/spm/</a>
FSL	FMRIB Software Library	<a href="https://fsl.fmrib.ox.ac.uk">https://fsl.fmrib.ox.ac.uk</a>
ANTS	<sup>76</sup>	<a href="http://stnava.github.io/ANTS/">http://stnava.github.io/ANTS/</a> ; RRID:SCR_004757
MATLAB	Mathworks	<a href="https://www.mathworks.com/products/matlab.html">https://www.mathworks.com/products/matlab.html</a> RRID:SCR_001622

**RESOURCES AVAILABILITY**

**Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Nathalie Mandairon ([nathalie.mandairon@cnrs.fr](mailto:nathalie.mandairon@cnrs.fr)).

**Materials availability**

This study did not generate new unique reagents.

**Data and code availability**

The datasets and codes generated during this study have been deposited: <https://github.com/Neuropop/Reward2021>.



## EXPERIMENTAL MODEL AND SUBJECT DETAILS

For all *in vivo* animal studies, we used 145 adult male C57Bl6/J mice (Charles River Laboratories, L'Arbresle, France) aged of 2 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 h light/dark cycle (22°C) with food and water *ad libitum*. Mouse assignment to the various experimental groups was randomized. Mice were housed by group of 5, then individually after surgery. All experiments were conducted after at least 1 week of habituation and handling.

For human studies, the data were collected and treated according to the ethical guidelines of the Declaration of Helsinki and approved by French IdF-IV ethics committee (CPP study) and by the Lyon Sud-Est ethics committee (fRMI study). All participants were recruited from the Lyon area in France. Their olfactory performances were verified using the European Test of Olfactory Capabilities.<sup>77</sup> All participants received financial compensation for the experiment and provided written informed consent prior to participation. Participant assignment to the various experimental groups was randomized. For CPP study, 72 participants were tested in total but 2 were excluded due to olfactory pollution and 1 left the living lab during the experiment. Thus, only 69 participants (34 women, 35 men) between the ages of 18 and 30 years (mean  $\pm$  SD, 21.5  $\pm$  2.6 years) provided data from April to May 2018. For fRMI study, 30 participants were tested (15 women, 15 men) between the ages of 19 and 34 years (means  $\pm$  SD, 22.4  $\pm$  3.5 years).

## METHOD DETAILS

### Cell analysis

Mice were sacrificed 1 h after odor stimulation or behavioral test using pentobarbital (0.2 mL/30 g) or dolethal (0.1 mL/30 g) and intracardiac perfusion of 50 mL of fixative (PFA 4%, pH = 7.4). This 1 h delay has previously been shown to enable the expression of the immediate early gene *c-Fos* in response to odorant stimulation,<sup>78,79</sup> allowing the mapping of neuronal activation.<sup>6,78–83</sup> The brains were removed, post-fixed overnight, cryoprotected in sucrose (20%), frozen rapidly, and then stored at  $-20^{\circ}\text{C}$  before sectioning with a cryostat (14  $\mu\text{m}$  thick; 210  $\mu\text{m}$  intervals). After immunohistochemistries using fluorescent secondary antibodies, sections were then coverslipped in Vectashield 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.

### Optogenetic intracranial self-stimulation

Electric intracranial self-stimulation has been extensively used to determine brain areas belonging to the reward system and unravel the neural bases of motivation.<sup>10,11</sup> This technic consists in an operant conditioning in which the stimulation of a specific brain area serves as reinforcer.

### Lentivirus injection and optical fiber implantation in the olfactory bulb

Prior to surgery, 24 mice 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) were injected bilaterally in pOB (with respect to the bregma: AP, +4.3 mm; ML,  $\pm$  0.75; DV,  $-2$  mm;  $n = 7$ ), and in the aOB (with respect to the bregma: AP, +5 mm; ML,  $\pm$  0.75; DV,  $-2$  mm;  $n = 9$ ). 300 nL of control Lenti-hSyn-EYFP lentivirus ( $1.1 \times 10^6$  IU/mL, expressing only EYFP;  $n = 9$ ) were injected bilaterally in pOB 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. The pLenti-hSyn-eNpHR 3.0-EYFP was a gift from Karl Deisseroth<sup>84</sup> and obtained through Addgene (plasmid #26775). Generation of the control Lenti-hSyn-EYFP lentivirus (empty virus containing only the EYFP insert) has been described previously.<sup>6</sup>

### Optical self-stimulation in freely moving animals

8 weeks after surgery, mice underwent the conditioning phases in which they were allowed to move freely on the one-hole-board apparatus for 2-min trial during 5 days. 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 lasted as long as the nose poke, mimicking odor investigation. This phase was followed by an extinction phase in which the nose presence in the zone did not trigger light stimulation anymore (5 trials on 3 days) and one last session in which light stimulation was available again similarly as in the previous conditioning phase.<sup>85</sup> Total duration of time spent by the nose in the zone was recorded.

### Cellular analysis

Mice were sacrificed one h 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') (Bonferroni corrected Two-Tailed Wilcoxon for difference between session of the day of sacrifice and Light1', NpHR post group:  $p = 0.094$ ; EYFP post group:  $p = 1$ ; NpHR ant group  $p = 1$ ).

OB sections were double labeled after immunohistochemistries against 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) and Alexa 488 goat anti-chicken (1:250; Molecular Probes) antibodies. The total density of EYFP<sup>+</sup> and the percentage of double-labeled cells (EYFP<sup>+</sup>/*c-Fos*<sup>+</sup>) were assessed in the ventral OB at the level of the

injection site (2 sections/animal) and at 0.96 mm from the injection site along the antero-posterior axis (2 sections/animal) (aBO; coordinates from the bregma [+5.33 to +4.73]; pOB: coordinates from the bregma [+4.61 to +3.89]).

To investigate the activity of the VTA (coordinate from the bregma [-3.3]) after self-stimulation, immunohistochemistries against TH and c-Fos were performed. 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) and Alexa 488 goat anti-rabbit (1:250; Vector Laboratories, Burlingame, CA, USA) antibodies and the percentage of double-labeled cells (TH<sup>+</sup>/c-Fos<sup>+</sup>) was counted (one section/animal; 10-32 cells/animal).

To investigate the activity of medium spiny neurons of the OT (coordinates from the bregma [+2.09 to -0.11]) after self-stimulation behavior, immunohistochemistries against DARPP-32 and c-Fos were performed. DARPP-32 has been used as a marker of medium spiny neurons of the striatum and the OT.<sup>17</sup> 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) and Alexa 546 goat anti-mouse (1:250; Molecular Probes) antibodies. The percentage of double-labeled cells (DARPP-32<sup>+</sup>/c-Fos<sup>+</sup>) was counted on five OT sections per animal (200-285 cells/animal).

### Statistics

Because of the lack of sphericity on behavioral data, we used Friedman test, non-parametric alternative to the repeated-measures ANOVA, for trial effect, and Wilcoxon, non-parametric test equivalent to the t-test, for specific trial comparisons. The location and the efficiency of viral infection were controlled using Bonferroni corrected One-Tailed Paired t-tests. Mann-Whitney tests were used for VTA and OT activities between group comparisons.

## DISCO

### Viral injection

Prior to surgery, 16 mice were anaesthetized and secured in a stereotaxic instrument as described previously. 150 nL of Lenti-hSyn-eNpHR3.0-EYFP lentivirus ( $9,22 \times 10^6$  IU/mL, expressing halorhodopsin and the yellow fluorescent protein) was injected bilaterally in pOB (with respect to the bregma: AP, +4.3 mm; ML,  $\pm 0.75$ ; DV, -2,8 mm; n = 8) or in aOB (with respect to the bregma: AP, +5 mm; ML,  $\pm 0.75$ ; DV, -2 mm; n = 8) at the rate of 150 nL/min.

### DISCO+ processing and light-sheet microscopy

Six brains were dehydrated in baths of methanol in ddH<sub>2</sub>O in a concentration gradient (20%, 40% 60%, 80% and 100% twice), and then put in methanol / DiChloroMethane (DCM) 1:2 overnight. Samples were then washed all day in methanol, and then bleached with 5% H<sub>2</sub>O<sub>2</sub> (1 volume of 30% H<sub>2</sub>O<sub>2</sub> for 5 volumes of methanol, ice cold) at 4°C overnight. Then samples were re-equilibrated at room temperature slowly and re-hydrated in baths of methanol in H<sub>2</sub>O at different concentration during one h each (80%, 60%, 40% and 20%) and finally in PBS/0.2% Triton X-100 for 1 h twice.

Pre-treated samples were then incubated in PBS/0.2% Triton X-100/20% DMSO/0.3M glycine at 37°C for 36 h, then blocked in PBS/0.2% Triton X-100/10% DMSO/2% Porc Skin Gelatin at 37°C for 2 days. Samples were then incubated in primary antibodies: chicken anti-GFP (1:2000, Aves GFP-1020) in PBS-Tween 0.2% with heparin 10 mg/mL (PTwH)/5% DMSO/3% donkey serum at 37°C for 4 to 7 days. Samples were then washed in PTwH for 24 h (five changes of the PTwH solution over that time), then incubated in secondary antibody donkey anti-chicken (Jackson ImmunoResearch at 1:500 in PTwH/3% donkey serum) at 37°C for 4 to 7 days. Samples were finally washed in PTwH for 1 day before clearing and imaging. Immunolabelled brains were dehydrated in baths of methanol in ddH<sub>2</sub>O at different concentrations (20%, 40% 60%, 80% and 100% twice). Samples were incubated overnight in 1 vol of methanol/2 vol of dichloromethane (DCM, Sigma-Aldrich 270997-12 x 100 ML) (Eppendorf tubes were used throughout the process).

The methanol was then washed for 20 min twice in 100% DCM. Finally, samples were incubated (without shaking) in dibenzyl ether (DBE, Sigma-Aldrich 108014-1 KG) until clear and then stored in DBE at room temperature.

Cleared samples were imaged in sagittal orientation (right lateral side up) on a light-sheet microscope (Ultramicroscope II, LaVision Biotec, Germany) equipped with a sCMOS camera (Andor Neo, UK) and 4X/0.3 objective. A numerical aperture of 0.6 was used for the light sheet, with a fixed 3-sources illumination. The microscope is equipped with LED lasers (488 nm and 640 nm).

Emission filters used are 525/50 and 680/30. The samples were scanned with a step-size of 3  $\mu$ m.

3D projections were performed using Imaris (Bitplane, <http://www.bitplane.com/imaris/imaris>). Images in Figures 2A and 2B were obtained from two brains.

### Quantification of OB-OT neural projections

On the 10 remaining brains, coronal sections were performed. OB sections were double labeled by immunohistochemistries against Tbx21 (marker of mitral cells) and EYFP. Briefly, guinea pig anti-Tbx21 (1:5000, gift from Y. Yoshihara<sup>72</sup>) and rabbit anti-EYFP (1:1000; Merk, ref: AB3080) primary antibodies were combined respectively with Alexa 546 goat anti-guinea pig (1:250; Molecular Probes) and Alexa 488 goat anti-rabbit (1:250; Vector Laboratories, Burlingame, CA, USA) antibodies. The percentage of double-labeled cells (Tbx21<sup>+</sup>/EYFP<sup>+</sup>) was assessed in the ventral OB over 3 sections at the injection site and at 0.96 mm from the injection site along the antero-posterior axis.

OT and PirCX sections (aPirCX: coordinates from the bregma [+2.33 to +1.09]; pPirCX: coordinates from the bregma [-0.23 to -2.79]) were labeled by immunohistochemistry of EYFP proteins as described previously. ImageJ was used to define a threshold for each image acquired by AxioVision and we calculated the percentage of labeled area (% labeled area = labeled area/total area\*100).

### Statistics

Bonferroni corrected Mann-Whitney tests were performed for pOB versus aOB labeled area comparisons. The percentage of Tbx21<sup>+</sup>/c-Fos<sup>+</sup> cells was compared between the injection site in the OB and the antero-posterior opposite side using Bonferroni corrected Wilcoxon tests and between groups comparison was done using Mann-Whitney. One-Tailed Unpaired t-test was performed for parametric data.

### c-Fos mapping of the olfactory secondary areas

#### Odorants

Six monomolecular odorants were used: three highly attractive (LIM, CAM and CITRO) versus three poorly attractive (PYR, GUA and CRE) diluted at 1Pa in mineral oil. Mineral oil was used as control<sup>6,86,87</sup> (Table S1).

#### Behavioral assessment of odor attraction

Odor attraction was measured in 30 mice using odor investigation time.<sup>6,19,20</sup> As previously described,<sup>6</sup> we used a computer-assisted one-hole-board apparatus fitted with sensors to automatically monitor the duration of nose poking into the central hole.<sup>20,21</sup> Total duration of nose poking into the hole was used as a measure of odor attraction. A polypropylene swab impregnated with 60  $\mu$ L of odorant was placed at the bottom of the hole, under a grid covered with clean bedding. The bedding was replaced after each trial. Every animal was allowed to explore each of the 6 odorants and a no odor condition (60  $\mu$ L of mineral oil) for 2 min. Each animal tested one odorant per day. Odorants were randomly presented and animals performed no more than 2 consecutive days of testing.

#### Odor stimulation and sacrifice

Mice were submitted to odor stimulation one h before sacrifice. 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 $\mu$ L, 1Pa) was then placed in the tea ball for a further h.

#### c-Fos immunohistochemistry

A rabbit anti-c-Fos primary antibody was used (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and combined to biotinylated goat anti-rabbit (1:200; Vector Laboratories, Burlingame, CA, USA). 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).

#### c-Fos-positive cell mapping

We used a method consisting in a high-accuracy matching of experimental brain sections with a reference brain atlas.<sup>22</sup> It allows precise, automatic assignment of the labeled cells to a brain structure and permits between-group comparisons. Briefly, for each animal, c-Fos immunohistochemistry was performed on brain adjacent sections ( $n = 6-7$  mice for each hedonic condition; 210  $\mu$ m interval inter sections). For each section analyzed, the external contour of the right hemisphere was outlined and c-Fos<sup>+</sup> cells were automatically counted using mapping software (Mercator, Explora Nova, La Rochelle, France) coupled to a Zeiss microscope. Then, the coordinates of each labeled cell (c-Fos<sup>+</sup>) and the contour point were exported. All cell counts were conducted blind with regard to the experimental group.

A digitized Paxinos atlas was used as a common anatomical space (« The mouse brain », Paxinos and Franklin's, 4th edition). The extracted contour and labeling of experimental sections were precisely matched to the corresponding atlas sections. More precisely, the extracted sections were automatically stacked, realigned, and reoriented between them and along the antero-posterior axis. Then, a nonlinear elastic registration based on a computed vector field is applied to the labeling and contour allowing a readjustment of the section size and a precise matching in the atlas. At the end of this computation, each labeled c-Fos<sup>+</sup> cell is correctly located in the common Paxinos Atlas and is thus spatially assigned to one region or sub-region. Since the experimental sections have been matched to the atlas, we selected regions of interest and extracted the density of labeled c-Fos<sup>+</sup> cells in this particular region.

### Analyses

We analyzed the neural representation of attractive and unattractive odorants in olfactory brain structures directly targeted by the OB: the Accessory Olfactory Nucleus (AON; coordinates from the bregma [+3.53 to +1.97]), the anterior Piriform Cortex (aPirCX; coordinates from the bregma [+2.33 to +1.09]), the posterior Piriform Cortex (pPirCX; coordinates from the bregma [-0.23 to -2.79]), the Olfactory Tubercle (OT; coordinates from the bregma [+2.09 to -0.11]), the postero-lateral Cortical Amygdala (plCoA; coordinates from the bregma [-1.23 to -3.15]) and the Entorhinal Cortex (EntCX; coordinates from the bregma [-1.91 to -3.87]). The OT was divided in medial (mOT) and lateral (lOT) domains. Then, c-Fos<sup>+</sup> cells were automatically counted using mapping software (Mercator, Explora Nova, La Rochelle, France) as previously described.

By considering the odorant treatment given before the sacrifice, 2 groups of mice were formed and compared: attractive group ( $n = 6$ ) and unattractive group ( $n = 7$ ). We extracted the density of labeled c-Fos<sup>+</sup> cells for each brain structure and compared it between groups.

#### Double labeling analysis

To investigate the activity of VTA and OT after free exploration of the odorants, we performed and analyzed immunohistochemistries against TH/c-Fos and DARPP-32/c-Fos as previously described.

### Statistics

For behavioral analyses, Friedman test, a non-parametric alternative to the repeated-measures ANOVA, was used to assess odorant attraction effect. The effect was confirming by permutation test (10x100 000 permutations). For c-Fos mapping between group comparisons, Bonferroni corrected One-Tailed Unpaired t-tests were performed. One-Tailed Unpaired t-tests were used for VTA and OT activities between group comparisons.

## Olfactory tubercle in vivo electrophysiology

### Animals

C57BL/6 male mice ( $n = 15$ , 2–4 months of age) originating from Envigo were bred and maintained within the Case Western Reserve University School of Medicine animal facility. Mice were housed on a 12 h light/dark cycle with food and water available *ad libitum*. Up to five mice were cohoused in a cage before experimentation, but all postsurgical animals were housed individually. All experimental procedures were conducted in accordance with the guidelines of the National Institutes of Health and were approved by the Case Western Reserve University Institutional Animal Care and Use Committee.

### Surgical procedures

Mice were anesthetized with isoflurane (2–4% in oxygen; Abbott Laboratories) and mounted in a Kopf stereotaxic frame with a water-filled heating pad (38°C) beneath to maintain body temperature. Anesthesia depth was verified by the absence of the toe-pinch reflex. An injection of a local anesthetic (10% marcaine, 0.05 ml, s.c.) was administered into the wound margin site before exposing the dorsal skull. A craniotomy was made to access the right medial OT (+1.7 mm from bregma, +0.5 mm lateral). An eight-channel tungsten electrode array, as previously described<sup>16</sup> was implanted within the right medial OT (4.7 mm ventral) and cemented in place. A second craniotomy was made over the contralateral cortex for placement of a stainless steel ground wire. Animals were allowed to recover for 5 days with food and water *ad libitum*. During the first 3 post-operative days, animals received a daily injection of carprofen (5 mg/kg, s.c.; Pfizer Animal Health).

### In vivo electrophysiology

The output of the electrode array was amplified, digitized at 24.4 kHz and filtered (bandpass filter, 300–3000 Hz) using an Intan 16 channel headstage and an RHD2000 interface board. One electrode wire was selected to serve as a local reference. Given the small dorsal–ventral extent of the OT (~300  $\mu\text{m}$ ), the electrode arrays were fixed in place, and no attempt was made to record from unique populations of neurons on different sessions. Indeed, it is possible that the same neuron was recorded across multiple days. On average,  $5.56 \pm 0.61$  single neurons were recorded per mouse per session, with an average of  $1.96 \pm 0.08$  neurons recorded per viable electrode wire per mouse per session. After all recording sessions were completed, mice were given (intraperitoneally) an overdose of Fatal-plus and were transcardially perfused with 0.9% saline and 10% formalin, and brains stored in 30% sucrose formalin at 4°C. OT recording sites (targeting the medial portion of the OT) were verified by histological examinations of slide-mounted, 40  $\mu\text{m}$  coronal sections stained with a 1% cresyl violet solution. 5 animals did not contribute data since the recording sites were not located in the mOT.

### Analysis of odor-evoked activity

Single units were identified offline in Spike2 (Cambridge Electronic Design), using a combination of template matching and cluster cutting based on principal component analysis.<sup>16</sup> Single units were further defined as those having < 2% of the spikes occurring within a refractory period of 2 ms. Among these identified single units, spike times associated with each odor condition were subsequently extracted for analysis.

For each neuron, we quantified the number of spikes during the 120 s of the trial. The numbers of spikes obtained for the three attractive and for the three unattractive odorants were averaged across neurons to reveal the activity reflecting attraction to odorants independently of their identity, as we did for the c-Fos data. To assess modulations in firing rate according to the attraction condition, spiking was aligned to the first odor investigation ( $t_0$ , when the mouse's nose enters the odorized zone). Mean firing rates across trials were measured in 100 ms bins. The mOT neurons fired at a low rate of 3.35 spikes/s (range, 0.05–33 spikes/s across the sampled population), in line with previous studies.<sup>16,88</sup> This firing rate was used as a reference background activity whereas mean odor firing rate for each neuron was calculated over the 3 s time-interval preceding the first investigation, referring to the first approaching phase (–3 to 0 s relative to the onset ( $t_0$ ) of odor investigation), of attractive and unattractive odorants. Trials in which the animal was already approaching the odorant source at the start of the trial (incomplete –3 s to 0 s period) were removed from the analysis.

### Statistics

Between group comparison was performed using Mann-Whitney and difference between Odorized Hole and background was compared using Bonferroni corrected Wilcoxon.

## pOB-mOT optogenetic inhibition

### Lentivirus injection and optical fiber implantation

Prior to surgery, 17 mice were anesthetized 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 pLenti-hSyn-eNpHR3.0-EYFP lentivirus ( $9.22 \times 10^6$  IU/mL, expressing halorhodopsin and the yellow fluorescent protein;  $n = 9$ ) or 300 nL of control pLenti-hSyn-EYFP lentivirus ( $1.1 \times 10^6$  IU/mL, expressing only EYFP;  $n = 8$ ) were injected bilaterally in mitral cells layer of the posterior olfactory bulb (with respect to the bregma: AP, +4.3 mm; ML,  $\pm 0.75$ ; DV, –3 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 mOT with respect to the bregma: AP, +1.7 mm; ML,  $\pm 0.5$ ; DV, –4.7 mm).

### Optical inhibition of pOB-mOT pathway

4 weeks after surgery, odorant attraction (LIM, CAM, CITRO, CRE, GUA and PYR) was measured as previously described. The bilateral continuous light stimulation (crystal laser, 561 nm, 10–15 mW) was automatically triggered by mice nose poking within 1 cm (light-triggering zone) of the odorized hole (VideoTrack, Viewpoint) and stopped automatically when the mouse's nose exited the zone.



### **Control of virus injection location**

A few days after optogenetic experiment, mice were sacrificed one h after a 15 min stimulation session (5 s of light stimulation every 15 s for 2 min at 10–15 mW, mimicking the light stimulation received during the olfactory test) as previously described. Double labeling for transfected mitral cells was performed using Tbx21<sup>6,89</sup> and EYFP immunohistochemistry. Briefly, guinea-pig anti-Tbx21 (1:15, 000; gift from Y. Yoshihara<sup>90</sup>) and rabbit anti-EYFP (1:1, 000; Merk, ref: AB3080) primary antibodies were combined respectively with Alexa 546 goat anti-guinea-pig (1:250; Molecular probes) and Alexa 488 goat anti-rabbit (1:250; Vector Laboratories, Burlingame, CA, USA) antibodies.

The percentage of double-labeled cells (Tbx21<sup>+</sup>/EYFP<sup>+</sup>) was assessed in the ventral OB over 3 sections at the injection site using AxioVision (Zeiss) software coupled to a fluorescent pseudo-confocal Zeiss microscope. Counting was performed blind with regards to the identity of the animal.

### **Control of pOB-mOT light-inhibition on the OT**

To investigate the activity of the OT after optogenetic inhibition of pOB-mOT pathway, we performed and analyzed immunohistochemistry against DARPP-32/c-Fos in mOT and IOT as previously described.

### **Statistics**

Between groups comparisons were performed using Mann-Whitney and Spearman test was used for correlation between OT activity and odorant attraction.

### **Odor conditioned place preference**

#### **Odorants**

We used here CITRO and LIM as attractive odorants; PYR and CRE as unattractive odorants diluted at 10Pa in mineral oil. Mineral oil served as control.

#### **Experimental design**

To avoid neophobic response, mice were exposed twice a day (one h each time) during 10 days with either CITRO (n = 8), LIM (n = 10), PYR (n = 8), CRE (n = 9) or no odor (NO; mineral oil, n = 10) before CPP test. Then, mice were all trained in CPP using the same odor as the one used for the exposure.

#### **CPP protocol**

Mice were trained in a CPP apparatus consisting of two visually different conditioning chambers (60 cm wide x 30 cm high x 30 cm long): one with orange stripes and large mesh on the wall and the other one with blue circles and short mesh on the wall, both connected by a smaller central chamber. A tea ball was placed on the top of each chamber. On day 1 (habituation), mice were allowed to move freely between the three chambers for 15 min. On day 2 (pre-conditioning test), mice had again free access to the chambers for 15 min and the time spent in the 2 conditioning chambers was video recorded using Volcan<sup>91,92</sup> to assess if there is a preferred chamber. Then from Day 3 to Day 7, mice were trained. Training consisted in confining the mouse alternatively in one compartment in the presence of the odorant (10 $\mu$ l at 10 Pa in the tea ball) and in the other compartment with no odorant (15 min per session, 4 sessions per day) in a random order. Control mice were confined alternatively in one and the other compartment with no odor in any compartment. Attractive odorants were paired to the less pleasant compartment whereas unattractive odorants were paired to the preferred compartment. At the end of the conditioning period (on Day 8), all mice have been tested without odorant, similarly to the pre-conditioning test: animals were allowed to freely explore all the chambers for 15 min and the time spent in each compartment was video recorded.

#### **Cell assessment**

VTA and OT activities were assessed with respectively TH/c-Fos and DARPP-32/c-Fos immunohistochemistry performed and analyzed as previously described.

#### **Statistics**

For each experimental group, a CPP score was calculated (time in second spent in the odor paired compartment during post conditioning – pre conditioning) to determine the effect of odor conditioning on place preference.<sup>24</sup> Average scores were calculated within each experimental group. One-Tailed One-Sample t-tests were used to compare CPP score to 0 (meaning no time variation between the pre and the post conditioning). One-Tailed Unpaired t-tests were used for VTA and OT activities between group comparisons.

### **D1 receptor blockade during CPP**

#### **CPP protocol**

To determine the implication of the dopaminergic system during odor CPP, 10 mice have been injected with a specific D1 receptor antagonist (SCH23390; 0.03mg/kg intraperitoneally) 15 min before each mice confinement in the compartment containing the odorant (LIM). Saline solution has been injected 15 min before each mice confinement in the compartment containing no odor. The interval between each session was set at 2h30 to allow body elimination of the drug (half-life 40 min<sup>90</sup>). The protocol of CPP and its analysis were similar as previously. Another group of mice was injected with saline solution 15 min before each LIM and no odor confinement (n = 13). A last group received SCH23390 injection but without any odorant during the conditioning (n = 10).

#### **Test of olfactory detection**

Because the OB contains dopaminergic neurons, we verified that the use of dopaminergic antagonist at the dose of 0.03mg/kg does not alter olfactory detection. We thus used habituation/dishabituation paradigm (n = 8). Mice were tested on 3 trials of 2 min in which

mineral oil (no odor condition, NO) was placed in the tea ball (habituation phase); during each trial, investigation time of the tea ball was recorded (active sniffing 1 cm around the tea ball, using Volcan). For the test trial (dishabituation phase), the odorant used for CPP was placed at the same concentration into the tea ball.

#### **Locomotion test**

Because dopamine is known to have an important role on locomotor activity, we analyzed the mice locomotion during 2 min, 20 min after SCH 23390 injection (n = 9) or saline injection (n = 10) using Volcan software.<sup>91,92</sup>

#### **Statistics**

Two-Tailed Paired t-tests were used to compare CPP score to 0. The effect of SCH-23390 on LIM detection and locomotion was assessed respectively with repeated-measure ANOVA (NO habituation effect) with Two-Tailed Paired t-test (NO3 versus LIM) and Mann-Whitney.

### **Conditioned place preference in humans**

#### **Conditioned place preference**

Participants were told that they were going to take part in a study on the relationship between odors, emotions and learning. Experiments took place over 5 days. On the first day, participants were guided to a first room in which they were asked to perform an ETOC test to evaluate their olfactory capacities (no exclusion) and to rate their emotional state (anxious, disgusted, angry, calm, happy, arousing, scared, relaxed, stressed, and sad) using Pie-Pie software. Participants were then asked to wait in another room composed of two compartments. They were instructed to wait in this room, before a new rating test (fake survey). The aim of this new rating test was to distract participants from the CPP test that was assessed during their wait.

The CPP facility consisted in a room separated in two equal but visually different compartments equipped with webcams allowing the observation of the participant. Videos were analyzed using A2V Volcan software.<sup>91,92</sup> Two passive diffusers were placed in the middle of other objects on a shelf present in each compartment. On the first day (pre-conditioning test), no chair was placed in any compartment and no odor was diffused (only mineral oil placed in the diffuser). The participants were asked to wait in this room and they were allowed to visit the two compartments. Time spent in each compartment was recorded during 10 min and participant's trajectory was calculated. The next three days consisted in the conditioning phase, with 2 sessions of 10 min per day. During the conditioning phase, an odorant (50 Pa, dilution in mineral oil) was diffused in one compartment whereas no odor (mineral oil) was introduced in the diffuser of the second compartment. Note that the presence or not of the odorant was not specified to the participants. To control that the odorant was mostly perceived in the room in which the diffuser is present, we performed a preliminary experiment. In this separate experiment, after sitting for 2 min in each compartment, volunteers (n = 12) rated odorant intensity (from 1 to 9). Results indicated a highest perceived intensity in the odorized compartment (odor intensity rating: n = 12; Odorized compartment  $6.000 \pm 0.663$  Non-odorized compartment  $3.083 \pm 0.583$ ; Two-Tailed paired t-test  $t = 4.696$   $p < 0.001$  Cohen's  $d = 1.356$ ). The highest perceived intensity in the odorized compartment was confirmed by participants in the CPP experiment. Indeed, at the end of the experiment they were asked to rate perceived intensity and reported a significant difference in favor of the odorized compartment (odor intensity rating: n = 38; Odorized compartment  $6.105 \pm 0.269$  Non-odorized compartment  $3.579 \pm 0.347$ ; Two-Tailed paired t-test  $t = 6.173$   $p < 0.001$  Cohen's  $d = 1.001$ ). During the first session of the first conditioning day, participants were asked to enter in one compartment, to sit on a chair and wait. During the second session, they were asked to sit and wait in the other compartment (10 min in each compartment; odorized and non-odorized compartment were randomly presented). The order of the presented compartments (left or right) was changed every day and randomized between participants, but for a given participant, the same compartment was always paired to the odorant. The compartments were ventilated between each participant. The aim of the conditioning phase was to implicitly train participants to associate one compartment to one odorant without any instruction. The paradigm of the post-conditioning test was similar to the pre-conditioning one. No odorant and no chair were present in any compartment and participants were allowed to freely circulate in both compartments. Time spent in each compartment was recorded and participant's trajectory was analyzed.

For this experiment, three groups of 24 participants were constituted. One group performed the CPP with a compartment odorized with CAR, another group with THIO and the last group was the control group without any odorant (mineral oil) associated to any of the two compartments. Odorants were selected based on their pleasantness rating assessed in a pilot study.

At the end of the last day, participants had to express their own hypothesis regarding the aim of the study. 64.2% of the participants agreed with the aim that we announced related to the effect of odors on emotions and learning. 28.4% of participants hypothesized behavioral and/or motor analyses. 7.5% hypothesized others intentions. They were also asked whether they detected an odorant during the conditioning sessions and if yes, they were asked to rate its intensity and pleasantness perception.

### **QUANTIFICATION AND STATISTICAL ANALYSIS**

2 participants were excluded of data analyses because they were considered as outliers (superior or inferior to the means+3SD). As in mice CPP, a CPP score was calculated as the time in second spent in the odor paired compartment during post-conditioning – pre-conditioning, in order to determine the effect of odor conditioning on place preference. One-Tailed Paired t-tests were used to compare CPP score to 0. Intensity and pleasantness rating were compared between group using Mann-Whitney tests.

## fMRI study

### fMRI acquisition

A 3 Tesla MR-scanner was used for the experiment (AWP66012 SIEMENS Prisma). The fMRI data were collected during four sessions (285 volumes/session, interleaved, AC/AP acquisition) with a 45 axial-slice 2D EPI sequence (matrix: 78x78; TR: 2,500 ms; TE: 30 ms; FA: 90; voxel size: 2.70x2.70x2.70 mm; FOV: 270). The first seven volumes of each functional session were discarded to allow for T1 equilibration. To reduce distortion in the sinus, orbitofrontal, and temporal areas during pre-processing, a field map was acquired before fMRI data collection. For coregistration, a high-resolution T1-weighted brain image was recorded (3D MPR sequence – TR: 3500ms; TE: 3.86 ms; voxel size: 0.88x0.88x0.88 mm).

### Odorants

7 odorants were selected based on previous psychophysics experiment in order to cover the entire dimension of attraction BUT, HMHA, MSH, CIS3, TER, LIM and ISO (Table S1).<sup>31</sup> Pure odorants (liquid) were provided by Sigma-Aldrich except for HMHA and MSH, which were synthesized for the purpose of this study, and dilutions were made using mineral oil (Sigma-Aldrich). Odor concentrations were adjusted to reach iso-intensity, using HMHA concentration as a reference. The airflow (air) was used as control condition. A computer-controlled olfactometer described in detail in Sezille et al.<sup>93</sup> was used to diffuse olfactory stimulation into both nostrils. The tubes were replaced every 2-3 consecutive participants.

## EXPERIMENTAL PROCEDURE

An event-related design was used with 8 conditions (7 odorants + 1 clean air). The odorants were presented to participants during 5 s with 20 s interstimulus interval in 16 trials. The 8x16 trials were divided in 4 fMRI scans (sessions). For each session, the order of odorants presentation was pseudo-randomized (each of the 8 conditions were presented 4 times with no twice the same condition consecutively) and for each participant the order of sessions was pseudo-randomized. Participants were asked to breathe naturally, and odorants were diffused synchronously with the subject's nasal respiration. We chose a stimulus duration of 5 s because all the odorants were released during exhalation and had to be maintained during at least the whole duration of the subsequent inhalation (2 s). During the experiment, we recorded respiratory signal, odor valve opening and time repetition (TR) signal from the fMRI scanner, enabling event-related statistical analysis. An airflow sensor connected to a nasal cannula (Cardinal Health, OH; 2.8mm inner diameter tube) was positioned in both nostrils to measure participant's respiratory signal. A microbridge mass airflow (AWM2100V, Honeywell, MN) allowed acquisition of both inhalation and exhalation phases. Sniffing was digitally recorded at 200 Hz and stored in a computer.

During each odorant stimulation, subjects rated odor attraction using a button-box from 1 to 5 (1 not at all attractive to 5 extremely attractive or 1 extremely attractive to 5 not at all attractive in random way). For each subject, the evaluation of all repetitions was average for each odorant.

### Preprocessing

Results included in this manuscript come from preprocessing performed using FMRIPREP latest version 1.4.1<sup>74</sup>; RRID:SCR\_016216), a Nipype<sup>75</sup>; RRID:SCR\_002502) based tool. Each T1w (T1-weighted) volume was corrected for INU (intensity non-uniformity) using N4BiasFieldCorrection v2.1.0<sup>77</sup> and skull-stripped using antsBrainExtraction.sh v2.1.0 (using the OASIS template). Spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c,<sup>94</sup> RRID:SCR\_008796) was performed through nonlinear registration with the antsRegistration tool of ANTs v2.1.0,<sup>76</sup> RRID:SCR\_004757), using brain-extracted versions of both T1w volume and template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using fast<sup>95</sup> (FSL v5.0.9, RRID:SCR\_002823).

Functional data were slice time corrected using 3dTshift from AFNI v16.2.07,<sup>96</sup> RRID:SCR\_005927] and motion corrected using mcflirt (FSL v5.0.9; Jenkinson et al., 2002). Distortion correction was performed using fieldmaps processed with fugue<sup>97</sup> (FSL v5.0.9). This was followed by co-registration to the corresponding T1w using boundary-based registration<sup>98</sup> with six degrees of freedom, using flirt(FSL). Motion correcting transformations, field distortion correcting warp, BOLD-to-T1w transformation and T1w-to-template (MNI) warp were concatenated and applied in a single step using antsApplyTransforms (ANTs v2.1.0) using Lanczos interpolation.

Physiological noise regressors were extracted applying CompCor.<sup>99</sup> Principal components were estimated for the two CompCor variants: temporal (tCompCor) and anatomical (aCompCor). A mask to exclude signal with cortical origin was obtained by eroding the brain mask, ensuring it only contained subcortical structures. Six tCompCor components were then calculated including only the top 5% variable voxels within that subcortical mask. For aCompCor, six components were calculated within the intersection of the subcortical mask and the union of CSF and WM masks calculated in T1w space, after their projection to the native space of each functional run. Frame-wise displacement<sup>100</sup> was calculated for each functional run using the implementation of Nipype.

Many internal operations of FMRIPREP use Nilearn,<sup>73</sup> SCR\_001362 principally within the BOLD-processing workflow. For more details of the pipeline see <https://fmriprep.readthedocs.io/en/latest/workflows.html>.

### Statistical analysis

Before statistical analysis preprocessed functional images were smoothed (8 × 8 × 8 mm<sup>3</sup> FWHM Gaussian kernel) to take account of between-subject anatomical variation. Statistical analysis used SPM12 software (Statistical Parametric Mapping; Wellcome

Department of Cognitive Neurology, London, UK) implemented with MATLAB 9.4 (r2018a, Mathworks). The first-level statistical analysis was modeled using a canonical hemodynamic response function. Eight regressors of interest were included in the model corresponding to the eight conditions. Motion parameters (3 rotations, 3 translations), frame displacement, 6 aCompCor components, a discrete cosine transform basis set acting as high-pass filter (with 128 s cutoff) and nasal respiration signal were also included in the model as regressors of no interest. For nasal respiration signal, before it was included as covariate, the raw signal (200Hz) was down-sampled into the fMRI frequency (1/RT) using the Fourier method implemented in the “resample” function from Scipy software (Python Library). Finally, for each subject, the seven contrast of interest consisted in comparing each of the odor condition with the “air” condition.

Here we used fMRI to investigate patterns of activity in olfactory areas. For this purpose, we conducted region of interest (ROI) approach because of the important anatomical variability in these areas. The neural activity (mean signal ( $\beta$ ) amplitude) in the OT, aPirCX and pPirCX were extracted for each contrast of interest (BUT versus air, HMHA versus air, MSH versus air, CIS3 versus air, TER versus air, LIM versus air and ISO versus air) and each participant. PirCX has already been investigated in humans, so we used standard preexisting templates. Studies on the OT are more rare, only one group has clearly studied it.<sup>42,101</sup> Therefore, based on these publications and using the May Atlas (May, 2014) as a support, we drew individually each region of interest using MRICron software (<https://people.cas.sc.edu/rorden/mricron/index.html>). Each region was drawn individually for each participant from coronal slices in both hemispheres before functional analysis and thus any error was unrelated to functional condition. Mean images and binary masks were calculated using Imcalc in SPM. To keep a voxel in the binary mask, at least 30% of subjects had to have the voxel in their ROI. For OT, aPirCX and pPirCX, brain activity of both hemispheres were averaged.

To test whether odorant attraction modulate OT activity, we formed for each subject a group of “attractive odorants” and a group of “unattractive odorants” based on odorant attraction evaluation collected during MRI (attractive and unattractive for odorants rated  $\geq 3$  and  $< 3$  respectively). Since, one odorant can be considered as pleasant for one subject (attraction  $\geq 3$ ) while it is not for another (attraction  $< 3$ ), we formed the attraction-based odorant groups for each subject for taking account of inter individual variability in attraction ratings.

We used repeated-measure ANOVA for hemisphere\*attraction interaction analyses and compared brain activity in respond to attractive versus unattractive odorants using Bonferroni corrected One-Tailed Paired t-tests.

## QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed using Statistica. Kolmogorov-Smirnov test was used to assess normality of the data and variance was assessed using Levene test and parametric or non-parametric tests were performed according to that. One-Tailed tests were performed when an assumption about the direction of the variation from a reference value was made; otherwise Two-Tailed tests were used. Bonferroni corrections were performed for multiple comparisons. Significant result was set at  $p < 0.05$ . No statistical methods were used to predetermine sample sizes, but our sample sizes were similar to those reported in previous publications.<sup>6,102–105</sup> Effect size were calculated using Cohen’s d and Rank-Biserial Correlation for parametric and non-parametric tests respectively. In addition, fMRI statistical analysis used SPM12 software (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK) implemented with MATLAB 9.4 (r2018a, Mathworks). Statistical tests used can be found in the star methods and result sections. The exact value of n as well as the precision measure can be found in the figure legends. In the manuscript, n represent the number of individuals or mice depending on the experiment.



**ÉTUDE 2 :** "Impact du vieillissement normal sur la valeur  
hédonique des odeurs chez la souris et circuits neuronaux  
sous-jacents." (en préparation)

## **Impact of normal aging on odor hedonics in mice and the underlying neural circuits**

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## **ABSTRACT**

Hedonic value of odorants is a primary criterion used to drive approach or avoidance behavior essential when facing food stimulus, partners or danger. Odor hedonics is represented along the antero-posterior axis of olfactory bulb. This hedonic signature is transferred to the olfactory tubercle and ventral tegmental area to lead to an approach behavior. The goal of this study is to determine whether odor hedonic perception becomes aberrant with aging and the brain mechanisms responsible for this change. We first revealed that while the perception of unpleasant odorants is unchanged, some but not all pleasant odorants are perceived as less pleasant. In line with these behavioral results, we found that neural activity in the anterior olfactory bulb, in which pleasant odorants are represented, as well as in the olfactory tubercle and the ventral tegmental area, parts of the brain reward system, was altered when odorants lost their attractive power while activity was maintained in the anterior olfactory bulb and olfactory tubercle when the odorants kept their power of attraction. Finally, using optogenetics, we report that self-stimulation of the olfactory bulb is still rewarding in old mice even though less efficiently than in young mice and not accompanied by ventral tegmental area activation. These data revealed how aging degrades behavioral and neural response to some pleasant odorants but also that the rewarding properties of olfactory bulb stimulation persisted in aging, providing new insights into developing olfactory remediation strategy to counteract the effect of aging on motivated behaviors.

## **KEYWORDS**

Aging, Hedonics, reward, olfaction, olfactory tubercle, self-stimulation, optogenetics

## 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, impaired hedonic responses to food odorants may decrease food intake (Joussain et al. 2017) and perpetuate bodily wasting disorders (Ali et Garcia 2014; Frontera et al. 2000; Madsen et al. 1997) – which are common in aging and age-related dementias (Joussain et al. 2013; Joussain et al. 2016) and represent a major public health problem.

Our team uncovered evidence that odor hedonics is represented along the antero-posterior axis of the olfactory bulb (OB) (Kermen et al. 2016) – the first cortical level for processing odor information. This neural signature of hedonic value present in the OB is transferred into downstream brain structures. The OB projects to the piriform cortex, the cortical amygdala, the olfactory tubercle (OT, which is part of the ventral striatum), the anterior olfactory nucleus and the entorhinal cortex. Among all these structures, we revealed recently that the activity of the OT is the only one to be higher in response to pleasant odorants than to unpleasant ones in young adult mice (Midroit et al. 2020). We also observed an increased activity in the ventral tegmental area (VTA) specifically in response to spontaneously pleasant odorants. Intracranial self-stimulation has been extensively used to determine brain areas belonging to the reward system and unravel the neural bases of motivation (Olds et Milner 1954; Olds et Fobes 1981; Carlezon et Chartoff 2007). This technic consists in an operant conditioning in which the stimulation of a specific brain area serves as 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 et al. 2020). This explained how a sensory system solicits the reward system to drive motivated behavior. Altogether these data showed that odorants are differentially represented in the forebrain depending upon whether they are pleasant versus unpleasant, and that spontaneous attraction induced by pleasant odorants is dependent upon the solicitation of the reward circuit.

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 DAergic neurons and their receptors in the midbrain decline (Collier, Kanaan, et 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 implicated 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; Jouslain et al. 2013; Vieillard et al. 2020). In this context, the overall goal of this project is to determine the respective roles of the olfactory and reward circuits in the alterations of odor hedonics with aging.

## **MATERIAL AND METHODS**

### Animals

92 adult male C57Bl6/J mice (Charles River Laboratories, L'Arbresle, France) aged of 22 months at the beginning of the experiments were used for all 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 were conducted after at least 1 week of habituation.

### Statistics

All statistical analyses were performed using Statistica. Kolmogorov-Smirnov and Levene tests were used to assess respectively normality of the data and variance homogeneity, and followed by parametric or non-parametric tests. Bonferroni corrections for multiple comparisons. were applied when needed No statistical methods were used to predetermine sample sizes, but sample sizes were similar to those reported in previous publications (Midroit et al. 2020; Kermen et al. 2016; Moreno et al. 2009; Mandairon et al. 2011; Sultan et al. 2011; Moreno et al. 2012).

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

#### *Odorants.*

Six monomolecular odorants were used: three known to be attractive in young adult mice (camphor: CAM; citronellol: CITRO; +limonene: LIM) and three known to be unattractive (pyridine: PYR; guaiacol: GUA; p-cresol: CRE). Odorants were diluted at 1Pa in mineral oil ([Supplementary Table 1](#)) (Midroit et al. 2020; Kermen et al. 2016; Kermen et al. 2011).

#### *Odorant detection*

To ensure that old mice can detect odorants at 1Pa, two other groups of mice were submitted to a preliminary detection test session. 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 (n=10) or GUA (n=10) at 1Pa (cross habituation). Time spent to investigate the tea ball was recorded (active sniffing 1cm around the tea ball, using Volcan software (Hegoburu et al. 2009; Richard et al. 2017)). 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).

#### *Assessment of odor attraction (or preference test)*

Odor attractiveness was measured in old mice (n=25) using odorant investigation time (Kobayakawa et al. 2007; Mandairon, Poncelet, et al. 2009; Kermen et al. 2016). As previously described (Kermen 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  $\mu$ L of odorant at 1Pa 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 (one odorant/day; 2 min/trial). Odorants were presented in random order and animals performed no more than two consecutive days of testing. A Friedman test was used to test odor effect and Bonferroni corrected One-Tailed Wilcoxon for odorant comparisons. Behavioral data from young adult mice ([Supplementary Figure 1](#)) were obtained in Midroit et al. 2020.

#### *Odorant stimulation and sacrifice*

Mice were submitted to odorant stimulation one hour before sacrifice. This 1-hour delay has previously been shown to enable the expression of the immediate early gene *c-Fos* in response to odorant stimulation (Kermen et al. 2016; Dielenberg, Hunt, et McGregor 2001; Kovács 2008). Briefly, mice were first placed in individual clean cages for 1 hour, with an empty tea ball hanging from the top of the cage. A polypropylene swab impregnated with one odorant (60 $\mu$ L, 1Pa) was then placed in the tea ball for an additional hour. Mice were sacrificed using dolethal (2g/Kg) and intracardiac perfusion of 50 ml of fixative (PFA 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  $\mu$ m thick; 210  $\mu$ m intervals).

#### *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  $\mu$ m 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<sup>+</sup> cells were automatically counted 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<sup>+</sup>) 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  $\mu$ m of the layer (missing sections were interpolated; for more method explanations see Midroit et al. 2018). We first calculated c-Fos<sup>+</sup> cell density per bin, then a z score (normalization of the matrix to mean = 0 and SD = $\pm$ 1) for each animal to further compare spatial patterns of labelled cells, and finally z-scored maps were averaged within groups. Between-groups comparisons were achieved by one-tailed Mann-Whitney tests performed on these z scored arrays. We compared maps of three groups of mice based on the behavioral results: unattractive (n=5), attractive in young but no more in old mice called previously attractive (n=5) and attractive (n=5) odorants. The effect was confirmed by permutation test (252 permutations).

### *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<sup>+</sup> among DARPP-32<sup>+</sup> = DARPP-32<sup>+</sup>/c-Fos<sup>+</sup>) was assessed (5 sections/animal; 5 animals/group). One-Way ANOVA was used to test attractiveness effect followed by Bonferroni corrected *post hoc* t-Tests for group 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 :2, 000 ; AbCam, ref: 76442) and rabbit anti-c-Fos (1 :2, 000 ; 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<sup>+</sup> among TH<sup>+</sup>=TH<sup>+</sup>/c-Fos<sup>+</sup>) was calculated and Kruskal-Wallis ANOVA was used to test the attractiveness effect. The percentage of TH/c-Fos<sup>+</sup> cells was also calculated and One-Way ANOVA was used to assess the attractiveness effect.

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

## Experiment 2. Optogenetic intracranial self-stimulation.

### *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,  $\pm$  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 this time window after surgery, resulting in 16 NpHR mice and 11 Ctrl mice available for the behavioral experiment.



### *Optical self-stimulation in freely moving animals*

8 weeks after surgery, NpHR and Ctrl mice were submitted to the conditioning phase which they were allowed to move freely on the one-hole-board apparatus for a 2-min trial per day, during 5 days (Midroit et al. 2020). 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 (5 trials per day during 3 days) and one last trial (Light1') in which light stimulation was available again. 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.

### *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 :5,000 ; Santa Cruz Biotechnology, Santa Cruz, CA, USA, ref : Sc-189) and chicken anti-EYFP (1 :1,000 ; 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<sup>+</sup> and the percentage of double-labelled cells (EYFP<sup>+</sup>/c-Fos<sup>+</sup>) were assessed in the ventral granule cell layer of the OB at the level of the injection site (ventro-posterior OB, 2 sections/animal; 27 animals) and at 0.96 mm anterior to the injection (2 sections/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<sup>+</sup> density between the injection site (ventro-posterior OB) and ventro-anterior OB. Two-Tailed Mann-Whitney was used to

compare the percentage of EYFP<sup>+</sup>/c-Fos<sup>+</sup> between groups. Neural activity in the VTA and OT was assessed by calculating the percentage of c-Fos-TH and cFos-DARPP-32 double-labelled cells as described in Experiment 1 (Two-Tailed Mann-Whitney for between group comparisons).

## RESULTS

### 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 odor attractiveness (Kobayakawa et al. 2007; Mandairon, Poncelet, et al. 2009; Kermen et al. 2016; Midroit et al. 2020) (Figure 1A) in 22-month old mice. We tested 6 unfamiliar odorants (Supplementary Table 1), 3 known to be attractive (CAM, CITRO, LIM) and three known to be unattractive (PYR, GUA, CRE) in young adult mice (Kermen et al. 2016; Midroit et al. 2020). We showed that these 6 odorants elicited diverse investigation times (Friedman Odor effect  $F_{(5,15)}=27.89$   $p<0.001$ ; Figure 1B). As expected, PYR, GUA, CRE showed similarly low levels of investigation in old mice ( $n=25$ ) (Bonferroni corrected One-Tailed Wilcoxon for odorants comparison,  $p>0.05$ ; Figure 1B) that were as low as in young adult mice ( $n=23$ ) (Bonferroni corrected Two-Tailed Mann-Whitney for between age comparison, PYR=1 GUA=0.266 CRE=0.974; Supplementary Figure 1A). Interestingly, CAM, CITRO and LIM showed different levels of investigation with only LIM remaining attractive for old mice (Bonferroni corrected One-Tailed Wilcoxon for odorants comparison, comparison to LIM: PYR  $p=0.03$  GUA  $p=0.002$  CRE  $p=0.001$  CAM  $p=0.04$  CITRO  $p=0.001$ ; comparisons between other odorants  $p>0.05$ ; Figure 1B). In old mice, LIM remained as attractive as in young adult mice (Bonferroni corrected Two-Tailed Mann-Whitney for between age comparison, LIM  $p=1$ ; Supplementary Figure 1A). CAM and CITRO, two odorants that were attractive for young adult mice (Kermen et al. 2016; Midroit et al. 2020) (Supplementary Figure 1A), were no longer attractive for old mice (Bonferroni corrected Two-Tailed Mann-Whitney for between age comparison, CAM  $p=0.003$  CITRO  $p<0.001$ ; Supplementary Figure 1A).

To ensure that the changes in hedonic perception observed in aged mice was not due to a lack of odor detection, we performed a cross habituation test. In this task, a tea ball containing odorless mineral oil was presented 3 times before one presentation of CITRO (Figure Ci) or 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 both odorants were detected at 1 Pa (Friedman trial effect, CITRO  $F_{(3,6)}=13.163$   $p=0.001$ , GUA  $F_{(3,6)}=9.000$   $p=0.029$ ; Two-Tailed Wilcoxon for difference between NO3 and odorant, CITRO  $p=0.013$ , GUA  $p=0.037$ ; [Figure 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).

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 old mice (PA; CAM CITRO) and that were still attractive in aged mice (A; LIM). C-Fos assessment 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 et al 2020).

Regarding the OB, we focused our analysis on the antero-posterior axis of the ventral granule cell layer ([Figure 2A](#)) as the neural signature of odor hedonics was previously identified in this region (Kermen et al. 2016b). Indeed, 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 et al. 2016). As in young mice, c-Fos positive cell density was higher in the ventro-anterior OB in response to still attractive compared to unattractive odorants (A>UnA, [Figure 2A-B](#)) or to odorants that lost their attractive power with aging (A>PA, [Figure 2A-B](#)). In addition, we showed a higher c-Fos+ cell density in the ventro-posterior OB in response to unattractive odorants compared to the attractive odorant (UnA>A, [Figure 2A-B](#)). Interestingly, unattractive odorants and odorants that lost their attractiveness showed similar patterns (UnA>A and A>UnA, [Figure 2A-B](#)). These results were confirmed by permutation tests ([Supplementary Figure 1B](#)). Our current data confirmed the existence of a neural signature of odor hedonics within the granule cell layer, characterized by a representation of attractive odorants in the ventro-anterior OB and of unattractive odorants in the ventro-posterior OB in old mice. The change of odorant driven-attraction observed with aging supports the functionality of this neural trace, even in old mice.

Enhanced activity in the OT only when an odorant remains attractive.

We thus observed the neural signature of hedonic value in the OB of young adult (Kermen et al. 2016) and aged mice (present results). 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 attractiveness effect,  $F_{(2,3)}=13.146$   $p<0.001$ ; [Figure 2C](#)). Indeed, we observed an increase of double labelled DARPP-32<sup>+</sup>/c-Fos<sup>+</sup> cells in A group compared to UnA (Bonferroni corrected *post hoc* T-Test  $p<0.001$ ) or compared to PA (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 are still attractive.

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<sup>+</sup> cells) of the VTA in the three odorant groups. Surprisingly, we found no difference in TH<sup>+</sup>/c-Fos<sup>+</sup> cells between the three groups (Kruskal-Wallis ANOVA attractiveness effect,  $H_{(2,3)}=1.078$   $p=0.583$ ; [Figure 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<sup>-</sup>) VTA neurons was neither observed between the groups (One-Way ANOVA attractiveness effect,  $F_{(2,3)}=0.205$   $p=0.818$ ; [Figure 2E](#)).

Altogether, these results revealed that when attraction to odorants was altered in old 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.

#### The olfactory bulb remains a rewarding site for intracranial self-stimulation in old mice

We previously revealed that the ventral granule cell layer of the ventro-posterior OB is a site for intracranial self-stimulation in young adult mice (Midroit et al. 2020). We investigated here whether in old mice, optogenetic stimulation in the ventro-posterior OB would retain the ability to support operant conditioning. 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). This optogenetic manipulation leads to a reduction of granule interneuron activity and thus to a disinhibition of OB relay cells (mitral/tufted cells) (Kermen et al. 2016).

A control group was injected with 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 (Figure 3A). Nose poke duration of NpHR mice increased across days of training (Friedman trial effect  $F_{(4,10)}=28.45$   $p<0.001$ , Wilcoxon Light1 vs Light5  $p<0.001$ ; Figure 3B), revealing that old 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 poke 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 poke 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 old mice. In Ctrl mice, a modulation of nose poke duration was observed during the initial sessions of light stimulation (Friedman trial effect  $F_{(4,10)}=10.109$   $p=0.039$ ; Figure 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 poke duration in NpHR mice was lower than in Ctrl mice suggesting a neophobic behavior induced by the optogenetic manipulation (Bonferroni corrected One-Tailed Mann-Whitney, Light1  $p=0.039$ ). It is worth noting that nose poke duration differed between NpHR and Ctrl mice from the 4<sup>th</sup> trial of training (Bonferroni corrected One-Tailed Mann-Whitney, Light2  $p=0.812$ , Light3  $p=0.081$ , Light4  $p=0.010$ , Light5  $p=0.008$ , Figure 3B). This difference 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<sup>+</sup> cells and analyzed the percentage of EYFP<sup>+</sup> cells expressing c-Fos in the ventral granule cell layer of the OB. We found a higher density of EYFP<sup>+</sup> 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  $p=0.007$ ; Figure 3C-D). The percentage of EYFP<sup>+</sup>/c-Fos<sup>+</sup> cells was lower in NpHR than in Ctrl mice (Two-

Tailed Mann-Whitney,  $p=0.032$ ; [Figure 3E](#)), indicating that the optogenetic manipulation efficiently induced an inhibition of postero-ventral 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 DARPP-32<sup>+</sup>/c-Fos<sup>+</sup> cells in NpHR compared to Ctrl mice in the OT (Two-Tailed Mann-Whitney,  $p<0.001$ ; [Figure 3F](#)). Remarkably, no difference between NpHR and Ctrl groups was observed for TH<sup>+</sup>/c-Fos<sup>+</sup> cells (Two-Tailed Mann-Whitney,  $p=0.904$ ; [Figure 3G](#)) and TH<sup>-</sup>/c-Fos<sup>+</sup> cells (Two-Tailed Mann-Whitney,  $p=1$ ; [Figure 3H](#)) in the VTA. These data indicated that ventro-posterior OB self-stimulation increased activity in the OT but not in the VTA.

## DISCUSSION

Age-related alteration of odor hedonics (Wang et al. 2005; Markovic et al. 2007; Jousain et al. 2013; Vieillard et al. 2020) is a major issue for the quality of life in human elderly (Jousain et al. 2017). While modifications of olfactory perception such as changes in odor detection (Bodyak et Slotnick 1999; Kraemer et Apfelbach 2004; Patel et Larson 2009), discrimination (Mandairon, Peace, et al. 2011; Moreno et al. 2014; Yoder et al. 2017) or learning and memory (Dardou, Datiche, et Cattarelli 2008; Kraemer et Apfelbach 2004; LaSarge et al. 2007) have been already shown during aging in rodents, the present study revealed that as in humans (Jousain et al. 2013), an hedonic dysfunction specific to pleasant odorants is observed in old 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. This result offers a good model to look for neural mechanisms underlying odor hedonic alteration during aging.

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 et Kamath 2014; Naessen 1971; Nakashima, Kimmelman, et Snow 1984; Morrison et Costanzo 1990; Paik et al. 1992), number of olfactory sensory neurons (Conley et al. 2003; Weiler et Farbman 1997) and expression of some olfactory receptor genes have been observed with aging in humans (Cavallin et al. 2010; Khan, Vaes, et 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 et McNelly 1981; Rey et al. 2012; Moreno et al. 2014) and human (Bhatnagar et al. 1987; Yousem et al. 1998;

Sama-ul-Haq, Tahir, et Lone 2008; Segura et al. 2013), connections (Richard, Taylor, et Greer 2010; Gocel et 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 unravel this question, we analyzed odor-evoked activity in the OB, OT and VTA, a relevant pathway involved in odor-guided behavior (Midroit et al. 2020).

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 et al. 2016). Moreover, optogenetic manipulation of these hot spots changed hedonic perception (Kermen et al. 2016). Here, we confirmed the significance of these bulbar hot spots using the ecological model of aging. Indeed, we confirmed in old mice increased neural activity in the ventro-anterior OB in response to attractive odorants compared to unattractive odorants and in the ventro-posterior OB in response to unattractive compared to attractive odorants. 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, et Schneider 1980; Schwob et Price 1984). In line with this, we found in aged mice 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 et Wilson 2011). In addition, the OT is closely connected to the VTA (Ikemoto 2007). While pleasant odorants induced an increased activity in the VTA in young adult mice (Midroit et al 2020), no activation of DAergic VTA neurons was observed in response to an odorant that remained attractive in old mice. In addition, since VTA glutamatergic and GABA neurons were recently involved in motivated behaviors (Wang et al. 2015; Morales et Margolis 2017), we also investigated the activity of non DAergic VTA neurons. However, no difference in activity depending on the hedonic value of the odorant 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 old mice and that the lack of



odor-evoked activity in the VTA may not reflect the lack of odorant positive appreciation in old mice.

The OB has been recently described as a core component of the reward system, driving motivated behavior (Midroit et al. 2020). Indeed, young adult mice are able to learn to self-stimulate their posterior OB. Here, we found that aged mice were also able to perform ventro-posterior OB self-stimulation. This conditioning induced an increase of OT activity, indicating that the ventro-posterior OB was still able to recruit the reward system. Remarkably, the self-stimulation behavior is not accompanied by an increase of neural activity in the VTA. The lack of evoked activity in VTA after optogenetic self-stimulation of the OB or in response to pleasant odorants could be due to a reduction in the number of VTA neurons and their impairment to synthesize DA that arises during 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, et Kordower 2017). To our knowledge, the present study is the first to provide evidence that self-stimulation behavior can be achieved without the recruitment of the VTA. However, it is unknown if this observation reflects a general pattern of the reward system activation in aging or if it is specific to the model of OB self-stimulation. Here, we revealed that the OT alone, or more likely the OT-related network, is sufficient to induce operant conditioning. Indeed, 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 et Wilson 2011; Xiong et Wesson 2016), suggesting that it could solicit by itself the neural network that underlies self-motivation without recruiting the VTA.

The present study highlights the key role of OB-OT network in olfactory perception and odor-motivated behaviors. It provides 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 provides new insight into developing a remediation strategy to counteract the effect of aging on olfactory hedonics. It also 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, et Kordower 2017).

## REFERENCES

- Adjei, Stacey, Alexandra L. Houck, Katherine Ma, et Daniel W. Wesson. 2013. « Age-Dependent Alterations in the Number, Volume, and Localization of Islands of Calleja within the Olfactory Tubercle ». *Neurobiology of Aging* 34 (11): 2676-82. <https://doi.org/10.1016/j.neurobiolaging.2013.05.014>.
- Ali, Sumbul, et Jose M. Garcia. 2014. « Sarcopenia, Cachexia and Aging: Diagnosis, Mechanisms and Therapeutic Options - a Mini-Review ». *Gerontology* 60 (4): 294-305. <https://doi.org/10.1159/000356760>.
- Araki, T., H. Kato, K. Shuto, T. Fujiwara, et Y. Itoyama. 1997. « Effect of Aging on Dopaminergic Receptors and Uptake Sites in the Rat Brain Studied by Receptor Autoradiography ». *Journal of the Neurological Sciences* 148 (2): 131-37. [https://doi.org/10.1016/s0022-510x\(96\)05343-9](https://doi.org/10.1016/s0022-510x(96)05343-9).
- Barili, P., G. De Carolis, D. Zaccheo, et F. Amenta. 1998. « Sensitivity to Ageing of the Limbic Dopaminergic System: A Review ». *Mechanisms of Ageing and Development* 106 (1-2): 57-92. [https://doi.org/10.1016/s0047-6374\(98\)00104-3](https://doi.org/10.1016/s0047-6374(98)00104-3).
- Bhatnagar, K. P., R. C. Kennedy, G. Baron, et R. A. Greenberg. 1987. « Number of Mitral Cells and the Bulb Volume in the Aging Human Olfactory Bulb: A Quantitative Morphological Study ». *The Anatomical Record* 218 (1): 73-87. <https://doi.org/10.1002/ar.1092180112>.
- Bodyak, N., et B. Slotnick. 1999. « Performance of Mice in an Automated Olfactometer: Odor Detection, Discrimination and Odor Memory ». *Chemical Senses* 24 (6): 637-45. <https://doi.org/10.1093/chemse/24.6.637>.
- Carlezon, William A., et Elena H. Chartoff. 2007. « Intracranial Self-Stimulation (ICSS) in Rodents to Study the Neurobiology of Motivation ». *Nature Protocols* 2 (11): 2987-95. <https://doi.org/10.1038/nprot.2007.441>.
- Cavallin, Melissa Ann, Katelyn Powell, K. C. Biju, et Debra Ann Fadool. 2010. « State-Dependent Sculpting of Olfactory Sensory Neurons Is Attributed to Sensory Enrichment, Odor Deprivation, and Aging ». *Neuroscience Letters* 483 (2): 90-95. <https://doi.org/10.1016/j.neulet.2010.07.059>.
- Collier, Timothy J., Nicholas M. Kanaan, et Jeffrey H. Kordower. 2017. « Aging and Parkinson's Disease: Different Sides of the Same Coin? » *Movement Disorders: Official Journal of the Movement Disorder Society* 32 (7): 983-90. <https://doi.org/10.1002/mds.27037>.
- Conley, David B., Alan M. Robinson, Michael J. Shinnars, et Robert C. Kern. 2003. « Age-Related Olfactory Dysfunction: Cellular and Molecular Characterization in the Rat ». *American Journal of Rhinology* 17 (3): 169-75.
- Dardou, David, Frédérique Datiche, et Martine Cattarelli. 2008. « Memory Is Differently Impaired during Aging According to the Learning Tasks in the Rat ». *Behavioural Brain Research* 194 (2): 193-200. <https://doi.org/10.1016/j.bbr.2008.07.007>.
- Dielenberg, R. A., G. E. Hunt, et I. S. McGregor. 2001. « "When a Rat Smells a Cat": The Distribution of Fos Immunoreactivity in Rat Brain Following Exposure to a Predatory Odor ». *Neuroscience* 104 (4): 1085-97. [https://doi.org/10.1016/s0306-4522\(01\)00150-6](https://doi.org/10.1016/s0306-4522(01)00150-6).
- Doty, et Vidyulata Kamath. 2014. « The Influences of Age on Olfaction: A Review ». *Frontiers in Psychology* 5: 20. <https://doi.org/10.3389/fpsyg.2014.00020>.
- Frontera, W. R., V. A. Hughes, R. A. Fielding, M. A. Fiatarone, W. J. Evans, et R. Roubenoff. 2000. « Aging of Skeletal Muscle: A 12-Yr Longitudinal Study ». *Journal of Applied Physiology (Bethesda, Md.: 1985)* 88 (4): 1321-26. <https://doi.org/10.1152/jappl.2000.88.4.1321>.

- Gocel, James, et John Larson. 2013. « Evidence for Loss of Synaptic AMPA Receptors in Anterior Piriform Cortex of Aged Mice ». *Frontiers in Aging Neuroscience* 5: 39. <https://doi.org/10.3389/fnagi.2013.00039>.
- Hegoburu, Chloé, Yannick Sevelinges, Marc Thévenet, Rémi Gervais, Sandrine Parrot, et Anne-Marie Mouly. 2009. « Differential Dynamics of Amino Acid Release in the Amygdala and Olfactory Cortex during Odor Fear Acquisition as Revealed with Simultaneous High Temporal Resolution Microdialysis ». *Learning & Memory (Cold Spring Harbor, N.Y.)* 16 (11): 687-97. <https://doi.org/10.1101/lm.1584209>.
- Hinds, J. W., et N. A. McNelly. 1981. « Aging in the Rat Olfactory System: Correlation of Changes in the Olfactory Epithelium and Olfactory Bulb ». *The Journal of Comparative Neurology* 203 (3): 441-53. <https://doi.org/10.1002/cne.902030308>.
- Ikemoto, Satoshi. 2007. « Dopamine Reward Circuitry: Two Projection Systems from the Ventral Midbrain to the Nucleus Accumbens-Olfactory Tubercle Complex ». *Brain Research Reviews* 56 (1): 27-78. <https://doi.org/10.1016/j.brainresrev.2007.05.004>.
- Irwin, I., L. E. DeLanney, T. McNeill, P. Chan, L. S. Forno, G. M. Murphy, D. A. Di Monte, M. S. Sandy, et J. W. Langston. 1994. « Aging and the Nigrostriatal Dopamine System: A Non-Human Primate Study ». *Neurodegeneration: A Journal for Neurodegenerative Disorders, Neuroprotection, and Neuroregeneration* 3 (4): 251-65.
- Joussain, Pauline, Marion Bessy, Arnaud Fournel, Camille Ferdenzi, Catherine Rouby, Floriane Delphin-Combe, Pierre Krolak-Salmon, et Moustafa Bensafi. 2016. « Altered Affective Evaluations of Smells in Alzheimer's Disease ». *Journal of Alzheimer's Disease: JAD* 49 (2): 433-41. <https://doi.org/10.3233/JAD-150332>.
- Joussain, Pauline, Camille Ferdenzi, Jelena Djordjevic, et Moustafa Bensafi. 2017. « Relationship Between Psychophysiological Responses to Aversive Odors and Nutritional Status During Normal Aging ». *Chemical Senses* 42 (6): 465-72. <https://doi.org/10.1093/chemse/bjx027>.
- Joussain, Pauline, Marc Thevenet, Catherine Rouby, et Moustafa Bensafi. 2013. « Effect of Aging on Hedonic Appreciation of Pleasant and Unpleasant Odors ». *PloS One* 8 (4): e61376. <https://doi.org/10.1371/journal.pone.0061376>.
- Kermen, A. Chakirian, C. Sezille, P. Joussain, G. Le Goff, A. Ziessel, M. Chastrette, et al. 2011. « Molecular Complexity Determines the Number of Olfactory Notes and the Pleasantness of Smells ». *Scientific Reports* 1: 206. <https://doi.org/10.1038/srep00206>.
- Kermen, Maëllie Midroit, Nicola Kuczewski, Jérémy Forest, Marc Thévenet, Joëlle Sacquet, Claire Benetollo, Marion Richard, Anne Didier, et Nathalie Mandairon. 2016. « Topographical Representation of Odor Hedonics in the Olfactory Bulb ». *Nature Neuroscience* 19 (7): 876-78. <https://doi.org/10.1038/nn.4317>.
- Khan, Evelien Vaes, et Peter Mombaerts. 2013. « Temporal Patterns of Odorant Receptor Gene Expression in Adult and Aged Mice ». *Molecular and Cellular Neurosciences* 57 (novembre): 120-29. <https://doi.org/10.1016/j.mcn.2013.08.001>.
- Kobayakawa, Ko, Reiko Kobayakawa, Hideyuki Matsumoto, Yuichiro Oka, Takeshi Imai, Masahito Ikawa, Masaru Okabe, et al. 2007. « Innate versus Learned Odour Processing in the Mouse Olfactory Bulb ». *Nature* 450 (7169): 503-8. <https://doi.org/10.1038/nature06281>.
- Kovács, K. J. 2008. « Measurement of Immediate-Early Gene Activation- c-Fos and Beyond ». *Journal of Neuroendocrinology* 20 (6): 665-72. <https://doi.org/10.1111/j.1365-2826.2008.01734.x>.
- Kraemer, Simone, et Raimund Apfelbach. 2004. « Olfactory Sensitivity, Learning and Cognition in Young Adult and Aged Male Wistar Rats ». *Physiology & Behavior* 81 (3): 435-42. <https://doi.org/10.1016/j.physbeh.2004.01.012>.

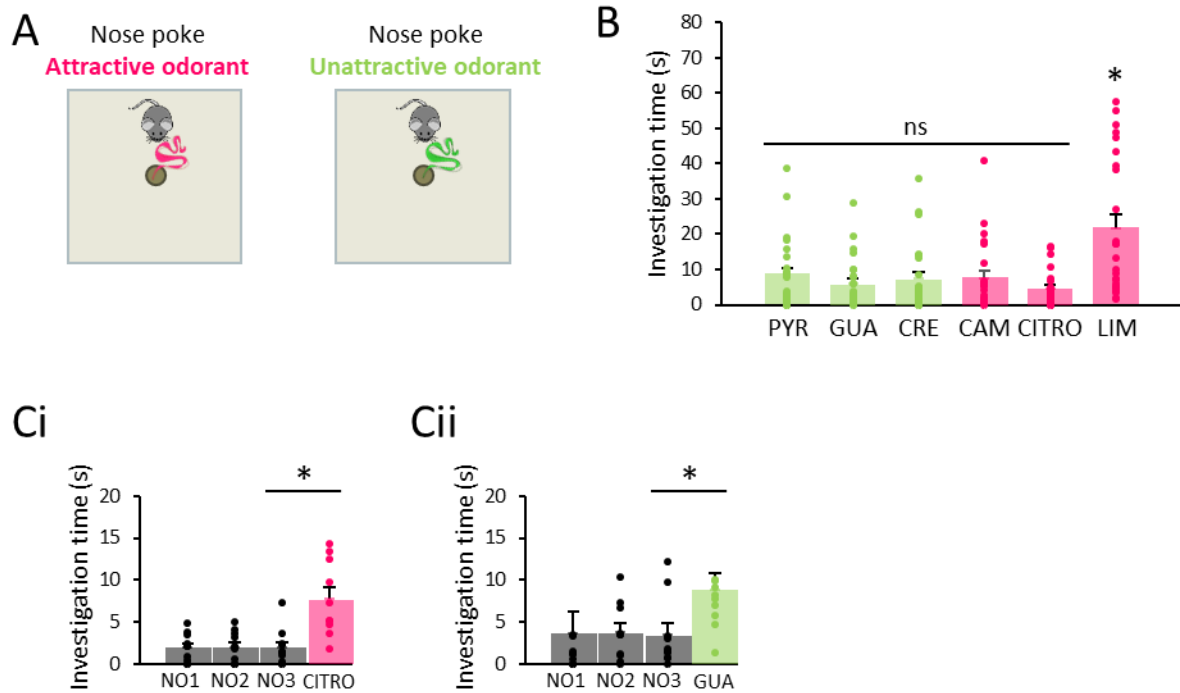
- Lapid, Hadas, Sagit Shushan, Anton Plotkin, Hillary Voet, Yehudah Roth, Thomas Hummel, Elad Schneidman, et Noam Sobel. 2011. « Neural Activity at the Human Olfactory Epithelium Reflects Olfactory Perception ». *Nature Neuroscience* 14 (11): 1455-61. <https://doi.org/10.1038/nn.2926>.
- LaSarge, Candi L., Karienn Souza Montgomery, Catherine Tucker, G. Simona Slaton, William H. Griffith, Barry Setlow, et Jennifer L. Bizon. 2007. « Deficits across Multiple Cognitive Domains in a Subset of Aged Fischer 344 Rats ». *Neurobiology of Aging* 28 (6): 928-36. <https://doi.org/10.1016/j.neurobiolaging.2006.04.010>.
- Madsen, O. R., U. B. Lauridsen, A. Hartkopp, et O. H. Sørensen. 1997. « Muscle Strength and Soft Tissue Composition as Measured by Dual Energy X-Ray Absorptiometry in Women Aged 18-87 Years ». *European Journal of Applied Physiology and Occupational Physiology* 75 (3): 239-45. <https://doi.org/10.1007/s004210050154>.
- Mandairon, Nathalie, Shane T. Peace, Karim Boudadi, Christine E. Boxhorn, Venkata Anupama Narla, Sara D. Suffis, et Thomas A. Cleland. 2011. « Compensatory Responses to Age-Related Decline in Odor Quality Acuity: Cholinergic Neuromodulation and Olfactory Enrichment ». *Neurobiology of Aging* 32 (12): 2254-65. <https://doi.org/10.1016/j.neurobiolaging.2009.12.024>.
- Mandairon, Nathalie, Johan Poncelet, Moustafa Bensafi, et Anne Didier. 2009. « Humans and Mice Express Similar Olfactory Preferences ». *PloS One* 4 (1): e4209. <https://doi.org/10.1371/journal.pone.0004209>.
- Mandairon, Nathalie, Sébastien Sultan, Morgane Nouvian, Joelle Sacquet, et Anne Didier. 2011. « Involvement of Newborn Neurons in Olfactory Associative Learning? The Operant or Non-Operant Component of the Task Makes All the Difference ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31 (35): 12455-60. <https://doi.org/10.1523/JNEUROSCI.2919-11.2011>.
- Mandairon, Nathalie, Sébastien Sultan, Nolwen Rey, Florence Kermen, Mélissa Moreno, Germain Busto, Vincent Farget, Belkacem Messaoudi, Marc Thevenet, et Anne Didier. 2009. « A Computer-Assisted Odorized Hole-Board for Testing Olfactory Perception in Mice ». *Journal of Neuroscience Methods* 180 (2): 296-303. <https://doi.org/10.1016/j.jneumeth.2009.04.008>.
- Markovic, Katrin, Udo Reulbach, Agapi Vassiliadu, Jens Lunkenheimer, Birgit Lunkenheimer, Rita Spannenberger, et Norbert Thuerauf. 2007. « Good News for Elderly Persons: Olfactory Pleasure Increases at Later Stages of the Life Span ». *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 62 (11): 1287-93. <https://doi.org/10.1093/gerona/62.11.1287>.
- Marschner, A., T. Mell, I. Wartenburger, A. Villringer, F. M. Reischies, et H. R. Heekeren. 2005. « Reward-Based Decision-Making and Aging ». *Brain Research Bulletin*, 2nd Conference on NeuroEconomics - ConNEcs 2004, 67 (5): 382-90. <https://doi.org/10.1016/j.brainresbull.2005.06.010>.
- Midroit, Maëllie, Marc Thevenet, Arnaud Fournel, Joelle Sacquet, Moustafa Bensafi, Marine Breton, Laura Chalençon, Matthias Cavalius, Anne Didier, et Nathalie Mandairon. 2018. « Non-Imaged Based Method for Matching Brains in a Common Anatomical Space for Cellular Imagery ». *Journal of Neuroscience Methods* 304: 136-45. <https://doi.org/10.1016/j.jneumeth.2018.04.004>.
- Morales, Marisela, et Elyssa B. Margolis. 2017. « Ventral Tegmental Area: Cellular Heterogeneity, Connectivity and Behaviour ». *Nature Reviews. Neuroscience* 18 (2): 73-85. <https://doi.org/10.1038/nrn.2016.165>.
- Moreno, Kevin Bath, Nicola Kuczewski, Joëlle Sacquet, Anne Didier, et Nathalie Mandairon. 2012. « Action of the Noradrenergic System on Adult-Born Cells Is Required for Olfactory Learning in Mice ». *The Journal of Neuroscience: The Official Journal of the*

- Society for Neuroscience* 32 (11): 3748-58. <https://doi.org/10.1523/JNEUROSCI.6335-11.2012>.
- Moreno, Christiane Linster, Olga Escanilla, Joëlle Sacquet, Anne Didier, et Nathalie Mandairon. 2009. « Olfactory Perceptual Learning Requires Adult Neurogenesis ». *Proceedings of the National Academy of Sciences of the United States of America* 106 (42): 17980-85. <https://doi.org/10.1073/pnas.0907063106>.
- Moreno, Marion Richard, Benoit Landrein, Joëlle Sacquet, Anne Didier, et Nathalie Mandairon. 2014. « Alteration of Olfactory Perceptual Learning and Its Cellular Basis in Aged Mice ». *Neurobiology of Aging* 35 (3): 680-91. <https://doi.org/10.1016/j.neurobiolaging.2013.08.034>.
- Morrison, E. E., et R. M. Costanzo. 1990. « Morphology of the Human Olfactory Epithelium ». *The Journal of Comparative Neurology* 297 (1): 1-13. <https://doi.org/10.1002/cne.902970102>.
- Naessen, R. 1971. « An Enquiry on the Morphological Characteristics and Possible Changes with Age in the Olfactory Region of Man ». *Acta Oto-Laryngologica* 71 (1): 49-62. <https://doi.org/10.3109/00016487109125332>.
- Nakashima, T., C. P. Kimmelman, et J. B. Snow. 1984. « Structure of Human Fetal and Adult Olfactory Neuroepithelium ». *Archives of Otolaryngology (Chicago, Ill.: 1960)* 110 (10): 641-46. <https://doi.org/10.1001/archotol.1984.00800360013003>.
- Norrara, Bianca, Felipe P. Fiuza, Ana Cristina Arrais, Ianara Mendonça Costa, Jose Ronaldo Santos, Rovenia Clara G. J. Engelberth, Jeferson Souza Cavalcante, Fausto Pierdoná Guzen, Jose Rodolfo Lopes P. Cavalcanti, et Marco Aurelio M. Freire. 2018. « Pattern of Tyrosine Hydroxylase Expression during Aging of Mesolimbic Pathway of the Rat ». *Journal of Chemical Neuroanatomy* 92: 83-91. <https://doi.org/10.1016/j.jchemneu.2018.05.004>.
- Olds, et Fobes. 1981. « The Central Basis of Motivation: Intracranial Self-Stimulation Studies ». *Annual Review of Psychology* 32: 523-74. <https://doi.org/10.1146/annurev.ps.32.020181.002515>.
- Olds, et Milner. 1954. « Positive Reinforcement Produced by Electrical Stimulation of Septal Area and Other Regions of Rat Brain ». *Journal of Comparative and Physiological Psychology* 47 (6): 419-27. <https://doi.org/10.1037/h0058775>.
- Paik, S. I., M. N. Lehman, A. M. Seiden, H. J. Duncan, et D. V. Smith. 1992. « Human Olfactory Biopsy. The Influence of Age and Receptor Distribution ». *Archives of Otolaryngology--Head & Neck Surgery* 118 (7): 731-38. <https://doi.org/10.1001/archotol.1992.01880070061012>.
- Patel, Roseanne C., et John Larson. 2009. « Impaired Olfactory Discrimination Learning and Decreased Olfactory Sensitivity in Aged C57Bl/6 Mice ». *Neurobiology of Aging* 30 (5): 829-37. <https://doi.org/10.1016/j.neurobiolaging.2007.08.007>.
- Rey, Nolwen L., Joëlle Sacquet, Alexandra Veyrac, François Jourdan, et Anne Didier. 2012. « Behavioral and Cellular Markers of Olfactory Aging and Their Response to Enrichment ». *Neurobiology of Aging* 33 (3): 626.e9-626.e23. <https://doi.org/10.1016/j.neurobiolaging.2011.03.026>.
- Richard, N. Aguilera, M. Thévenet, O. Dkhissi-Benyahya, et F. Flamant. 2017. « Neuronal Expression of a Thyroid Hormone Receptor  $\alpha$  Mutation Alters Mouse Behaviour ». *Behavioural Brain Research* 321: 18-27. <https://doi.org/10.1016/j.bbr.2016.12.025>.
- Richard, Marion B., Seth R. Taylor, et Charles A. Greer. 2010. « Age-Induced Disruption of Selective Olfactory Bulb Synaptic Circuits ». *Proceedings of the National Academy of Sciences of the United States of America* 107 (35): 15613-18. <https://doi.org/10.1073/pnas.1007931107>.

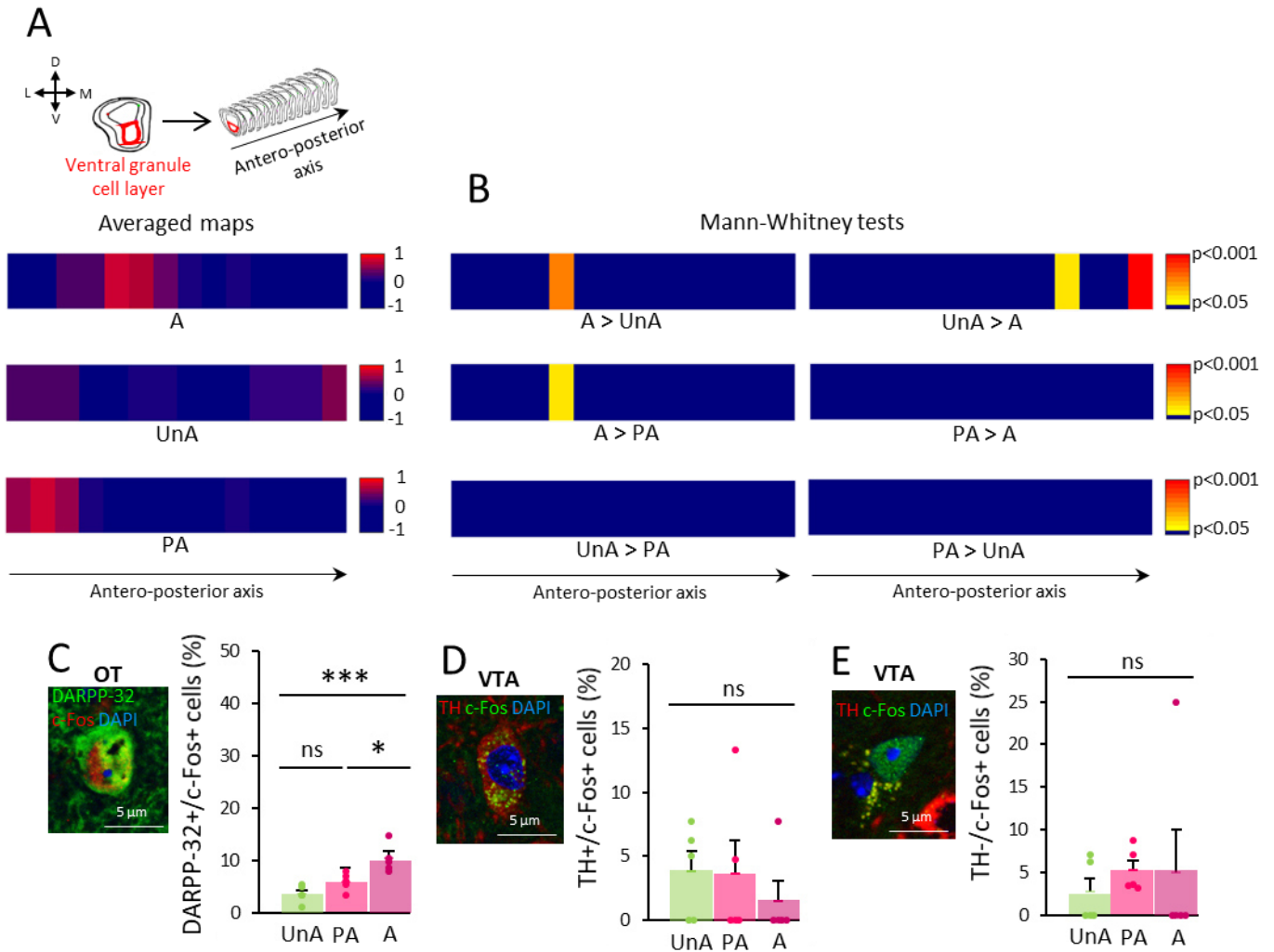
- Sama-ul-Haq, null, Mohammad Tahir, et Khalid P. Lone. 2008. « Age and Gender-Related Differences in Mitral Cells of Olfactory Bulb ». *Journal of the College of Physicians and Surgeons--Pakistan: JCPSP* 18 (11): 669-73. <https://doi.org/11.2008/JCPSP.669973>.
- Schuligoi, R., J. Fernandez, R. P. Heavens, et D. J. Sirinathsinghji. 1993. « Decreased Tyrosine Hydroxylase mRNA but Not Cholecystokinin mRNA in the Pars Compacta of the Substantia Nigra and Ventral Tegmental Area of Aged Rats ». *Brain Research. Molecular Brain Research* 19 (4): 333-38. [https://doi.org/10.1016/0169-328x\(93\)90135-c](https://doi.org/10.1016/0169-328x(93)90135-c).
- Schwob, J. E., et J. L. Price. 1984. « The Development of Axonal Connections in the Central Olfactory System of Rats ». *The Journal of Comparative Neurology* 223 (2): 177-202. <https://doi.org/10.1002/cne.902230204>.
- Scott, J. W., R. L. McBride, et S. P. Schneider. 1980. « The Organization of Projections from the Olfactory Bulb to the Piriform Cortex and Olfactory Tubercle in the Rat ». *The Journal of Comparative Neurology* 194 (3): 519-34. <https://doi.org/10.1002/cne.901940304>.
- Segura, Bàrbara, Hugo César Baggio, Elisabeth Solana, Eva M. Palacios, Pere Vendrell, Núria Bargalló, et Carme Junqué. 2013. « Neuroanatomical Correlates of Olfactory Loss in Normal Aged Subjects ». *Behavioural Brain Research* 246 (juin): 148-53. <https://doi.org/10.1016/j.bbr.2013.02.025>.
- Sojitra, Ravi B., Itamar Lerner, Jessica R. Petok, et Mark A. Gluck. 2018. « Age Affects Reinforcement Learning through Dopamine-Based Learning Imbalance and High Decision Noise-Not through Parkinsonian Mechanisms ». *Neurobiology of Aging* 68: 102-13. <https://doi.org/10.1016/j.neurobiolaging.2018.04.006>.
- Sultan, Sébastien, Nolwen Rey, Joelle Sacquet, Nathalie Mandairon, et Anne Didier. 2011. « Newborn Neurons in the Olfactory Bulb Selected for Long-Term Survival through Olfactory Learning Are Prematurely Suppressed When the Olfactory Memory Is Erased ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31 (42): 14893-98. <https://doi.org/10.1523/JNEUROSCI.3677-11.2011>.
- Suzuki, Y., H. D. Critchley, J. Suckling, R. Fukuda, S. C. Williams, C. Andrew, R. Howard, et al. 2001. « Functional Magnetic Resonance Imaging of Odor Identification: The Effect of Aging ». *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 56 (12): M756-760. <https://doi.org/10.1093/gerona/56.12.m756>.
- Vieillard, Sandrine, Lucas Ronat, Alessia Baccarani, Benoist Schaal, Jean-Yves Baudouin, et Renaud Brochard. 2020. « Age Differences in Olfactory Affective Responses: Evidence for a Positivity Effect and an Emotional Dedifferentiation ». *Neuropsychology, Development, and Cognition. Section B, Aging, Neuropsychology and Cognition*, août, 1-14. <https://doi.org/10.1080/13825585.2020.1799926>.
- Wang, Paul J. Eslinger, Michael B. Smith, et Qing X. Yang. 2005. « Functional Magnetic Resonance Imaging Study of Human Olfaction and Normal Aging ». *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 60 (4): 510-14. <https://doi.org/10.1093/gerona/60.4.510>.
- Wang, Jia Qi, Shiliang Zhang, Huikun Wang, et Marisela Morales. 2015. « Rewarding Effects of Optical Stimulation of Ventral Tegmental Area Glutamatergic Neurons ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 35 (48): 15948-54. <https://doi.org/10.1523/JNEUROSCI.3428-15.2015>.
- Watanabe, H. 1987. « Differential Decrease in the Rate of Dopamine Synthesis in Several Dopaminergic Neurons of Aged Rat Brain ». *Experimental Gerontology* 22 (1): 17-25. [https://doi.org/10.1016/0531-5565\(87\)90011-8](https://doi.org/10.1016/0531-5565(87)90011-8).

- Weiler, E., et A. I. Farbman. 1997. « Proliferation in the Rat Olfactory Epithelium: Age-Dependent Changes ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 17 (10): 3610-22.
- Wesson, Daniel W., et Donald A. Wilson. 2011. « Sniffing out the Contributions of the Olfactory Tubercle to the Sense of Smell: Hedonics, Sensory Integration, and More? » *Neuroscience and Biobehavioral Reviews* 35 (3): 655-68. <https://doi.org/10.1016/j.neubiorev.2010.08.004>.
- Xiong, Angeline, et Daniel W. Wesson. 2016. « Illustrated Review of the Ventral Striatum's Olfactory Tubercle ». *Chemical Senses* 41 (7): 549-55. <https://doi.org/10.1093/chemse/bjw069>.
- Yoder, Wendy M., Leslie S. Gaynor, Sara N. Burke, Barry Setlow, David W. Smith, et Jennifer L. Bizon. 2017. « Interaction between Age and Perceptual Similarity in Olfactory Discrimination Learning in F344 Rats: Relationships with Spatial Learning ». *Neurobiology of Aging* 53: 122-37. <https://doi.org/10.1016/j.neurobiolaging.2017.01.023>.
- Yousem, D. M., R. J. Geckle, W. B. Bilker, et R. L. Doty. 1998. « Olfactory Bulb and Tract and Temporal Lobe Volumes. Normative Data across Decades ». *Annals of the New York Academy of Sciences* 855 (novembre): 546-55. <https://doi.org/10.1111/j.1749-6632.1998.tb10624.x>.



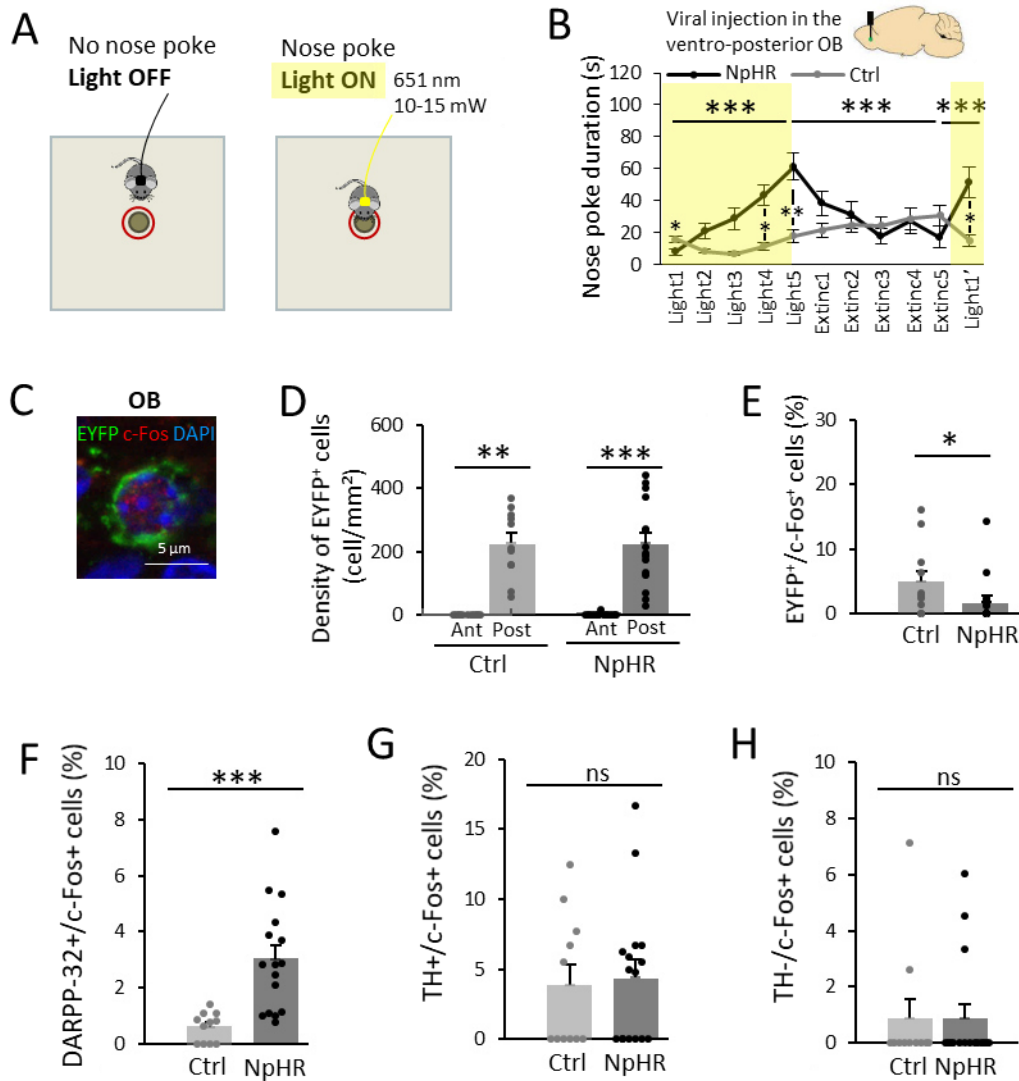


**Figure 1. Odor-driven attraction is altered during aging.** **A.** Experimental setup. **B.** Investigation time measured in old mice in response to 3 odorants known to be attractive (CAM, CITRO, LIM) and 3 unattractive in young adult mice (PYR, GUA, CRE). The level of attraction varied among odorants. Notably, among odorants that are attractive in young adult mice, only the LIM showed higher level of investigation time in old mice. **C.** To assess detection of odorants at 1Pa, an habituation/cross habituation test was used. The significant difference of investigation time between NO3 and CITRO (**Ci**) or NO3 and GUA (**Cii**) indicated efficient odor detection by old mice. For all graphs, bars represent means of individual data points  $\pm$ sem. \*:  $p < 0.05$   
 PYR: pyridine, GUA: guaiacol, CRE: p-cresol, CAM: camphor, CITRO: citronellol, LIM: +limonene, NO: no odor.



**Figure 2. Neural activity in the pOB, OT and VTA depends on the attractive power of odorants.**

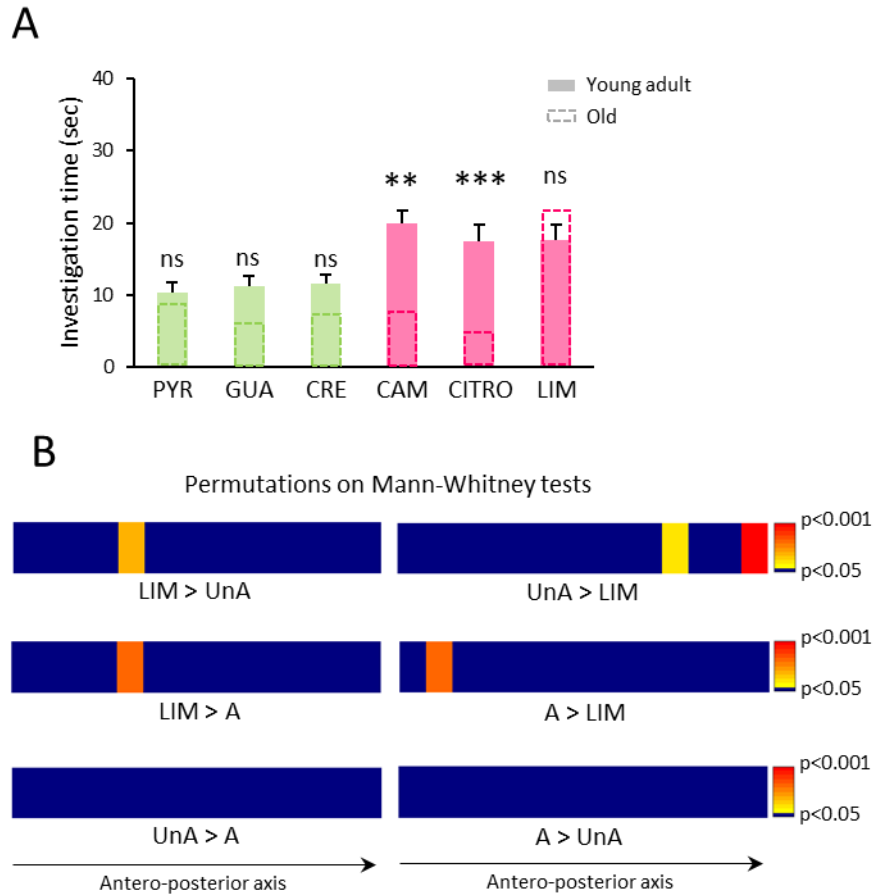
**A. Top.** Delineation of the ventral granule cell layer of in the OB. **Bottom.** Averaged maps of c-Fos density (cells/mm<sup>2</sup>) in the OB ventral granule cell layer represented along the antero-posterior axis in response to unattractive (UnA), previously attractive (PA) and attractive odorants (A). **B.** Mann-Whitney tests for between group comparison of the maps. Significance cutoff was set at  $p < 0.05$ . **C. Left.** Representative image of double labeled DARPP-32+/c-Fos+ cells in the OT. **Right.** The percentage of DARPP-32+/c-Fos+ cells was higher in response to A compared to PA and UnA odorants. There was no difference between PA and UnA groups. **D. Left.** Representative image of double labeled TH+/c-Fos+ cells in the VTA. **Right.** There was no difference in the percentage of TH+ cells expressing c-Fos in the VTA among experimental groups. **E. Left.** Representative image of TH- cells expressing c-Fos in the VTA. **Right.** There was no difference in the percentage of TH-/c-Fos+ cells in the VTA among experimental groups. For all graphs, bars represent means of individual data points  $\pm$  sem. \*:  $p < 0.05$  \*\*\*:  $p < 0.001$ .



**Figure 3. The pOB remains a site for optogenetic intracranial self-stimulation in old mice.** **A.** Experimental setup. **B. Up.** Injection of NpHR-EYFP (NpHR) or control virus (Ctrl) 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 poke started again. Nose pokes significantly decreased in Ctrl during the light stimulation phase, but no change was observed between Light 1 and Light 5. 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 4<sup>th</sup> trial and was no longer observed during the extinction phase. Values represent means  $\pm$  sem. **C.** Representative image of double labelled EYFP+/c-Fos+ cells 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. **E.** A lower percentage of EYFP+ cells expressing c-Fos was observed in ventro-posterior OB of NpHR compared to Ctrl mice. **F.** The percentage of DARPP-32+/c-Fos+ cells was higher in NpHR than Ctrl mice. **G.** No difference in TH+/c-Fos+ cells was observed between NpHR and Ctrl groups. **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.

<b>Odorant name</b>	<b>Abbreviation</b>	<b>CID</b>	<b>Dilution in mineral oil (vol/vol)</b>
+limonene	LIM	1133	0.20%
citronellol	CITRO	8842	17.80%
camphor	CAM	159055	0.46%
pyridine	PYR	1049	0.01%
p-cresol	CRE	2879	1.84%
guaiacol	GUA	460	2.09%

**Supplementary Table 1. Odorant names, abbreviations, CID and dilution for the first experiment.**



**Supplementary Figure 1. A.** Odorant investigation time measured in young adult mice. Only CAM and CITRO are less investigated by old mice (dotted bars) than by young adult mice (solid bars). There is no age-related modification of investigation times for LIM, PYR, GUA and CRE. Bars represent means of individual data points  $\pm$  sem. **B.** Same representation as in Figure 2B. Here, after permutations tests (252 permutations), the colored bins depict position along the antero-posterior axis of the OB in which the statistical Mann-Whitney difference found previously in Figure 2B is not due to chance. Significance cutoff was set at  $p < 0.05$ . \*:  $p < 0.05$ . \*\*:  $p < 0.01$  \*\*\*:  $p < 0.001$ .

**ÉTUDE 3 :** "Identification de nouveaux paramètres comportementaux pour évaluer la valeur hédonique des odorants chez l'Homme." (en préparation)

## **Identification of new behavioral parameters to assess hedonic value of odorants in humans**

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### **Acknowledgements**

This work was supported by the CNRS, Inserm, Lyon 1 University, Lyon 1 University doctoral fellowship (L.C).



## **Abstract**

As you smell an odorant, your first reaction will certainly be either I like or I dislike this smell. This primary reaction is a reflection of what is called the hedonic value of the odor. Very often, this hedonic value dominates the olfactory percept before that of olfactory identification or intensity. This component of olfactory perception is of primary importance for guiding behavior, allowing avoiding danger (smell of smoke, gas...), consuming food or seduction. Olfactory hedonics can be assessed using a large number of methods in human including psychophysical measures, autonomic responses, measurement of facial expressions or peripheral nervous activity. All of these technics present limitations going from subjective assessment, invasiveness to expertise requirement. The olfactory system is closely linked to the reward system which role is to mediate motivated behavior. In this context, we propose to use the property of odorants to recruit the reward system and thus induce motivated behavior to identify new behavioral parameters to assess odor hedonic value in humans. We here recorded freely moving human subjects exploring odors emanating from flasks and revealed that five parameters linked to motivated behavior (the approach and withdrawal speeds of the flask containing the odorant to the nose, the distance between the flask and the nose, the number of sniffs and distance of withdrawal corresponding to the maximal distance between the nose and the flask after odor sniffing) are closely linked to odor hedonics. We thus highlighted new, non-verbal and non-invasive parameters to evaluate olfactory hedonics in humans based on the assessment of odor motivated behavior.

## **Keywords**

Odor hedonics, human olfaction, behavioral response, preference, reward system

## **Introduction**

Olfactory perception includes different dimensions such as identity (what do I smell?), familiarity (do I know that smell?), intensity (Is the smell strong or weak?) and hedonics (Is the smell pleasant or not?). Among all these dimensions, odor hedonics appears to be the most important in Humans. Indeed, it is the primary odor feature spontaneously used by subjects to perform olfactory discrimination tasks (Schiffman 1974), categorize odorants into groups (Berglund et al. 1973; Schiffman, Robinson, et Erickson 1977) and to verbally describe odorants (Khan et al. 2007; Moskowitz et Barbe 1977; Zarzo 2008). Odor hedonics possess major implications in a large array of everyday life as food intake, social communication, increasing awareness of environmental hazards and experiencing pleasure (Stevenson 2010; Walliczek-Dworschak et Hummel 2017) .

Emotional responses to odorants are expressed at different levels (e.g. verbalization of emotional feelings, changes in physiological state or motor behavior) and thus hedonic perception can be assessed by a large number of methods depending on the explored level. The most direct approach use psychophysical measures with scales of measurements or verbal responses (Stevens 1958; Poncelet et al. 2010). The limitation of these techniques is that it requires active participation of the subjects and thus hedonic evaluation depends on the subjectivity of the participant. Autonomic responses that are known to be modulated by odor hedonics can also be used. This is the case of electrodermal response (Brauchli et al. 1995), heart rate activity (Alaoui-Ismaïli, Vernet-Maury, et al. 1997; Alaoui-Ismaïli, Robin, et al. 1997; Bensafi, Rouby, Farget, Bertrand, et al. 2002) and the motor startle reflex (Ehrlichman et al. 1995; Ehrlichman et al. 1997; Miltner et al. 1994). In addition, the motor sniff response can also be assessed as a reflect of odor hedonics (Bensafi et al. 2003; Bensafi, Sobel, et Khan 2007; Ferdenzi et al. 2015). These responses have the advantage to be more objective since they are not based on verbal reports from of the participant. However, they are more invasive in that various types of sensors are placed on the subject's body and/or face. This may prevent the volunteer from expressing spontaneous behavior in response to odorants. The measurement of facial expressions can also be used to assess olfactory preferences. It includes "mouthing" behavior (Soussignan et al. 1997; Mennella et Beauchamp 1998; Delaunay-El Allam et al. 2010), slight smile, and expressions of disgust (Soussignan et al. 1997; Schaal, Marlier, et Soussignan 2000; Li, Jia, et Wang 2020). These experiments are however dependent on the expertise of the experimenter to recognize and categorize facial expressions. Finally, recent software are able to measure peripheral vasomotor activity to assess emotional response to a stimulus but results

are not completely reliable and limitations of head movements is a constraint (Bousefsaf, Maaoui, et Pruski 2016).

Olfactory emotional responses represent a valuable source of information for decision-making and guiding motivated behaviors leading to attraction or aversion in response to an odorant source. This particularity can be explained, at least partly, by the anatomo-functional organization of the olfactory system and its close connections to the reward system (Wesson et Wilson 2011; Ikemoto 2007; Xiong et Wesson 2016). Indeed, the olfactory bulb, the first cortical relay of the olfactory information which carries the neural signature of odor hedonics (Kermen et al. 2011) projects directly on the olfactory tubercle (Wesson et Wilson 2011; Xiong et Wesson 2016; Price 1973). This last structure is part of the ventral striatum (with the accumbens nucleus) and the target of dopaminergic projections from the ventral tegmental area constituting the key structures of the motivated behaviors (Ikemoto 2007; Ikemoto 2003; Fitzgerald, Richardson, et Wesson 2014; Kornetsky, Huston-Lyons, et Porrino 1991). In this context, we propose to use the property of odorants to recruit the reward system and thus induce motivated behavior to identify new behavioral parameters to assess odor hedonic value of odorants in humans. The aim of the study is thus to investigate new, non-verbal and non-invasive parameters to evaluate olfactory hedonics in humans based on the assessment of odor motivated behavior.

## **Material and Methods**

### *Participants*

The data were collected and treated according to the ethical guidelines of the Declaration of Helsinki and approved by the French ethics committee (#CPP- IdF-IV). Twenty-four participants (12 women and 12 men) between the ages of 18 and 28 years (mean  $\pm$  SD, 21  $\pm$  2.1 years) were tested in March 2018. All were recruited from the Lyon area in France. Their olfactory performances were verified using the European Test of Olfactory Capabilities (Joussain et al. 2016). Participants received financial compensation for the experiment and provided written informed consent prior to participation.

### *Perceptual rating and statistical analysis*

23 monomeric odorants (Table 1) were selected to cover as much as possible the olfactory perceptual space (edible and non-edible odors, body-odors, odors of flowers, decay odors...).

The odorants were diluted to reach similar vapor pressure (1 Pa, diluted in mineral oil) on a polypropylene piece and were presented in a 15mL flask (opening diameter, 1.7 cm; height, 5.8 cm; filled with five ml of liquid; (Licon et al. 2018) (Table 1). Participants were allowed to freely smell each odorant during 1 minute. The order of odorant presentation was randomized between participants. During the odorant exploration, participants had to rate each odorant in term of liking (“Is the smell pleasant?”), wanting (“Do you want to smell the smell again?”), intensity (“Is the smell intense?”), familiarity (“Is the smell familiar?”) and irritation (“Is the smell irritant?”). Participants evaluated each odorant on Pie-Pie software (Licon et al. 2018), which allows to rate on a continuous scale (from 0 to 1). Using this software, all perceptual criterions, presented on a pie, were evaluated at the same time for a given odorant. Participants could not switch to the next odorant before the end of the trial (duration: 1 min).

All statistical analyses were performed using Statistica with a risk  $\alpha$  set at 5%. For all experiments, we verified normality (Kolmogorov-Smirnov) and variance (Levene Test) and parametric or non-parametric tests were performed according these criteria. For perceptual rating analyses (explicit assessments), we performed Friedman tests for odorant comparisons and Spearman test for correlations. Odorant groups were constituted based on principal component analysis (PCA) performed on odorant ratings.

#### *Assessment and analysis of odor motivated behaviors*

During the odorant exploration and rating phase, participants were video recorded with a webcam (Logitech C920) positioned on the side of the participant. Video measures were conducted with Volcan software developed under Labview (National Instrument) (Richard et al. 2017; Hegoburu et al. 2009). Each recording was stored in AVI file format with jpeg codec. Offline, the video recordings can be replayed using Volcan (A2V module) software and video track analyses were performed. Using this procedure, human’s behavior was encoded throughout each session and each trial in a file allowing measurement of speed, distance, locations of the flask containing the odorant and the nose. More specifically, we measured approach and withdrawal speeds of the flask containing the odorant to the nose, the distance between the flask and the nose (called sniff distance), the number of sniffs and distance of withdrawal corresponding to the maximal distance between the nose and the flask after odor sniffing (Figure 1). We compared these independent behavioral parameters in response to high pleasant versus low pleasant odorants or in response to high intense versus low intense using

each time two-tailed paired T-tests. Finally, we performed Person correlations between PC1 and the difference behavioral parameters.

## Results

### Explicit assessments and relationships between ratings

The use of motivational behavior to assess the hedonic value of odorants requires first to confirm that pleasant odorants are also motivating. One simple way to assess this motivational component is to directly ask to the participants whether they want to smell it again (Triscoli et al. 2014) (Figure 2A). Analysis of verbal reports first showed that both liking (Odorant effect Friedman  $F_{24,22}= 263.542$ ,  $p<0.001$ ; Figure 2Bi) and wanting ratings (Odorant effect Friedman  $F_{24,22}= 230.823$ ,  $p<0.001$ ; Figure 2Bii) varied among the odorants. Importantly, a significant correlation between these two ratings was observed: the more the odorant is liked, the more it is wanted (Spearman  $r=0.979$ ,  $p<0.0001$ ; Figure 2Biii).

We then explored other perceptual dimensions such as intensity (Odorant effect Friedman  $F_{24,22}= 213.513$ ,  $p<0.001$ ; Figure 2C), familiarity (Odorant effect Friedman  $F_{24,22}= 156.008$ ,  $p<0.001$ ; Figure 2D) and irritation (Odorant effect Friedman  $F_{24,22}= 217.639$ ,  $p<0.001$ ; Figure 2E) and showed that all these olfactory parameters varies among odorants.

Third, we considered human olfactory perception in its complexity by applying a Principal Component Analyses (PCA) on the 23 olfactory stimulations and showed that liking and wanting dimensions are very closely represented in the perceptual space (Figure 3A). As can be seen, the first Principal Component (PC) accounts for 71.4% of the total variance and underlies liking and wanting on the hand, and irritation dimension on the other hand (Figure 3A). The second PC accounts for 24.99% of the total variance and underlie to a less extent intensity and familiarity dimensions (Figure 3A).

We then built an odor space formed by the first two PCs in which the 23 odorants were projected (Figure 3B). Within this odor space, odorants that are understood to be close on the X axis are also found to be close in term of hedonics and reinforcing properties (Figure 3B).

In sum, we showed here that odorants that are perceived as pleasant are also described as wanted. This suggests that pleasant odorants possess rewarding properties involved in guiding motivated behavior.

We next analyzed the impact of odorants that are the most liked/wanted (high pleasant odorants) and the less liked/wanted (low pleasant odorants) on different parameters of the motivated behavior. This will allow determining if odorants with different hedonic values impact differently motivated behavior.

### Odor motivated behavior

We first selected odorants that are the closest on PC2 (to control for intensity and/or familiarity effects on behavior), but that are the most distant on PC1 (the most different in term of hedonic and reinforcing values). It appears that ISO, CAR and GER were the most “liked/wanted odorants” (“high pleasant odorants”, see in the right side of PC1 axis, [Figure 3B](#)), and BUT, THIO, CRE the most “unliked/unwanted odorants” (“low pleasant odorants”, left side of PC1 axis, [Figure 3B](#)). In the odorant rating experiment, the participants had 1 min to freely smell each odorant of the panel. During this time, their movements were video tracked (this technique avoids the use of devices that could affect movements; [Figure 2A](#)). Using Volcan software, we analyzed five independent steps of the kinetic of exploration of the 6 selected odorants: the approach and withdrawal speed of odor source, the number of sniffs, the closest distance between the flask containing the odorant and the nose (sniff distance) and the largest distance between the odor source and the nose after odor sampling (withdrawal distance). We found that participants exhibited different motor behaviors depending on the odorant group (high versus low pleasantness) (Interaction between odorant group and motor parameters  $F_{(4,115)}=5.7$   $p<0.001$ ). Participants approached faster ([Figure 4Ai](#), Paired T-Test  $p=0.027$ ) and closer to their nose ([Figure 4Aii](#), Paired T-Test  $p=0.013$ ) the odor source for high compared to low pleasant odorant group. They smelled the odor source more time ([Figure 4Aiii](#), Wilcoxon  $p=0.003$ ), and removed it slower ([Figure 4Aiv](#), Paired T-Test  $p=0.035$ ) for high pleasant compared to low pleasant odor group. Finally, the odor flask was brought at a smaller distance from the nose after sniffing for high pleasant compared to low pleasant odorants ([Figure 4Av](#), Paired T-Test  $p=0.015$ ). Using permutation tests (100 000 permutations), we found that the probability to have the five motor parameters significantly different between two odorant groups (constituted of three odorants each) is of  $p<0.00002$ .

To confirm that these results can be applied to the whole range of odorants, and not only to the extreme odorants, we performed correlations between PC1 including the 23 odorants and the five individual motor parameters. Whereas no correlation between the speed of approach and

PC1 coordinates (Figure 4Bi, Pearson correlation  $R=0.240$   $p=0.269$ ) were found, the sniff distance (Figure 4Bii, Pearson correlation  $R=-0.478$   $p=0.021$ ), the number of sniffs (Figure 4Biii, Pearson correlation  $R=0.627$   $p=0.001$ ), the speed of withdrawal (Figure 4Biv, Pearson correlation  $R=-0.510$   $p=0.013$ ) and the withdrawal distance (Figure 4Bv, Pearson correlation  $R=-0.490$   $p=0.018$ ) were significantly correlated to PC1 coordinates. These results revealed that high pleasant and low pleasant odorants elicit different motivated behaviors (Figure 4C) that can be implicitly assessed, without external intervention or invasive method.

To further confirm that motivated behavior reflects odor pleasantness and no other olfactory dimensions such as intensity or familiarity, we selected odorants that are the most distant on PC2 (the most different in term of intensity and/or familiarity), but that are the closest on PC1 (to control hedonic and reinforcing values effects on behavior). It appears that EUG, CIS3 and CITRA were the most “intense/familiar” (“high intensity odorants”, see on the top part of PC2 axis, Figure 3B), and PRO, LIM and TRANS, a group of three “unfamiliar/low intense odorants” (“low intensity odorants”, bottom part of PC2 axis, Figure 3B). Again, using Volcan software, we analyzed the same five independent steps of the kinetic of exploration of the 6 new selected odorants and found that participants exhibited similar behaviors between the two odor groups for all parameters (Figure 5, Interaction between odor group and motor parameters  $F_{(4,115)}=0.208$   $p=0.934$ ; Two-Tailed paired T-Test, Vmax approach  $p=0.872$ , sniff distance  $p=0.177$ , number of sniffs  $p=0.080$ , speed of withdrawal  $p=0.225$ , withdrawal distance  $p=0.189$ ).

All together, these results showed that the approach and withdrawal speed of odor source, the number of sniffs, the closest distance between the flask containing the odorant and the nose and the largest distance between the odor source and the nose after odor sampling depend on the odor hedonic value. In other words, it strongly suggests that motivated behavior reflect the odor hedonic value and can thus be used as an objective and non-invasive measure of odor hedonics.

## Discussion

In the present study, we sought to identify new measurements of odor hedonics based on motivated-approach behavior. Odor hedonics is the most salient aspect of olfactory perception. In affective neurosciences, the hedonic value of a stimulus and its induced motivation have been dissociated based on their neural bases (Berridge, Robinson, et Aldridge 2009). We found here in accordance with Berridge’s observations with food, that odorants liking and wanting



ratings are positively correlated: the more the odorant is liked, the more it is wanted. These findings highlight the close link between affective response and the motivation to smell the odorants and encouraged us to use the odor-induced motivated behavior to assess odor hedonics.

On a behavioral point of view, odor hedonics is a major source of information about environment and triggers approach or avoidance. Here, we revealed that participants approached pleasant odorants faster, closer to their nose, smelled it more time and removed it slower and less far than unpleasant ones. These data opens new possibility to measure odor-driven behaviors in freely moving humans. Such approach represents a real added value to the paradigms that are currently used and that suffer from some limitations such as invasiveness (assessment of skin conductance, heart rate, sniffing...), subjectivity (scales of measurements or verbal responses) or the requirement of high expertise (facial expressions).

The difference of motor behavior induced by pleasant and unpleasant odorants could allow adaptive behavior (Scherer, Ekman, and Schorr 1994; Keltner and Gross 1999). In animals, pleasant odorants can signal pleasant stimuli such as palatable food or sexual behavior. In this case, it seems relevant to quickly approach food before a congener eats it or to quickly approach sex-opposite congener to increase the likelihood of mating. Interestingly, in our procedure, while speed of approach was different between pleasant and unpleasant odorants, it did not correlate with olfactory perception (PC1). This can be explained by the fact that the odorant cannot be sampled before being close enough to the nose and that some participants may slowed down their movement before odor detection. However, when odor intensity is sufficiently high to allow detection far enough, speed of approach has been successfully used in rodents to assess odor hedonics (Kermen et al. 2016). Note that during the entire trial, the subject is allowed to approach the flask to its nose as many times as he/she wants. We measured the number of time that he/she did it (number of sniffs) and found that it was greater for pleasant odorants which is in line with previous studies showing that sniff volume and duration are larger/longer for appetitive smells (Warren et al. 1994; Bensafi et al. 2003; Bensafi, Sobel, and Khan 2007; Johnson et al. 2006). Beside the number of sniffs, we identified other novel motor parameters: the withdrawal speed, the distance between the flask and the nose and the distance of withdrawal corresponding to the maximal distance between the nose and the flask after odor sniffing. These motor behaviors are highly correlated with hedonics and they occur after sampling. They reflect the need of getting far away and faster from aversive odorant sources, or staying longer and closer when the source is pleasant.

In sum, the present methodological contribution suggests that measurements of motivated behaviors can be used to assess human odor hedonics. This could be achieved with a simple webcam placed next to the participant in order to automatically evaluate the speeds and trajectories of the odor flask and the participant's nose. In these conditions, participant's motor behavior is not constrained by any sensors. A singularity of our approach concerns the way in which participants are stimulated. Indeed, whereas most previous studies in the field use a stimulation mode managed by an automaton (olfactometer) or an external person (experimenter), our method allows a measurement of the emotional response to odorants in more ecological or naturalistic conditions since the subject stimulates himself. Thus, the collected response is closer to real conditions, much less influenced by the experimental setting. Another advantage is the cost of the experiment requiring a standard webcam. One limitation can come from the necessity of the subject to stay in the camera range although this can be solved by the use of a more powerful camera system that would allow following flasks and participant's nose trajectories in 3D. Finally, as a perspective, one may consider the use of these measures in populations with language alterations or when the use of sensors is difficult to implement.

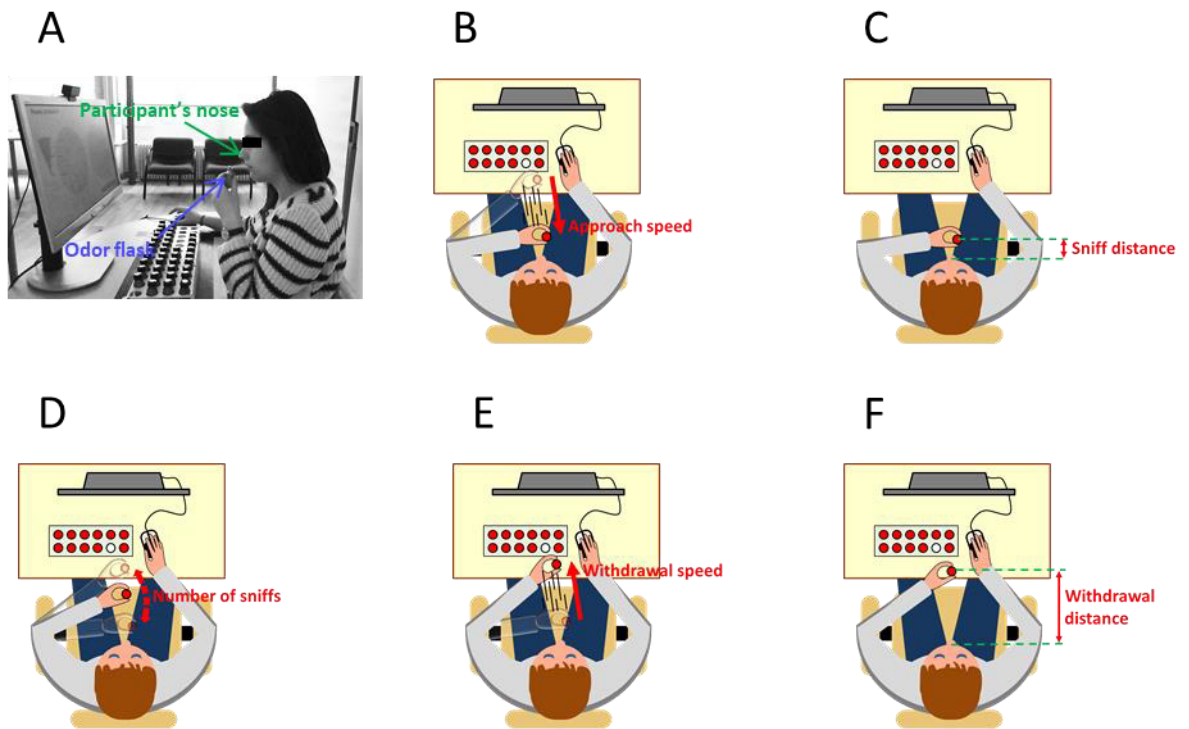
## References

- Alaoui-Ismaïli, O., O. Robin, H. Rada, A. Dittmar, et E. Vernet-Maury. 1997. « Basic Emotions Evoked by Odorants: Comparison between Autonomic Responses and Self-Evaluation ». *Physiology & Behavior* 62 (4): 713-20. [https://doi.org/10.1016/s0031-9384\(97\)90016-0](https://doi.org/10.1016/s0031-9384(97)90016-0).
- Alaoui-Ismaïli, O., E. Vernet-Maury, A. Dittmar, G. Delhomme, et J. Chanel. 1997. « Odor Hedonics: Connection with Emotional Response Estimated by Autonomic Parameters ». *Chemical Senses* 22 (3): 237-48. <https://doi.org/10.1093/chemse/22.3.237>.
- Bensafi, Jessica Porter, Sandra Pouliot, Joel Mainland, Bradley Johnson, Christina Zelano, Natasha Young, et al. 2003. « Olfactomotor Activity during Imagery Mimics That during Perception ». *Nature Neuroscience* 6 (11): 1142-44. <https://doi.org/10.1038/nn1145>.
- Bensafi, C. Rouby, V. Farget, B. Bertrand, M. Vigouroux, et A. Holley. 2002. « Autonomic Nervous System Responses to Odours: The Role of Pleasantness and Arousal ». *Chemical Senses* 27 (8): 703-9. <https://doi.org/10.1093/chemse/27.8.703>.
- Bensafi, Noam Sobel, et Rehan M. Khan. 2007. « Hedonic-Specific Activity in Piriform Cortex during Odor Imagery Mimics That during Odor Perception ». *Journal of Neurophysiology* 98 (6): 3254-62. <https://doi.org/10.1152/jn.00349.2007>.
- Berglund, B., U. Berglund, T. Engen, et G. Ekman. 1973. « Multidimensional Analysis of Twenty-One Odors ». *Scandinavian Journal of Psychology* 14 (2): 131-37. <https://doi.org/10.1111/j.1467-9450.1973.tb00104.x>.

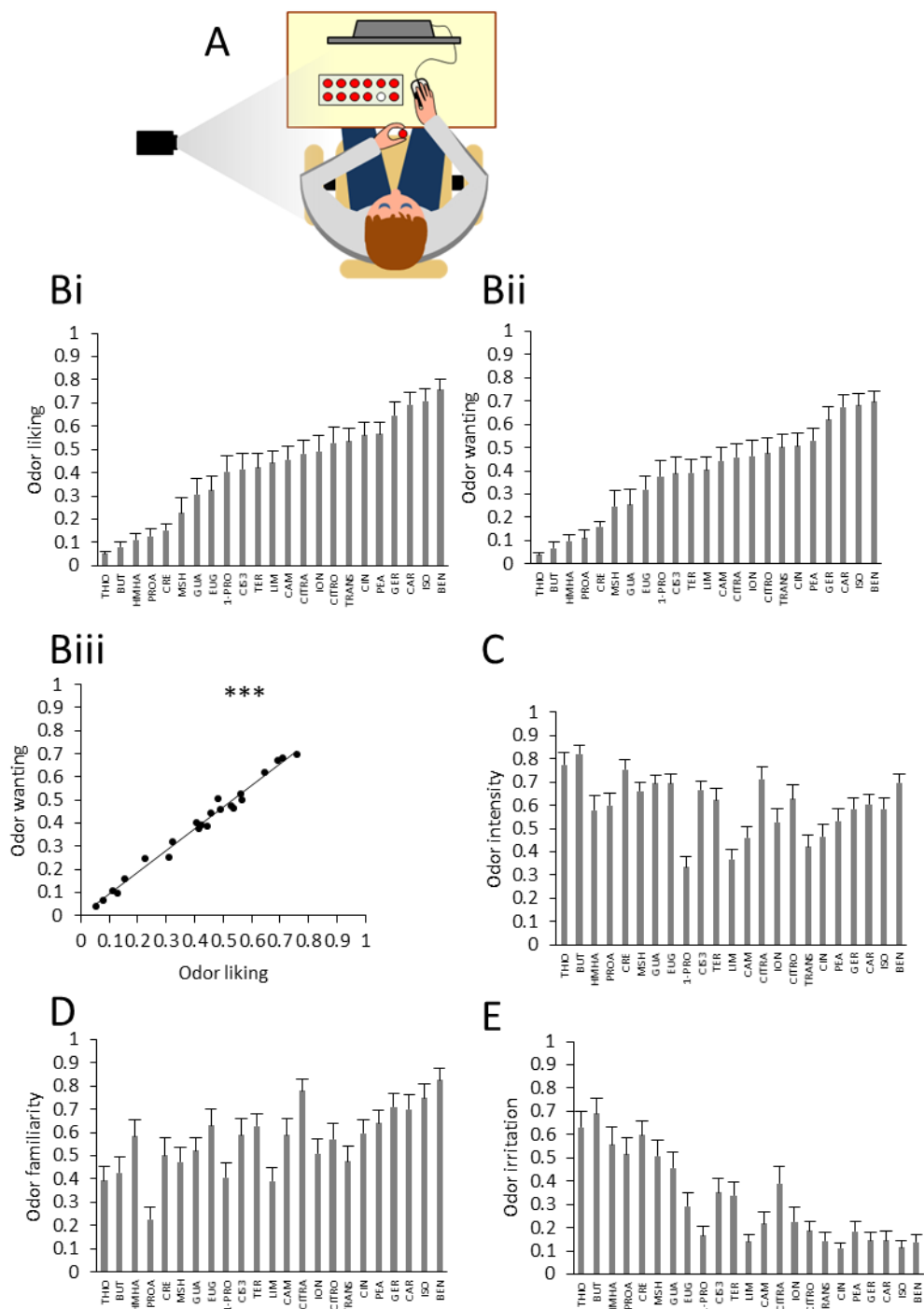
- Berridge, Terry E. Robinson, et J. Wayne Aldridge. 2009. « Dissecting Components of Reward: “Liking”, “Wanting”, and Learning ». *Current Opinion in Pharmacology* 9 (1): 65-73. <https://doi.org/10.1016/j.coph.2008.12.014>.
- Bousefsaf, F., C. Maaoui, et A. Pruski. 2016. « Peripheral Vasomotor Activity Assessment Using a Continuous Wavelet Analysis on Webcam Photoplethysmographic Signals ». *Bio-Medical Materials and Engineering* 27 (5): 527-38. <https://doi.org/10.3233/BME-161606>.
- Brauchli, P., P. B. Rüegg, F. Etzweiler, et H. Zeier. 1995. « Electrocortical and Autonomic Alteration by Administration of a Pleasant and an Unpleasant Odor ». *Chemical Senses* 20 (5): 505-15. <https://doi.org/10.1093/chemse/20.5.505>.
- Delaunay-El Allam, Maryse, Robert Soussignan, Bruno Patris, Luc Marlier, et Benoist Schaal. 2010. « Long-Lasting Memory for an Odor Acquired at the Mother’s Breast ». *Developmental Science* 13 (6): 849-63. <https://doi.org/10.1111/j.1467-7687.2009.00941.x>.
- Ehrlichman, H., S. Brown, J. Zhu, et S. Warrenburg. 1995. « Startle Reflex Modulation during Exposure to Pleasant and Unpleasant Odors ». *Psychophysiology* 32 (2): 150-54. <https://doi.org/10.1111/j.1469-8986.1995.tb03306.x>.
- Ehrlichman, H., S. B. Kuhl, J. Zhu, et S. Warrenburg. 1997. « Startle Reflex Modulation by Pleasant and Unpleasant Odors in a Between-Subjects Design ». *Psychophysiology* 34 (6): 726-29. <https://doi.org/10.1111/j.1469-8986.1997.tb02149.x>.
- Ferdenzi, Camille, Arnaud Fournel, Marc Thévenet, Géraldine Coppin, et Moustafa Bensafi. 2015. « Viewing Olfactory Affective Responses Through the Sniff Prism: Effect of Perceptual Dimensions and Age on Olfactomotor Responses to Odors ». *Frontiers in Psychology* 6: 1776. <https://doi.org/10.3389/fpsyg.2015.01776>.
- Fitzgerald, Brynn J., Kara Richardson, et Daniel W. Wesson. 2014. « Olfactory Tubercle Stimulation Alters Odor Preference Behavior and Recruits Forebrain Reward and Motivational Centers ». *Frontiers in Behavioral Neuroscience* 8: 81. <https://doi.org/10.3389/fnbeh.2014.00081>.
- Hegoburu, Chloé, Yannick Sevelinges, Marc Thévenet, Rémi Gervais, Sandrine Parrot, et Anne-Marie Mouly. 2009. « Differential Dynamics of Amino Acid Release in the Amygdala and Olfactory Cortex during Odor Fear Acquisition as Revealed with Simultaneous High Temporal Resolution Microdialysis ». *Learning & Memory (Cold Spring Harbor, N.Y.)* 16 (11): 687-97. <https://doi.org/10.1101/lm.1584209>.
- Ikemoto, Satoshi. 2003. « Involvement of the Olfactory Tubercle in Cocaine Reward: Intracranial Self-Administration Studies ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 23 (28): 9305-11.
- Ikemoto, Satoshi. 2007. « Dopamine Reward Circuitry: Two Projection Systems from the Ventral Midbrain to the Nucleus Accumbens-Olfactory Tubercle Complex ». *Brain Research Reviews* 56 (1): 27-78. <https://doi.org/10.1016/j.brainresrev.2007.05.004>.
- Joussain, P., M. Bessy, F. Faure, D. Bellil, B. N. Landis, M. Hugentobler, H. Tuorila, et al. 2016. « Application of the European Test of Olfactory Capabilities in Patients with Olfactory Impairment ». *European Archives of Oto-Rhino-Laryngology: Official Journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS): Affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery* 273 (2): 381-90. <https://doi.org/10.1007/s00405-015-3536-6>.
- Kermen, A. Chakirian, C. Sezille, P. Joussain, G. Le Goff, A. Ziessel, M. Chastrette, et al. 2011. « Molecular Complexity Determines the Number of Olfactory Notes and the Pleasantness of Smells ». *Scientific Reports* 1: 206. <https://doi.org/10.1038/srep00206>.
- Kermen, Maëllie Midroit, Nicola Kuczewski, Jérémy Forest, Marc Thévenet, Joëlle Sacquet, Claire Benetollo, Marion Richard, Anne Didier, et Nathalie Mandairon. 2016.

- « Topographical Representation of Odor Hedonics in the Olfactory Bulb ». *Nature Neuroscience* 19 (7): 876-78. <https://doi.org/10.1038/nn.4317>.
- Khan, Rehan M., Chung-Hay Luk, Adeen Flinker, Amit Aggarwal, Hadas Lapid, Rafi Haddad, et Noam Sobel. 2007. « Predicting Odor Pleasantness from Odorant Structure: Pleasantness as a Reflection of the Physical World ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 27 (37): 10015-23. <https://doi.org/10.1523/JNEUROSCI.1158-07.2007>.
- Kornetsky, C., D. Huston-Lyons, et L. J. Porrino. 1991. « The Role of the Olfactory Tubercle in the Effects of Cocaine, Morphine and Brain-Stimulation Reward ». *Brain Research* 541 (1): 75-81. [https://doi.org/10.1016/0006-8993\(91\)91076-d](https://doi.org/10.1016/0006-8993(91)91076-d).
- Li, Jiafeng Jia, et Xiaochun Wang. 2020. « Unpleasant Food Odors Modulate the Processing of Facial Expressions: An Event-Related Potential Study ». *Frontiers in Neuroscience* 14: 686. <https://doi.org/10.3389/fnins.2020.00686>.
- Licon, C. C., C. Manesse, M. Dantec, A. Fournel, et M. Bensafi. 2018. « Pleasantness and Trigeminal Sensations as Salient Dimensions in Organizing the Semantic and Physiological Spaces of Odors ». *Scientific Reports* 8 (1): 8444. <https://doi.org/10.1038/s41598-018-26510-5>.
- Mennella, J. A., et G. K. Beauchamp. 1998. « Smoking and the Flavor of Breast Milk ». *The New England Journal of Medicine* 339 (21): 1559-60. <https://doi.org/10.1056/NEJM199811193392119>.
- Miltner, W., M. Matjak, C. Braun, H. Diekmann, et S. Brody. 1994. « Emotional Qualities of Odors and Their Influence on the Startle Reflex in Humans ». *Psychophysiology* 31 (1): 107-10. <https://doi.org/10.1111/j.1469-8986.1994.tb01030.x>.
- Moskowitz, H. R., et C. D. Barbe. 1977. « Profiling of Odor Components and Their Mixtures ». *Sensory Processes* 1 (3): 212-26.
- Poncelet, Johan, Fanny Rinck, Fanny Bourgeat, Benoist Schaal, Catherine Rouby, Moustafa Bensafi, et Thomas Hummel. 2010. « The Effect of Early Experience on Odor Perception in Humans: Psychological and Physiological Correlates ». *Behavioural Brain Research* 208 (2): 458-65. <https://doi.org/10.1016/j.bbr.2009.12.011>.
- Price, J. L. 1973. « An Autoradiographic Study of Complementary Laminar Patterns of Termination of Afferent Fibers to the Olfactory Cortex ». *The Journal of Comparative Neurology* 150 (1): 87-108. <https://doi.org/10.1002/cne.901500105>.
- Richard, N. Aguilera, M. Thévenet, O. Dkhissi-Benyahya, et F. Flamant. 2017. « Neuronal Expression of a Thyroid Hormone Receptor  $\alpha$  Mutation Alters Mouse Behaviour ». *Behavioural Brain Research* 321: 18-27. <https://doi.org/10.1016/j.bbr.2016.12.025>.
- Schaal, B., L. Marlier, et R. Soussignan. 2000. « Human Foetuses Learn Odours from Their Pregnant Mother's Diet ». *Chemical Senses* 25 (6): 729-37. <https://doi.org/10.1093/chemse/25.6.729>.
- Schiffman. 1974. « Contributions to the Physicochemical Dimensions of Odor: A Psychophysical Approach ». *Annals of the New York Academy of Sciences* 237 (0): 164-83. <https://doi.org/10.1111/j.1749-6632.1974.tb49852.x>.
- Schiffman, David E. Robinson, et Robert P. Erickson. 1977. « Multidimensional Scaling of Odorants: Examination of Psychological and Physicochemical Dimensions ». *Chemical Senses* 2 (3): 375-90. <https://doi.org/10.1093/chemse/2.3.375>.
- Soussignan, R., B. Schaal, L. Marlier, et T. Jiang. 1997. « Facial and Autonomic Responses to Biological and Artificial Olfactory Stimuli in Human Neonates: Re-Examining Early Hedonic Discrimination of Odors ». *Physiology & Behavior* 62 (4): 745-58. [https://doi.org/10.1016/s0031-9384\(97\)00187-x](https://doi.org/10.1016/s0031-9384(97)00187-x).
- Stevens. 1958. « Problems and Methods of Psychophysics ». *Psychological Bulletin* 55 (4): 177-96. <https://doi.org/10.1037/h0044251>.

- Stevenson, Richard J. 2010. « An Initial Evaluation of the Functions of Human Olfaction ». *Chemical Senses* 35 (1): 3-20. <https://doi.org/10.1093/chemse/bjp083>.
- Triscoli, Chantal, Ilona Croy, Håkan Olausson, et Uta Sailer. 2014. « Liking and Wanting Pleasant Odors: Different Effects of Repetitive Exposure in Men and Women ». *Frontiers in Psychology* 5: 526. <https://doi.org/10.3389/fpsyg.2014.00526>.
- Walliczek-Dworschak, Ute, et Thomas Hummel. 2017. « The Human Sense of Olfaction ». *Facial Plastic Surgery: FPS* 33 (4): 396-404. <https://doi.org/10.1055/s-0037-1603828>.
- Wesson, Daniel W., et Donald A. Wilson. 2011. « Sniffing out the Contributions of the Olfactory Tubercle to the Sense of Smell: Hedonics, Sensory Integration, and More? ». *Neuroscience and Biobehavioral Reviews* 35 (3): 655-68. <https://doi.org/10.1016/j.neubiorev.2010.08.004>.
- Xiong, Angeline, et Daniel W. Wesson. 2016. « Illustrated Review of the Ventral Striatum's Olfactory Tubercle ». *Chemical Senses* 41 (7): 549-55. <https://doi.org/10.1093/chemse/bjw069>.
- Zarzo, Manuel. 2008. « Psychologic Dimensions in the Perception of Everyday Odors: Pleasantness and Edibility ». *Journal of Sensory Studies* 23 (3): 354-76. <https://doi.org/10.1111/j.1745-459X.2008.00160.x>.



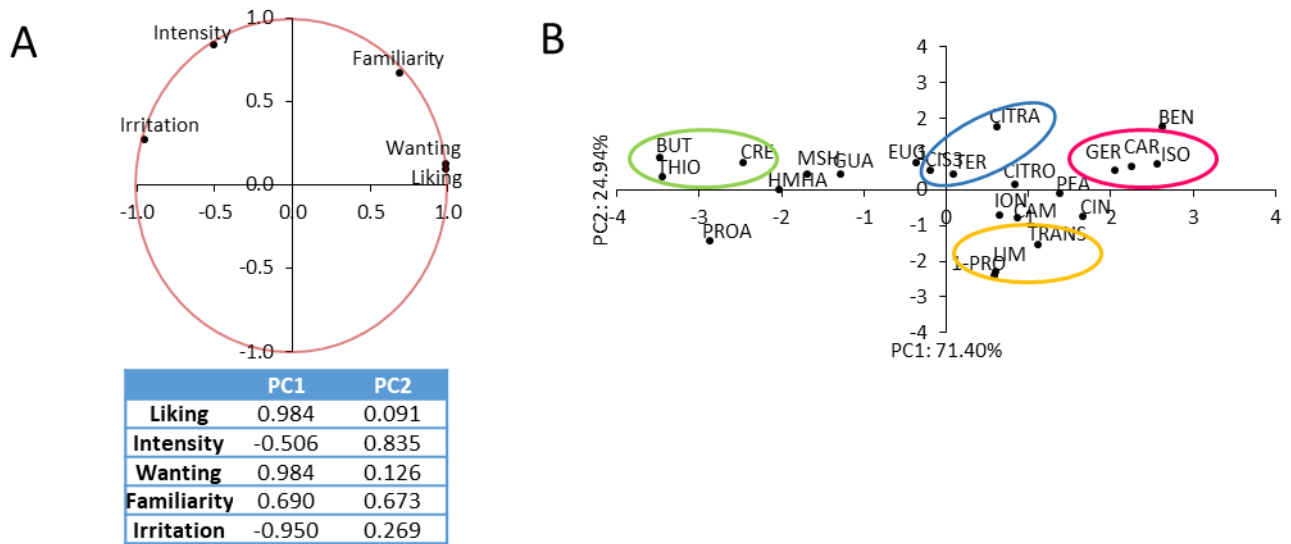
**Figure 1 : Motor parameters.** A. Example of a participant exploring odor flask. We measured the B. speed of approach of the flask containing the odorant to the nose C. the distance between the flask and the nose (called sniff distance), D. the number of sniffs, E. the withdrawal speed and F. the distance of withdrawal corresponding to the maximal distance between the nose and the flask after odor sniffing.



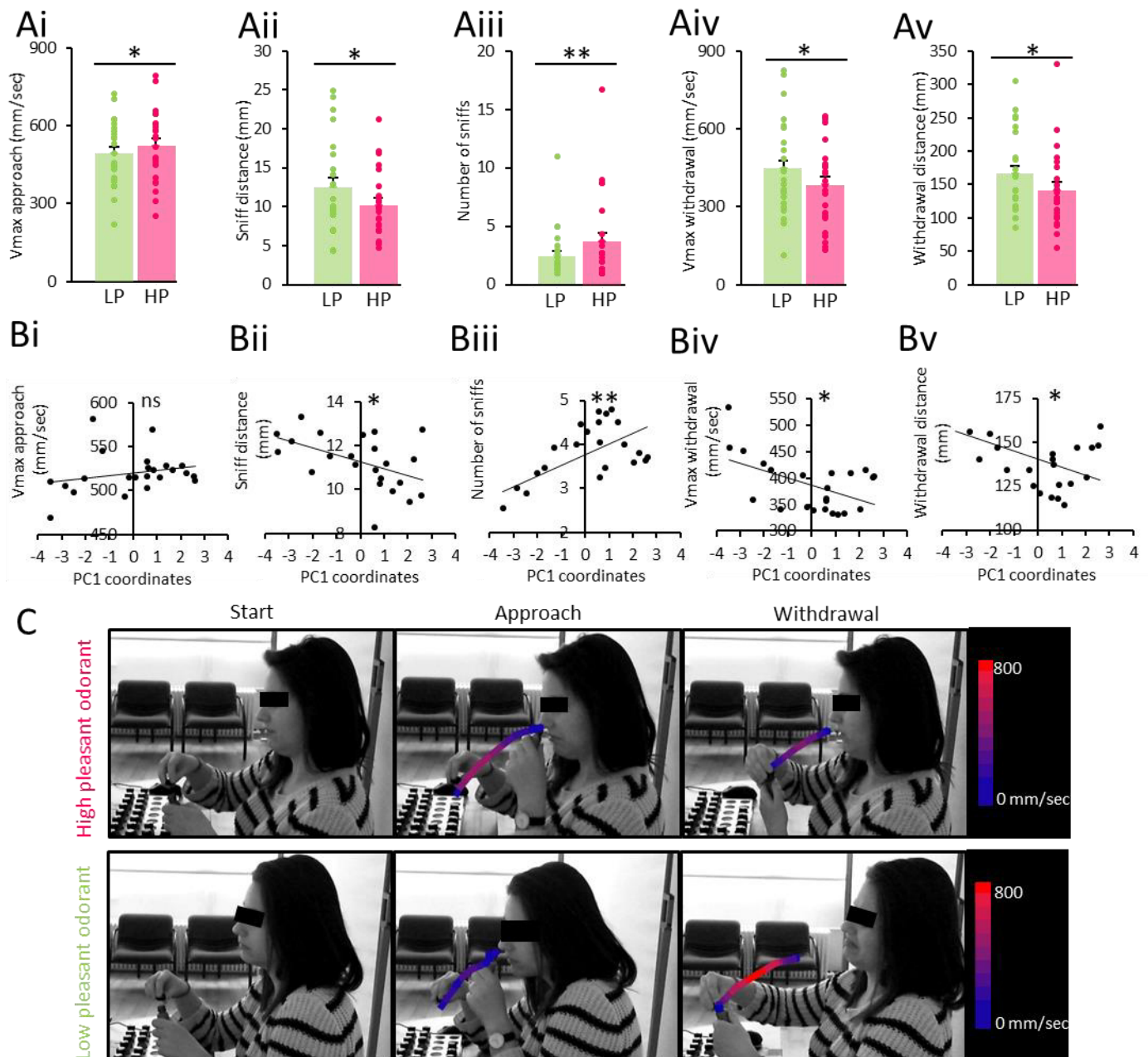
**Figure 2 : Subjective assessment of odorant perception by rating.** **A.** Subjects ( $n=24$ ) were instructed to rate the pleasantness, intensity, familiarity, irritation and desire to smell again of 23 odorants in one session. All odorant compounds were presented at the same vapor pressure and in random order during 1 min. **Bi.** Liking and **Bii.** Wanting were assessed for the 23 odorants (Friedman Odorant effect; Liking  $F_{(24,22)}=263.542$   $p<0.001$ , Wanting  $F_{(24,22)}=230.823$   $p<0.001$ ). **Biii.** There is a positive correlation between Liking and wanting (Spearman correlation  $r=0.979$ ,  $p<0.001$ ). **C.** Intensity, **D.** Familiarity and **E.** Irritation ratings varied among odorants (Intensity  $F_{(24,22)}=213.513$   $p<0.001$ , Familiarity  $F_{(24,22)}=156.008$   $p<0.001$ , Irritation  $F_{(24,22)}=217.639$   $p<0.001$ ).

BEN : Benzaldehyde, ISO : Isoamylacetate, CAR: L-Carvone, GER: Geraniol, PEA: 2-phenylethanol, CIN: Cineol, TRANS: Trans: trans-2-hexenylacetate, CITRO: Citronelol, ION:  $\beta$ -Ionone, CITRA: Citronelal, CAM: Camphor, LIM: R-Limonene, TER: (-)-Terpinen-4-ol, CIS3: Cis-3-hexenol, 1-Pro: 1-propanol, EUG: Eugenol, GUA: Guaïacol, MSH: 3-methyl-3-sulfanylhexan-1-ol, CRE: p-Cresol, PROA: Propanoic acid, HMHA: 3-hydroxy-3-methylhexanoic acid, BUT: Butanoic acid, Thio: Thioglycolic acid.

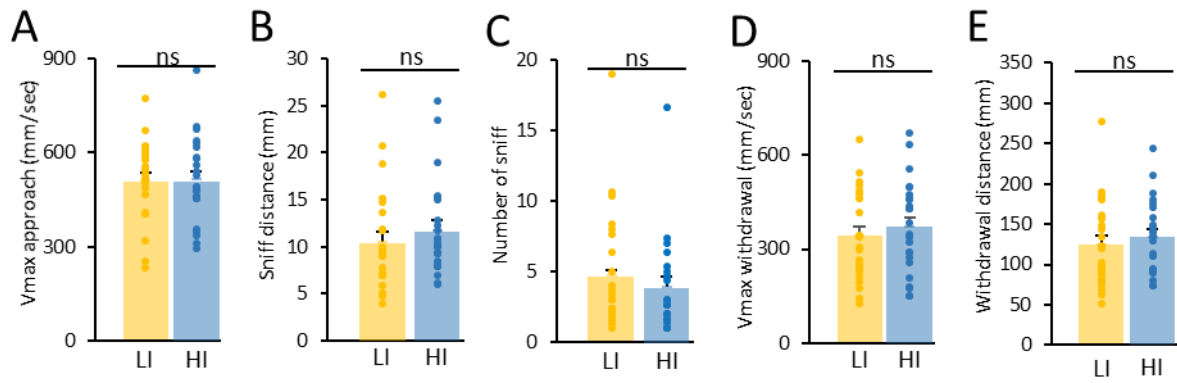




**Figure 3 : PCA on odorants rating. B.** The correlation coefficients between the two first principal components of the PCA (PC1 and PC2) and each of the five olfactory perceptual dimensions. **B.** The 23 odorants projected into a two-dimensional space made of the first and second PCs. For motor behavior analyses, we selected the 3 most extreme odorants on PC1 and closest on PC2 (pink: high pleasant; green: low pleasant) and the 3 most extreme odorants on PC2 and closest on PC1 (bleu: high intense; yellow: low intense).



**Figure 4 : Pleasant odors induce motor motivated behaviors. A.** We compared motor behavior between high pleasant (HP) and low pleasant (LP) odorant groups. **Ai.** Speed of approach toward subjects (n=24) is higher for high HP compared to LP odors (Two-Tailed paired T-Test,  $p=0.027$ ). **Aii.** The shorter distance between the nose and the odor source is shorter for HP compared to LP odors (Two-Tailed paired T-Test,  $p=0.013$ ). **Aiii.** HP odors are sniffed more time than LP ones (Two-Tailed paired T-Test,  $p=0.003$ ). **Aiv.** Speed withdrawal is slower for HP than LP odors (Two-Tailed paired T-Test,  $p=0.035$ ). **Av.** Withdrawal distance is shorter for HP than for LP odors (Two-Tailed paired T-Test,  $p=0.017$ ). **C.** Correlations between motor behavior and PC1 coordinates. **Bi.** There is no correlation between the speed of approach and PC1 coordinates (Pearson correlation,  $R=0.240$   $p=0.269$ ). **Bii.** The sniff distance (Pearson correlation,  $R=-0.478$   $p=0.021$ ), **Biii** the number of sniffs (Pearson correlation,  $R=0.627$   $p=0.001$ ), **Biv** the speed of withdrawal (Pearson correlation,  $R=-0.510$   $p=0.013$ ) and **Bv** the withdrawal distance (Pearson correlation,  $R=-0.490$   $p=0.018$ ) are correlated to PC1 coordinates. **C.** Example of odor exploration kinetic for HP and LP odors. Bars represent means of individual data points. \* $p<0.05$ , \*\* $p<0.01$ .



**Figure 5 : No effect of intensity on motor motivated behaviors.** We compared motor behavior between high intense (HI) and low intense (LI) odorant groups. There is no difference of **A.** speed of approach (Two-Tailed paired T-Test,  $p=0.872$ ), **B.** sniff distance (Two-Tailed paired T-Test,  $p=0.177$ ), **C.** number of sniff (Two-Tailed paired T-Test,  $p=0.080$ ), **D.** speed of withdrawal (Two-Tailed paired T-Test,  $p=0.225$ ) and **E.** withdrawal distance (Two-Tailed paired T-Test,  $p=0.189$ ) between HI compared to lower intense LI odorants..

Odorant name	Abbreviation	CID #	Dilution in mineral oil (vol/vol)
Thioglycolic acid	THIO	1133	1.55%
Butanoic acid	BUT	264	0.11%
3-hydroxy-3-methylhexanoic acid	HMHA	16666688	1%
Propanoic acid	PROA	1032	0.04%
p-cresol	CRE	2879	1.84%
3-methyl-3-sulfanylhexan-1-ol	MSH	10130039	1%
Guaiacol	GUA	460	2.09%
Eugenol	EUG	3314	13.44%
1-propanol	1-PRO	1031	0.007%
Cis3hexenol	CIS3	5281167	0.24%
(-)-terpinen-4-ol	TER	5325830	8.003%
d-limonene	LIM	440917	0.2%
(+)-camphor	CAM	159055	0.46%
Citronellal	CITRA	7794	1.42%
b ionone	ION	668014	7,27%
Citronellol	CITRO	8842	17.85%
Trans-2-hexenylacetate	TRANS	2733294	0,16%
Cineole	CIN	2758	0,17%
2-phenylethanol	PEA	6054	2,66%
Geraniol	GER	637566	11,29%
L-carvone	CAR	439570	1,93%
Isoamyl acetate	ISO	31276	0.03%
Benzaldehyde	BEN	240	1,54%

**Table 1.** Odorant names, abbreviations, CID and dilution for the experiment.

## **DISCUSSION**

La valeur hédonique, c'est-à-dire le caractère plus ou moins plaisant, est un paramètre primordial dans l'analyse des informations olfactives contenues dans notre environnement. Ce paramètre perceptif permet à l'individu d'exprimer une réponse comportementale appropriée en réponse à l'odeur sentie. Selon leurs valeurs hédoniques les odorants vont induire un comportement d'approche ou de retrait vis-à-vis de la source odorante.

L'objectif global de cette thèse était de déterminer les bases neurales de la valeur hédonique des odeurs et leurs altérations au cours du vieillissement normal puis de mettre à profit ces découvertes pour développer une nouvelle méthode de mesure de la valeur hédonique chez l'Homme. Comme cela a été décrit dans notre revue de la littérature scientifique, il a été précédemment montré que la valeur hédonique d'une odeur est codée dès le BO. Dans cette thèse, nous avons pu tout d'abord mettre en évidence que l'information hédonique codée au sein du BO est transférée au système de récompense, notamment au niveau du TO et de la VTA permettant ainsi d'induire un comportement motivé d'approche (étude 1). En effet, un comportement d'autostimulation intracérébrale est observé au sein du BO mettant ainsi en évidence la capacité de cette structure sensorielle à recruter le système de récompense. De plus, parmi toutes les structures olfactives secondaires, seul le TO montre une activation plus importante en réponse aux odorants attractifs comparé à des non attractifs. Ce phénomène est également observé pour la VTA. Le recrutement du système de récompense par les odorants attractifs semble avoir été conservé au cours de l'évolution puisque ces odorants possèdent également un pouvoir récompensant et induisent une activation du TO chez l'Homme. Nous avons ensuite mis en évidence qu'au cours du vieillissement la valeur hédonique des odorants plaisants est fortement altérée et que cette altération semble être sous-tendue par une modification de l'activité neurale du BO et du TO (étude 2). Dans cette même étude, nous avons observé chez la souris âgée un comportement d'autostimulation intracérébrale. Le TO directement, ou indirectement via les structures du système de récompense auxquelles il est interconnecté, semble compenser l'altération de l'activité neurale de la VTA observée afin de générer ce conditionnement opérant.

Enfin, nous avons mis à profit notre découverte de la capacité des odorants à recruter le système de récompense pour développer de nouvelles mesures de la valeur hédonique des odeurs (étude 3). Ainsi, nous avons décrit plusieurs paramètres moteurs liés à l'approche et au retrait de la source odorante par le sujet humain pouvant être utilisés comme mesure objective et non invasive de la valeur hédonique des odeurs.

# ***I. Une valeur hédonique de l'odeur innée mais pas immuable***

## **1- Existence d'une valeur hédonique innée des odorants**

La valeur hédonique est un paramètre dominant de la perception olfactive. Chez l'animal, le temps d'exploration de l'odeur est communément utilisé pour refléter la valeur hédonique de l'odeur (*Kobayakawa et al. 2007; Mandairon, Poncelet, et al. 2009; Devore, Lee, et Linster 2013; Kermen et al. 2016; Jagetia et al. 2018*). Cette mesure permet d'établir un continuum entre les odorants plaisants et déplaisants, en passant par les plus neutres. Bien que possédant une variabilité interindividuelle plus forte que pour des odeurs de prédateurs, de congénères du sexe opposé ou de nourriture (*Jagetia et al. 2018*), un tel continuum hédonique est observable pour des odorants n'ayant pas de signification éthologique particulière et sans apprentissage olfactif préalable (*Kermen et al. 2016*). Dans notre première étude, nous avons à nouveau constaté que le temps d'exploration d'odorants ne présentant pas de signification éthologique particulière pour l'animal varie en fonction des odorants, certains étant en effet beaucoup explorés par l'animal, et d'autre moins. Cette prédétermination de la valeur hédonique qui serait en partie portée par la structure de la molécule odorante (*Kermen et al. 2011; Khan et al. 2007*) aurait un intérêt évolutif majeur permettant aux odorants d'induire une réponse comportementale appropriée et universelle de la part des animaux. Or cette valeur hédonique n'est pas immuable, elle peut en effet être modifiée par un certain nombre de paramètres dont l'apprentissage, l'expérience ou le vieillissement. Dans ma thèse, je me suis intéressée à l'impact du vieillissement sur la perception hédoniques des odorants.

## **2- Modification de la valeur hédonique au cours du vieillissement**

Plusieurs études réalisées chez l'Homme ont constaté une altération du caractère hédonique des odeurs au cours du vieillissement normal (*Wang et al. 2005; Markovic et al. 2007; Jousain et al. 2013; Vieillard et al. 2020*). Nous avons ici révélé que le model murin présente lui aussi une altération de la valeur hédonique des odeurs liée à l'âge (étude 2). Nos résultats corroborent ceux obtenus par Jousain *et al.* (*Jousain et al. 2013*) en mettant en évidence une dépréciation spécifique des odorants plaisants, alors que le temps d'exploration des odorants déplaisants n'est pas impacté par le vieillissement. L'observation de résultats similaires entre l'Homme et le model murin permettent de valider le modèle murin pour l'étude des bases neurales de cette altération spécifique.



Il est largement admis que les déficits olfactifs liés à l'âge possèdent des effets néfastes sur le bien-être physique, la qualité de vie, la nutrition, le plaisir de manger et la sécurité des personnes âgées (*Doty et Kamath 2014; Attems, Walker, et Jellinger 2015; Lafreniere et Mann 2009*), allant jusqu'à augmenter de 36% le taux de mortalité chez des personnes ne présentant pourtant pas de démences (*Wilson, Yu, et Bennett 2011*). De façon intéressante, nous avons révélé chez la souris que tous les odorants plaisants ne voient pas leur valeur hédonique s'altérer au cours du vieillissement. C'est le cas du limonène qui possède une durée d'exploration identique chez les souris jeune et âgée (étude 2). Ce résultat est prometteur car il souligne une certaine variabilité au sein des odorants dans l'impact du vieillissement avec certains odorants toujours appréciés. Il serait intéressant de rechercher cette variabilité inter-odorants chez l'Homme (*Joussain et al. 2013*). Ainsi bien qu'une dépréciation générale des odorants plaisants soit observée au cours du vieillissement, ce phénomène n'est pas universel puisque certains odorants semblent conserver leur caractère plaisant. Ce phénomène de sélectivité dans l'altération du caractère hédonique des odorants pourrait être en partie expliqué par l'apparition de modifications d'ordre périphérique liées à l'âge. Il a en effet été mis en évidence chez l'Homme que certaines localisations de l'épithélium olfactif répondent préférentiellement aux odeurs plaisantes alors que d'autres répondent préférentiellement aux odeurs déplaisantes (*Lapid et al. 2011*). Or le taux d'expression de certains gènes des récepteurs olfactifs est modulé au cours du vieillissement (*Cavallin et al. 2010; Khan, Vaes, et Mombaerts 2013*). Alors qu'environ 30% des gènes sont sous-exprimés et 10% sont surexprimés chez l'animal âgé, 60% des gènes ne voient pas leur taux d'expression se modifier au cours du vieillissement (*Khan, Vaes, et Mombaerts 2013*). Il est important de noter que malgré l'altération du caractère plaisant de l'odorant, l'animal est toujours capable de le détecter (étude 2). Ainsi, il est possible de supposer que les modifications fonctionnelles des neurones sensoriels olfactifs puissent être à l'origine, du moins en partie, de l'altération de la valeur hédonique spécifique à certains odorants plaisants sans que cela n'impacte leur détection.

Ces résultats pourraient être utilisés pour mettre en place des stratégies afin d'améliorer les conditions de vie des personnes âgées. En effet, l'implémentation d'odorants toujours plaisants dans leur environnement, notamment au moment des repas, pourrait être envisagée afin d'améliorer leur plaisir olfactif et possiblement contrecarrer les effets néfastes du vieillissement sur leur statut nutritionnel. Ce type d'application a déjà fait ses preuves notamment chez des enfants atteints de troubles autistiques pour qui une forte néophobie

alimentaire est souvent observée (*Luisier et al. 2015; Luisier, Petitpierre, Béro, et al. 2019; Luisier, Petitpierre, Clerc Béro, et al. 2019*).

## ***II. Bases neurales de la valeur hédonique des odeurs dans le bulbe olfactif***

### **1- Représentation topographique de la valeur hédonique des odeurs selon l'axe antéro-postérieur du bulbe olfactif**

Le BO est la première structure corticale de traitement de l'information olfactive. Les colonnes d'activité présentes au sein du BO sont connues comme permettant un codage spatial de l'identité de l'odeur depuis l'épithélium olfactif jusqu'aux différentes couches du BO (*Guthrie et al. 1993; Willhite et al. 2006; Cummings et Belluscio 2008*). Or, les odorants semblent être codés spatialement dans le BO également en fonction de leur valeur hédonique. En effet, comme nous en avons fait la description dans notre revue de la littérature, les odeurs plaisantes possèdent des représentations topographiques différentes de celles des odeurs déplaisantes.

Un certain nombre d'études ont mis en évidence que les odorants ayant une valeur hédonique positive de par leur signification éthologique (e.g. urine de congénères du sexe opposé) activent préférentiellement la partie ventrale du BO alors que les odorants ayant une signification éthologique négative (e.g. odeur de prédateur) activent la partie dorsale (*Xu et al. 2005; Kang, Baum, et Cherry 2009; Kobayakawa et al. 2007; Cho et al. 2011; Saito et al. 2017*). En parallèle de ces travaux, il a récemment été montré que la valeur hédonique des odorants ne possédant pas de signification éthologique particulière est codée selon l'axe antéro-postérieur du BO (*Kermen et al. 2016*). Alors que les odorants plaisants activent la partie antérieure de la couche granulaire du BO, les odorants déplaisants activent quant à eux la partie postérieure. Cette étude a ainsi mis en évidence une nouvelle représentation topographique des odeurs selon leur valeur hédonique au sein du BO. Dans cette thèse, nous avons répliqué ces résultats chez la souris âgée (étude 2). En effet, nous avons constaté chez la souris âgée une augmentation de l'activité neurale dans la couche granulaire de la partie antérieure du BO en réponse aux odorants plaisants et dans la partie postérieure en réponse aux odorants déplaisants.

Ces résultats mettent ainsi en évidence que la représentation topographique de la valeur hédonique des odeurs selon l'axe antéro-postérieur du BO perdure au cours du vieillissement.

L'intégrité fonctionnelle de ces signatures neurales antérieures ou postérieures semble nécessaire au codage du caractère plus ou moins plaisant des odorants car leur manipulation optogénétique induit une modification de la valeur hédonique (*Kermen et al. 2016*). Dans cette thèse, nous avons montré qu'au cours du vieillissement, la modification physiologique de ces signatures neurales altère la valeur hédonique des odeurs. En effet, nous avons montré dans notre deuxième étude que la perte du caractère plaisant de l'odeur chez l'animal âgé est accompagnée d'une perte de l'activation neurale de la partie antérieure de la couche granulaire du BO. Ces résultats corroborent ceux obtenus par *Kermen et al. (Kermen et al. 2016)* en montrant de façon plus physiologique que la représentation topographique selon l'axe antéro-postérieur du BO est nécessaire au codage de la valeur hédonique des odeurs.

## 2- Modulation de la représentation topographique de la valeur hédonique par l'expérience ?

La valeur hédonique d'une odeur est modulable. Comme cela avait été mis en évidence chez l'Homme (*Joussain et al. 2013*) et comme nous l'avons confirmé chez la souris dans notre deuxième étude, la valeur hédonique des odeurs change au cours du vieillissement. Le caractère plus ou moins plaisant d'une odeur peut également changer en fonction de nos expériences, par apprentissage. Nous pouvons en effet changer notre appréciation d'une odeur en fonction de la bonne ou de la mauvaise expérience qui lui a été associée. Le model d'apprentissage associatif odeur/récompense ou odeur/punition est communément utilisé en neurosciences pour étudier les mécanismes mnésiques (*Wilson et Linster 2008; Wilson et Sullivan 1994; Wilson et Stevenson 2003; Martin et al. 2004; Doucette et Restrepo 2008; Doucette et al. 2011; Fletcher 2012; Ross et Fletcher 2019; Grelat et al. 2018; Roesch, Stalnaker, et Schoenbaum 2007*) mais peu d'auteurs ont pris en compte la valeur hédonique initiale de l'odeur et le fait que cet apprentissage permette l'acquisition d'une nouvelle valeur hédonique (*Yuan et al. 2002; Calu et al. 2007; Gadziola et al. 2015*). Il serait ainsi intéressant de déterminer comment est remodelée la signature neurale de la valeur hédonique de l'odeur au sein du BO lorsque celle-ci se modifie au cours d'un apprentissage associatif. Nous avons mis en évidence que la modification physiologique de la perception hédonique d'une odeur au cours du vieillissement est accompagnée d'une modification de la représentation neurale au sein du BO (étude 2). Le

lien entre perception hédonique et signature neurale bulbaire semble donc très fort. Ainsi, un odorant possédant une valeur hédonique initialement positive mais rendue négative par apprentissage associatif verra-t-elle son spot d'activation neurale dans la partie antérieure du BO disparaître comme dans le cas de la perte physiologique du caractère plaisant de l'odorant liée à l'âge ? L'acquisition de la nouvelle valeur hédonique négative de l'odorant induira-t-elle plutôt une inversion de la signature neurale bulbaire, c'est-à-dire une activation neurale de la partie postérieure du BO et non plus de la partie antérieure ? A l'inverse, une odeur initialement déplaisante, activera-t-elle la partie antérieure du BO après acquisition d'une valeur hédonique positive par association avec une récompense ? Les deux signatures neurales antérieure et postérieure peuvent-elles coexister au sein du BO dans un contexte d'apprentissage associatif ?

### 3- D'où provient cet axe antéro-postérieur et quelles en sont ses conséquences sur la connectivité du bulbe olfactif ?

Les informations relatives à l'existence de différences anatomo-fonctionnelles le long de l'axe antéro-postérieur du BO sont rares. Une étude réalisée par Imamura *et al.* (Imamura *et al.* 2011) a montré que les cellules mitrales de la partie postérieure du BO se forment plus tardivement que celles de la partie antérieure. Ainsi, il existerait un axe antéro-postérieur de formation séquentielle des cellules de projection du BO lors de l'embryogenèse.

La différenciation du BO selon son axe antéro-postérieur lors de l'embryogenèse possède de nombreuses conséquences anatomo-fonctionnelles à l'âge adulte. En effet, notre première étude a montré que la partie postérieure du BO, mais pas la partie antérieure, est un site d'autostimulation intracérébrale. En effet, ce conditionnement opérant n'est obtenu que lorsque la stimulation lumineuse est délivrée dans la partie postérieure du BO, permettant ainsi d'activer les cellules mitrales postérieures par inhibition des cellules granulaires (Kermen *et al.* 2016). De façon intéressante, la stimulation lumineuse de la partie antérieure du BO semble quant à elle induire un comportement aversif. Cette expérience montre que la partie postérieure du BO est fonctionnellement reliée au système de récompense alors que cela ne semble pas être le cas de la partie antérieure. De plus, l'étude d'Imamura *et al.* (Imamura *et al.* 2011) suggère que selon leur date de naissance, et donc selon leur position le long de l'axe antéro-postérieur, les connexions des cellules mitrales avec les structures olfactives secondaires seraient différentes. Nous avons montré dans cette thèse que les cellules mitrales de la partie postérieure du BO projettent plus vers le TO que les cellules mitrales de la partie antérieure (étude 1). Les

connexions du BO avec le TO avaient été établies depuis longtemps (*Scott, McBride, et Schneider 1980; Schwob et Price 1984*), mais pas selon l'axe antéro-postérieur du BO. Le BO postérieur possède donc des connexions plus importantes avec le TO et donc avec le système de récompense.

Il existe deux types de cellules relais dans le BO, les cellules mitrales et les cellules à panache. Dans notre première étude nous nous sommes focalisés sur les connexions des cellules mitrales du BO avec le TO et avons montré leur importance dans le codage de la valeur hédonique des odeurs. Nous avons choisi de nous concentrer sur les cellules mitrales car elles ne projettent pas dans les mêmes parties du TO que les cellules à panache (*Igarashi et al. 2012*). Les cellules à panache projettent en effet préférentiellement sur la partie cap du TO, dont le rôle n'est encore pas très bien compris à ce jour (*Igarashi et al. 2012*), alors que les cellules mitrales projettent sur la partie corticale du TO qui a été reliée aux comportements motivés induits par les odeurs (*Gadziola et al. 2015; Murata et al. 2015; DiBenedictis et al. 2015; Agustín-Pavón, Martínez-García, et Lanuza 2014*). De plus, les cellules à panaches semblent plutôt coder pour la concentration de l'odeur alors que les cellules mitrales codent pour des comportements olfactifs plus complexes dont font partis les comportements d'approche ou de retrait (*Igarashi et al. 2012*). De plus, l'activité des cellules mitrales est modulée dans un contexte d'apprentissage associatif odeur/récompense ou odeur/punition (*Kay et Laurent 1999; Doucette et Restrepo 2008; Doucette et al. 2011; Liu et al. 2017*). Toutefois, nous ne pouvons pas exclure une possible distribution différentielle des connexions des cellules à panaches avec le TO ou avec d'autres structures olfactives secondaire selon l'axe antéro-postérieur du BO.

Ainsi, les différences d'activités entre la partie antérieure et postérieure du BO dans le codage de la valeur hédonique pourraient ensuite être transmises à des réseaux au moins en partie différents, induisant ainsi des comportements différents.

#### 4- Transfert de l'information hédonique de l'odeur du bulbe olfactif vers le circuit de la récompense ?

Les expériences d'autostimulation intracérébrale ont été utilisées à partir des années 50 et ont permis de découvrir les structures appartenant au système de récompense (*Olds et Milner 1954; Olds et Fobes 1981; Bielajew et Harris 1991*). Elles permettent aujourd'hui encore de disséquer les mécanismes neuronaux sous-tendant la motivation (*Carlezon et Chartoff 2007;*

*Steinberg et al. 2014; Wang et al. 2015; Johnson et al. 2020*). A la fin des années 60, Philips et Mogenson (*Phillips et Mogenson 1969*) ont suggéré que la stimulation électrique du BO pourrait être capable d'induire un comportement d'autostimulation intracérébrale chez un petit nombre de rats mais aucune étude statistique n'a été réalisée afin de démontrer l'existence de l'effet comportemental observé. Cette étude n'ayant jamais été répliquée ou approfondie, la possibilité que le BO puisse être un site d'autostimulation intracérébrale est tombée dans l'oubli. En utilisant l'optogénétique, notre première étude a ainsi démontré plus de 50 ans plus tard que le BO constitue effectivement un site d'autostimulation intracérébrale. En effet, les souris augmentent de façon drastique la durée de leurs nose pokes au cours des essais afin d'obtenir la stimulation lumineuse et ce comportement perdure après plusieurs essais d'extinction durant lesquels les nose pokes n'induisent plus de stimulation lumineuse. Nous avons répliqué ce comportement d'autostimulation du BO dans une seconde étude, mais cette fois chez l'animal âgé (étude 2). Nous avons pris en compte deux éléments essentiels dans notre expérience : nous avons fait la distinction entre la partie antérieure et postérieure du BO à la suite des données de l'étude de Kermen *et al.* (*Kermen et al. 2016*) et nous avons utilisé la technique de l'optogénétique. Concernant l'utilisation de stimulations optogénétiques, plutôt qu'électriques ou pharmacologiques, elle est de plus en plus fréquente dans les études car elle a une meilleure résolution à la fois spatiale et temporelle (*Nieh et al. 2013*). Dans notre étude, la stimulation lumineuse permet d'activer de manière indirecte les cellules mitrales par inhibition des cellules granulaires (*Kermen et al. 2016*). Cette méthodologie a été choisie car il a été montré que la modulation de l'activité des cellules de projections est assurément plus efficace car plus physiologique lorsqu'elle est effectuée de façon indirecte en modulant l'activité des interneurons (*Wiegert et al. 2017*). Pour ce qui est de l'axe antéro-postérieur du BO, il apparaît que cette distinction anatomique est un élément clef dans l'induction de ce conditionnement opérant car l'autostimulation n'est observée que dans la partie postérieure du BO. Dans leur étude Phillips et Mogenson (*Phillips et Mogenson 1969*) ont remarqué que les rats semblaient plus s'autostimuler lorsque la stimulation était réalisée dans la partie moyenne du BO par rapport à la partie la plus antérieure. Ces résultats sont difficilement comparables aux nôtres car nous ne connaissons pas le type de neurones modulés dans l'expérience de Philips et Mogenson. Cependant ces résultats préliminaires obtenus dans les années 60 vont dans le sens de l'importance de l'axe antéro-postérieur du BO dans l'induction d'un comportement de renforcement positif. Ainsi, cette thèse enrichie les connaissances relatives au système de récompense en caractérisant le BO postérieur comme nouveau site d'autostimulation

intracérébrale. Mais cette propriété que possède le BO à être un site d'autostimulation intracérébrale est-elle singulière à l'odorat ?

De nombreux stimuli sont connus pour être considérés comme des récompenses chez l'Homme. C'est notamment le cas pour le goût sucré, l'argent, un visage souriant, les œuvres d'art ou la musique (*Berridge, Robinson, et Aldridge 2009; Salimpoor et al. 2011; Ferreri et al. 2019; Lacey et al. 2011*). Une récente étude TEP a en effet montré que la musique induisait une libération de dopamine dans les régions striatales (*Salimpoor et al. 2011*) et que l'administration d'un précurseur ou d'un antagoniste de la dopamine augmentait ou diminuait respectivement les réponses émotionnelles induites par la musique (*Ferreri et al. 2019*). Malgré l'existence de récompenses sensorielles, aucun cortex sensoriel primaire n'a à notre connaissance été décrit comme site d'autostimulation intracérébrale. Alors qu'il ne reçoit et n'envoie pas de projections directes à la VTA, le BO constituerait donc la première structure sensorielle primaire à être un site d'autostimulation intracérébrale. Dans les autres modalités sensorielles, les informations sensorielles passent par un relai thalamique avant d'atteindre le cortex sensoriel primaire. Or le thalamus constitue au demeurant un site d'autostimulation intracérébrale (*Clavier et Gerfen 1982; Johnson et al. 2020*). Il a notamment été mis en évidence que l'activation par optogénétique des neurones intralaminaires du thalamus projetant sur le striatum est capable d'induire un conditionnement opérant (*Johnson et al. 2020*). En comparaison avec les autres informations sensorielles, les informations olfactives ne convergent pas directement vers le thalamus mais vers le BO, qui est aussi un site d'autostimulation intracérébrale. Ces données suggèrent que le système olfactif possède un traitement spécifique des propriétés récompensantes des stimuli sensoriels.

En ce qui concerne les bases neurales du comportement d'autostimulation intracérébrale du BO, nous avons mis en évidence que ce conditionnement opérant induit une activation des neurones dopaminergiques de la VTA et du TO chez la jeune souris (étude 1). Ces données sont en accord avec la libération de dopamine par la VTA au niveau de ses structures cibles lors de comportements d'autostimulation intracérébrale. Ainsi, ces données suggèrent que le conditionnement opérant obtenu par stimulation du BO serait médié, comme pour les autres sites d'autostimulation intracérébrale, par un recrutement du système de récompense. Nous avons également montré dans notre deuxième étude que le BO postérieur constitue toujours un site d'autostimulation intracérébrale chez l'animal âgé. Cependant, ce conditionnement opérant induit une activation neurale du TO mais pas de la VTA, révélant ainsi un recrutement seulement partiel du système de récompense. En effet, ni les neurones dopaminergiques, ni les



neurones non-dopaminergiques de la VTA ne présentent une augmentation de leur activité suite à la stimulation du BO. De plus, si l'on compare les résultats comportementaux obtenus chez le jeune adulte (étude 1) et chez l'animal âgé (étude 2), on s'aperçoit que l'autostimulation semble moins robuste et moins persistante chez les souris âgées. En effet, alors que le conditionnement opérant est acquis dès le deuxième essai chez le jeune, il faut attendre le quatrième essai pour observer ce comportement chez l'animal âgé. De même, les animaux âgés cessent leur comportement d'autostimulation dès le premier essai de la phase d'extinction, lorsque les nose pokes n'induisent plus de stimulation lumineuse, alors que ce comportement est observé tout au long de la phase d'extinction chez le jeune adulte. L'absence d'augmentation d'activité de la VTA pourrait être due à la dégénérescence du système dopaminergique (*Araki et al. 1997; Irwin et al. 1994; Schuligoi et al. 1993; Barili et al. 1998; Watanabe 1987; Norrara et al. 2018; Collier, Kanaan, et Kordower 2017*) et pourrait au moins en partie expliquer la plus faible robustesse et persistance du conditionnement opérant. En effet, l'altération du système dopaminergique liée au vieillissement entraîne des anomalies dans les comportements motivés (*Marschner et al. 2005; Sojitra et al. 2018*). À notre connaissance, notre étude est la première à fournir la preuve qu'un comportement d'autostimulation puisse être obtenu sans changement d'activité de la VTA. Cependant, il n'est pas possible de déterminer si cette observation est courante au cours du vieillissement ou si elle est spécifique au modèle d'autostimulation du BO.

### ***III. Le pouvoir récompensant des odeurs***

#### **1- Mise en évidence du pouvoir récompensant des odeurs**

Comme présenté précédemment, un certain nombre de stimuli sensoriels sont considérés comme des récompenses chez l'Homme (*Berridge, Robinson, et Aldridge 2009; Salimpoor et al. 2011; Ferreri et al. 2019; Lacey et al. 2011*). Le test de préférence de place conditionnée est couramment utilisé pour étudier le pouvoir récompensant des drogues (*McReynolds et al. 2017; Tahsili-Fahadan et al. 2006; Calcagnetti, Quatrella, et Schechter 1996*) ou de stimuli plus naturels tels que la nourriture ou les comportements sexuels chez l'animal (*Domínguez-Salazar, Naser, et Velázquez-Moctezuma 2014; Golden et al. 2016; Vrontou et al. 2013; Imaizumi, Takeda, et Fushiki 2000*) et a récemment été adapté à l'Homme (*Astur, Carew, et Deaton 2014; Astur et al. 2016; Childs, Astur, et de Wit 2017*). Tout comme n'importe quel

aliment, les récompenses alimentaires possèdent une odeur, néanmoins l'implication respective du goût et de l'odorat dans ce pouvoir récompensant reste méconnu. Par l'expérience de préférence de place conditionnée, nous avons montré dans notre première étude que certains odorants agissent comme des récompenses chez la souris et également chez l'Homme. Certaines études ont montré par le test de préférence de place conditionnée que certaines phéromones sexuelles, ayant une forte signification positive pour l'animal, agissent comme des récompenses (*Martínez-Ricós et al. 2007; Bell, Meerts, et Sisk 2010; Roberts et al. 2012*). Ainsi les stimuli traités par système olfactif principal et le système olfactif secondaire semblent avoir la capacité de recruter le système de récompense. Nous avons également mis en évidence que la préférence de place conditionnée observée chez la souris est dépendante du système dopaminergique (étude 1). En effet, l'injection d'un antagoniste dopaminergique durant le conditionnement à l'odeur induit une perte du pouvoir récompensant des odorants plaisants. De plus, suite à la préférence de place conditionnée on observe une augmentation de l'activité de la VTA et du TO.

Il convient de noter que tous les stimuli sensoriels ne sont pas considérés comme des récompenses. Par exemple, chez l'Homme seule la musique préférée (et non n'importe quelle musique) recrute le système de récompense (*Salimpoor et al. 2011*). De la même façon, nous avons constaté que seules les odeurs attractives (et non toutes les odeurs) sont considérées comme des récompenses par la souris et par l'Homme. Par ailleurs, certains odorants semblent également capables d'induire une aversion de place conditionnée chez l'animal (étude 1). Cela suggère que l'implication du système de récompense est basée sur les propriétés hédoniques intrinsèques des stimuli sensoriels et non sur un effet de récompensant global de la saillance sensorielle (*Xu et al., 2019*).

## 2- Lien entre valeur hédonique, attraction et pouvoir récompensant

A ce stade, il convient de faire le point sur les liens existant entre les concepts de valeur hédonique, d'attraction et de récompense. Selon sa valeur hédonique un stimulus induit un comportement d'approche ou de retrait. Comme nous l'avons vu dans l'introduction, ces réponses comportementales sont essentielles à la survie de l'animal. Dans notre troisième étude, nous avons mis en évidence que ces comportements d'approche et de retrait peuvent être utilisés comme mesure objective et non invasive de la valeur hédonique de l'odeur. Un odorant possédant une valeur hédonique positive induira donc une attraction pour la source odorante

(étude 1, 2 et 3). Cependant le comportement d'approche exécuté par l'animal ne dépend pas seulement de la composante émotionnelle de l'odorant, mais également de sa composante motivationnelle.

Dans le cas d'autres stimuli attractifs, comme la nourriture par exemple, la composante émotionnelle et la composante motivationnelle de l'attraction ont été définies respectivement comme le "liking" (plaisir) et le "wanting" (désir) (*Berridge, Robinson, et Aldridge 2009*). Nous désirons généralement ce que nous aimons et inversement. D'un point de vue psychologique, il est donc assez difficile d'établir une distinction précise entre ces deux composantes de l'attraction. Or le "liking" et le "wanting" possèdent des bases neurales distinctes. Alors que le "liking" est médié par plusieurs systèmes neuromodulateurs (e.g. opioïdes, endocannabinoïdes et benzodiazépines) libérés spécifiquement dans des zones très restreintes du système de récompense appelées "hedonic hotspots" (*Berridge et Kringelbach 2015*), le "wanting" est quant à lui médié par la libération de dopamine au niveau du système de récompense (*Berridge et Robinson 1998*). D'un point de vue comportemental, le "liking" et le "wanting" sont assez facilement distinguables dans le cas de la nourriture. En effet, Berridge et ces collaborateurs ont mis en évidence l'expression des mimiques faciales différentes en réponse à un goût sucré (e.g. passage de la langue sur les lèvres) par rapport à un goût amer (e.g. ouverture de la bouche) (*Berridge, Robinson, et Aldridge 2009*). Ces mimiques sont utilisées comme reflet du "liking" et sont observables chez l'Homme comme chez l'animal. Le "wanting" est quant à lui mesuré par la quantité de nourriture consommée. Ainsi la stimulation des "hedonic hotspots" induit à la fois une augmentation des mimiques relatives au "liking" et une augmentation de la quantité de nourriture consommée, alors que lorsque la même stimulation est réalisée à l'extérieur d'un "hedonic hotspot" seul le "wanting" augmente (*Castro et Berridge 2014*). Dans le cas des odorants, aucune réponse comportementale ne permet à ce jour de distinguer le "liking" du "wanting" chez l'Homme comme chez l'animal. En effet, dans notre troisième étude réalisée chez l'Homme, nous avons mis en évidence que l'évaluation du "liking" et du "wanting" olfactif sont très fortement corrélés, ne rendant pas possible leur dissociation comportementale.

Ainsi, l'attraction induite par les odeurs plaisantes pourrait être à la fois sous-tendue par le système dopaminergique qui coderait pour la composante motivationnelle de l'attraction, le "wanting", et des systèmes neuronaux différents qui coderaient pour la composante émotionnelle, le "liking". Qu'en est-il des réseaux de la composante émotionnelle olfactive ? Existe-t-il dans le système de récompense des "hedonic hotspots" codant pour le "liking" olfactif ? Le "liking" olfactif est-il médié par des structures extérieures au système de

récompense ? Il est intéressant de noter qu'alors que la recherche d'"hedonic hotspots" a été réalisée dans la majorité des structures du système de récompense (e.g. NAc, VP, OFC), aucune étude ne semble à ce jour avoir tenté d'identifier d'"hedonic hotspots" au sein du TO.

### 3- Tubercule olfactif : structure clef de l'attraction induite par les odorants

Parmi l'ensemble des structures olfactives secondaires, seul le TO semble répondre préférentiellement aux odorants attractifs par rapport aux odorants non attractifs (étude 1). Chez l'animal, la cartographie c-Fos réalisée dans les structures olfactives secondaires a montré que ni l'aPirCX, le pPirCX, le noyau olfactif antérieur, l'amygdale corticale ou le cortex entorhinal ne voient leur activité être modifiée en fonction de la valeur hédonique de l'odorant. L'activation neurale du TO a été confirmée par enregistrement électrophysiologique. En effet, le taux de décharge des neurones du TO augmente en réponse aux odorants plaisants et cette augmentation n'est pas retrouvée pour les odorants déplaisants. Ainsi, le TO semble posséder un rôle important dans le pouvoir attractif de certaines odeurs. Dans les modèles animaux, le rôle du TO dans le traitement des odeurs attractives ou aversives ayant une signification éthologique pour l'animal telles que les odeurs de nourriture, de prédateur ou de congénères du sexe opposé (*Fitzgerald, Richardson, et Wesson 2014; Agustín-Pavón, Martínez-García, et Lanuza 2014; DiBenedictis et al. 2014; 2015*) ou les odeurs prédisant une récompense (*Murata et al. 2015; Wesson et Wilson 2011; Gadziola et al. 2015; Zhang, Liu, et al. 2017*) est bien documenté. Nous apportons ici l'évidence d'un nouveau rôle du TO dans le traitement de l'attractivité des odeurs non apprises. Le rôle du TO dans le codage de la valeur hédonique reste très controversé chez l'Homme (*Zelano et al. 2007; de Araujo et al. 2005*) du fait du peu d'étude ayant porté sur le sujet. En utilisant une analyse par ROI afin de prendre en compte les variabilités anatomiques entre les sujets, nous avons pu étendre nos résultats à l'Homme et montrer une augmentation du signal BOLD dans le TO en réponse aux odeurs jugées comme attractives par les participants. Il est intéressant de noter que le PirCX antérieur et postérieur n'ont montré aucun changement d'activité en fonction de la valeur hédonique des odeurs.

En ce qui concerne les odeurs déplaisantes, nous n'avons pas retrouvé de modulation de l'activité neurale du TO ou de VTA en réponse à ces odeurs chez l'animal (étude 1). D'autres structures olfactives semblent être importantes dans le codage de la valeur hédonique négative des odorants ayant une signification éthologique ou dans le cas d'un apprentissage odeur/punition. C'est notamment le cas de l'amygdale corticale qui est notamment activée en

réponse à l'odeur de la TMT (*Takahashi 2014*) et pour laquelle il a été montré que son inhibition induit une altération du comportement de répulsion vis-à-vis de la TMT et que ce comportement qui est rétabli par activation de cette structure (*Root et al. 2014*). On pourrait supposer que la valeur hédonique spontanément négative des odorants ne possédant pas de signification éthologique forte soit sous-tendue par les mêmes mécanismes neuronaux. Cependant, notre étude de cartographie c-Fos ne va pas dans ce sens puisque nous n'observons pas d'augmentation d'activité de l'amygdale corticale en réponse aux odorants non attractifs versus attractifs (étude 1). Les mécanismes neuronaux permettant le codage de la valeur hédonique des odorants ne possédant pas, ou peu, de signification éthologique particulière semblent donc être sensiblement différents de ceux des odorants ayant une forte signification comme l'odeur de prédateur. De plus, dans nos études réalisées chez l'Homme et chez l'animal, aucune autre structure olfactive secondaire n'a vu son activité augmenter en réponse aux odorants non attractifs (étude 1). Une possibilité est que le caractère spontanément déplaisant d'une odeur résulte d'une moindre activation du système de récompense et notamment du TO. Selon cette hypothèse, l'activation du système de récompense conduirait à un comportement d'approche vis-à-vis de l'odorant alors qu'une réduction de son d'activation induirait un comportement de répulsion. Cependant des études complémentaires se focalisant sur le codage de la valeur hédonique spontanément négative seraient nécessaires afin de tester cette hypothèse et de mieux en appréhender les mécanismes neuronaux.

Ainsi nous avons mis en évidence par cartographie d'expression de la protéine c-Fos chez l'animal et par IRMf chez l'Homme le rôle important du TO dans le codage de la valeur hédonique des odeurs. Bien que possédant une forte résolution spatiale, nos techniques de cartographie c-Fos et d'IRMf ont une résolution temporelle très restreinte. En effet, la cartographie c-Fos permet de révéler les cellules présentant une activité environ une heure avant la fixation des tissus (*Dielenberg, Hunt, et McGregor 2001; Kovács 2008*) et l'IRMf permet de visualiser une modification du signal BOLD avec une résolution temporelle de l'ordre d'environ cinq secondes (*Rodden et Stemmer 2008*). Une analyse électrophysiologique dans les autres structures olfactives secondaires permettrait de compléter les données déjà acquises. Par ailleurs, notre technique de cartographie c-Fos ne nous permet pas non plus de catégoriser le type de neurone activé. Il est donc envisageable que l'absence de différence d'activité globale au niveau des structures olfactives secondaires autre que le TO résulte en réalité de la sommation d'une augmentation d'activité dans un type neuronal et à une diminution d'activité dans un autre type neuronal. Il serait intéressant de compléter ces données par une analyse

phénotypique. Chaque méthodologie possède des avantages et des inconvénients. Celles que nous avons choisi d'utiliser ont permis de révéler le rôle singulier que semble posséder le TO dans l'attraction induite par les odeurs.

#### 4- Utilisation des propriétés motivationnelles des odorants pour mesurer la valeur hédonique chez l'Homme

Comme nous l'avons vu, l'attraction induite par les odorants est capable d'induire une motivation forte chez l'Homme comme chez l'animal (étude 1). Dans notre troisième étude, nous avons cherché à déterminer si cette composante motivationnelle pouvait être utilisée comme mesure de la valeur hédonique de l'odeur. Nous avons mesuré de façon automatique, sur la base en partie du modèle souris (*Kermen et al. 2016*), la vitesse d'approche de la source odorante vers le nez par le sujet, la distance entre la source et le nez, le nombre de sniff, la vitesse de retrait de la source ainsi que la distance de retrait maximale des odorants. Ces paramètres sont différents entre les odorants plaisants et déplaisants. La nécessité de l'apport d'une nouvelle méthode d'évaluation de la valeur hédonique trouve son origine dans les limites que présentent les techniques standards. Ces techniques sont en effet soit invasives de par la présence de nombreux capteurs sur le corps et/ou le visage (mesure de la réponse électrodermale, du rythme cardiaque, du sniff...), soit subjectives (échelles de mesure ou réponses verbales), soit dépendantes de l'expertise de certains experts (expressions faciales). La mesure des comportements motivés comme évaluation de la valeur hédonique possède l'avantage de ne pas dépendre du jugement du participant ou de l'avis d'un quelconque expert. De plus, une simple webcam est placée à côté de l'individu afin de mesurer les vitesses et les trajectoires réalisées par le flacon contenant l'odorant et le nez du participant. Enfin, une singularité de notre approche concerne le fait que le sujet se stimule olfactivement par lui-même.

La mesure de ces comportements moteurs pourrait ainsi permettre de réaliser des mesures objectives la valeur hédonique d'une odeur dans des conditions plus écologiques ou naturalistiques. De plus, il est possible d'envisager l'utilisation de ces mesures chez des participants possédant des troubles du langage et où l'utilisation de capteur est difficile à mettre en place.

## IV. Conclusion et perspectives

Cette thèse a permis d'apporter de nouvelles connaissances quant aux mécanismes neuronaux responsables de la valeur hédonique des odeurs. Le pouvoir récompensant des odorants et leur capacité à recruter le système de récompense apparaît comme un mécanisme essentiel au comportement d'approche vis-à-vis de l'odeur. Parmi les différentes structures impliquées dans cette composante motivationnelle de la perception olfactive, le TO apparaît comme une structure primordiale qui pourrait convertir l'information hédonique présente dans le BO en comportement motivé grâce au recrutement du système de récompense. Afin de confirmer ce rôle clef du TO, il serait intéressant de moduler son activité durant l'exploration de l'odorant par l'animal par exemple afin de déterminer de façon plus causale son rôle dans l'attraction olfactive. Il apparaît également comme nécessaires que les études futures aient pour ambition de disséquer plus finement les interactions entre le BO, le TO, la VTA et le reste du système de récompense. Quels réseaux ou sous-circuits sont nécessaires au pouvoir récompensant des odeurs ? En utilisant le model du vieillissement, nous avons mis en évidence que l'activité de VTA est impliquée dans ces comportements mais qu'elle peut être compensée. Ainsi, un comportement d'autostimulation intracérébrale serait-il observable malgré une inhibition de la VTA ? Par ailleurs, bien que possédant une composante innée, la valeur hédonique d'une odeur est également fortement plastique. Comment est modulée la signature neurale du caractère plus ou moins plaisant d'une odeur au sein du BO, du TO et de la VTA après l'acquisition d'une nouvelle valeur hédonique par apprentissage ?

Le pouvoir récompensant des odorants plaisants ouvre la voie à une possible utilisation des odeurs comme stimuli capables de stimuler le système de récompense. Un dysfonctionnement du système de récompense est constaté dans de nombreuses pathologies. C'est notamment le cas dans la dépression pour laquelle l'inhibition des neurones dopaminergiques de la VTA induit un phénotype type de cette pathologie chez l'animal (*Tye et al. 2013*). Un second exemple est celui de la maladie de Parkinson. Les troubles moteurs présents dans la maladie de Parkinson sont connus comme étant notamment sous-tendus par une dégénérescence des neurones dopaminergiques de la substance noire. Or la dégénérescence des neurones dopaminergiques de la VTA, qui est également observée dans cette maladie neurodégénérative (*Alberico, Cassell, et Narayanan 2015*), induit un syndrome d'apathie chez les patients (*McGuigan et al. 2019*). Ainsi, l'exposition quotidienne à des odeurs récompensantes pourrait être envisagée afin de stimuler de façon physiologique le système



dopaminergique et d'ainsi contrecarrer les troubles motivationnels présents dans ces pathologies. Des thérapies olfactives basées sur une exposition quotidienne à des odeurs sont déjà utilisées chez les personnes présentant une hyposmie (*Hummel et al. 2009; Konstantinidis et al. 2013; Haehner et al. 2013*). Qu'en est-il de l'utilisation des propriétés motivationnelles des odeurs dans des protocoles d'enrichissement olfactif ? Afin que de telles applications puissent être envisagées, il apparaît comme nécessaire de caractériser dans un premier temps comment l'information hédonique est traitée au niveau de la réponse comportementale et d'un point de vue cérébral dans ces pathologies.

# RÉFÉRENCES BIBLIOGRAPHIQUES

- Abriat, A., S. Barkat, M. Bensafi, C. Rouby, et C. Fanchon. 2007. « Psychological and Physiological Evaluation of Emotional Effects of a Perfume in Menopausal Women ». *International Journal of Cosmetic Science* 29 (5): 399-408. <https://doi.org/10.1111/j.1468-2494.2007.00398.x>.
- Adjei, Stacey, Alexandra L. Houck, Katherine Ma, et Daniel W. Wesson. 2013. « Age-Dependent Alterations in the Number, Volume, and Localization of Islands of Calleja within the Olfactory Tubercle ». *Neurobiology of Aging* 34 (11): 2676-82. <https://doi.org/10.1016/j.neurobiolaging.2013.05.014>.
- Agustín-Pavón, Carmen, Fernando Martínez-García, et Enrique Lanuza. 2014. « Focal Lesions within the Ventral Striato-Pallidum Abolish Attraction for Male Chemosignals in Female Mice ». *Behavioural Brain Research* 259 (février): 292-96. <https://doi.org/10.1016/j.bbr.2013.11.020>.
- Aiello, et Dean. 1991. « An Introduction to Human Evolutionary Anatomy ». *International Journal of Primatology* 12 (5): 529-32. <https://doi.org/10.1007/BF02547638>.
- Alaoui-Ismaïli, O., O. Robin, H. Rada, A. Dittmar, et E. Vernet-Maury. 1997. « Basic Emotions Evoked by Odorants: Comparison between Autonomic Responses and Self-Evaluation ». *Physiology & Behavior* 62 (4): 713-20. [https://doi.org/10.1016/s0031-9384\(97\)90016-0](https://doi.org/10.1016/s0031-9384(97)90016-0).
- Alaoui-Ismaïli, O., E. Vernet-Maury, A. Dittmar, G. Delhomme, et J. Chanel. 1997. « Odor Hedonics: Connection with Emotional Response Estimated by Autonomic Parameters ». *Chemical Senses* 22 (3): 237-48. <https://doi.org/10.1093/chemse/22.3.237>.
- Alberico, Stephanie L., Martin D. Cassell, et Nandakumar S. Narayanan. 2015. « The Vulnerable Ventral Tegmental Area in Parkinson's Disease ». *Basal ganglia* 5 (2-3): 51-55. <https://doi.org/10.1016/j.baga.2015.06.001>.
- Alheid, G. F., et L. Heimer. 1988. « New Perspectives in Basal Forebrain Organization of Special Relevance for Neuropsychiatric Disorders: The Striatopallidal, Amygdaloid, and Corticopetal Components of Substantia Innominata ». *Neuroscience* 27 (1): 1-39. [https://doi.org/10.1016/0306-4522\(88\)90217-5](https://doi.org/10.1016/0306-4522(88)90217-5).
- Allen, Paul D., et David A. Eddins. 2010. « Presbycusis Phenotypes Form a Heterogeneous Continuum When Ordered by Degree and Configuration of Hearing Loss ». *Hearing Research* 264 (1-2): 10-20. <https://doi.org/10.1016/j.heares.2010.02.001>.
- Altman, J. 1969. « Autoradiographic and Histological Studies of Postnatal Neurogenesis. IV. Cell Proliferation and Migration in the Anterior Forebrain, with Special Reference to Persisting Neurogenesis in the Olfactory Bulb ». *The Journal of Comparative Neurology* 137 (4): 433-57. <https://doi.org/10.1002/cne.901370404>.
- Amoore, J. E., J. W. Johnston, et M. Rubin. 1964. « THE STEROCHEMICAL THEORY OF ODOR ». *Scientific American* 210 (février): 42-49. <https://doi.org/10.1038/scientificamerican0264-42>.
- Anderson, A. K., K. Christoff, I. Stappen, D. Panitz, D. G. Ghahremani, G. Glover, J. D. E. Gabrieli, et N. Sobel. 2003. « Dissociated Neural Representations of Intensity and Valence in Human Olfaction ». *Nature Neuroscience* 6 (2): 196-202. <https://doi.org/10.1038/nn1001>.
- Araki, T., H. Kato, K. Shuto, T. Fujiwara, et Y. Itoyama. 1997. « Effect of Aging on Dopaminergic Receptors and Uptake Sites in the Rat Brain Studied by Receptor Autoradiography ». *Journal of the Neurological Sciences* 148 (2): 131-37. [https://doi.org/10.1016/s0022-510x\(96\)05343-9](https://doi.org/10.1016/s0022-510x(96)05343-9).

- Araujo, Ivan E. de, Edmund T. Rolls, Maria Inés Velazco, Christian Margot, et Isabelle Cayeux. 2005. « Cognitive Modulation of Olfactory Processing ». *Neuron* 46 (4): 671-79. <https://doi.org/10.1016/j.neuron.2005.04.021>.
- Astur, Robert S., Andrew W. Carew, et Bonnie E. Deaton. 2014. « Conditioned Place Preferences in Humans Using Virtual Reality ». *Behavioural Brain Research* 267 (juillet): 173-77. <https://doi.org/10.1016/j.bbr.2014.03.018>.
- Astur, Robert S., Alexandra N. Palmisano, Andrew W. Carew, Bonnie E. Deaton, Franchesca S. Kuhney, Rachel N. Niezrecki, Ellie C. Hudd, Kelly L. Mendicino, et Christopher J. Ritter. 2016. « Conditioned Place Preferences in Humans Using Secondary Reinforcers ». *Behavioural Brain Research* 297 (janvier): 15-19. <https://doi.org/10.1016/j.bbr.2015.09.042>.
- Atanasova, Borianna, Wissam El-Hage, Claire Chabanet, Philippe Gaillard, Catherine Belzung, et Vincent Camus. 2010. « Olfactory Anhedonia and Negative Olfactory Alliesthesia in Depressed Patients ». *Psychiatry Research* 176 (2-3): 190-96. <https://doi.org/10.1016/j.psychres.2008.11.016>.
- Attems, Johannes, Lauren Walker, et Kurt A. Jellinger. 2015. « Olfaction and Aging: A Mini-Review ». *Gerontology* 61 (6): 485-90. <https://doi.org/10.1159/000381619>.
- Aungst, J. L., P. M. Heyward, A. C. Puche, S. V. Karnup, A. Hayar, G. Szabo, et M. T. Shipley. 2003. « Centre-Surround Inhibition among Olfactory Bulb Glomeruli ». *Nature* 426 (6967): 623-29. <https://doi.org/10.1038/nature02185>.
- Bachelard, Gaston, et Suzanne Bachelard. 1988. *Fragments d'une poétique du feu*. Presses Universitaires de France.
- Barili, P., G. De Carolis, D. Zaccheo, et F. Amenta. 1998. « Sensitivity to Ageing of the Limbic Dopaminergic System: A Review ». *Mechanisms of Ageing and Development* 106 (1-2): 57-92. [https://doi.org/10.1016/s0047-6374\(98\)00104-3](https://doi.org/10.1016/s0047-6374(98)00104-3).
- Barkai, E., et D. Saar. 2001. « Cellular Correlates of Olfactory Learning in the Rat Piriform Cortex ». *Reviews in the Neurosciences* 12 (2): 111-20. <https://doi.org/10.1515/revneuro.2001.12.2.111>.
- Barkat, S., J. Poncelet, B. N. Landis, C. Rouby, et M. Bensafi. 2008. « Improved Smell Pleasantness after Odor-Taste Associative Learning in Humans ». *Neuroscience Letters* 434 (1): 108-12. <https://doi.org/10.1016/j.neulet.2008.01.037>.
- Barnes, Dylan C., Julie Chapuis, Dipesh Chaudhury, et Donald A. Wilson. 2011. « Odor Fear Conditioning Modifies Piriform Cortex Local Field Potentials Both during Conditioning and during Post-Conditioning Sleep ». *PloS One* 6 (3): e18130. <https://doi.org/10.1371/journal.pone.0018130>.
- Becker, Susanne, Anne-Kathrin Bräscher, Scott Bannister, Moustafa Bensafi, Destany Calma-Birling, Raymond C. K. Chan, Tuomas Eerola, et al. 2019. « The Role of Hedonics in the Human Affectome ». *Neuroscience and Biobehavioral Reviews* 102: 221-41. <https://doi.org/10.1016/j.neubiorev.2019.05.003>.
- Bekkers, John M., et Norimitsu Suzuki. 2013. « Neurons and Circuits for Odor Processing in the Piriform Cortex ». *Trends in Neurosciences* 36 (7): 429-38. <https://doi.org/10.1016/j.tins.2013.04.005>.
- Bell, Margaret R., Sarah H. Meerts, et Cheryl L. Sisk. 2010. « Male Syrian Hamsters Demonstrate a Conditioned Place Preference for Sexual Behavior and Female Chemosensory Stimuli ». *Hormones and Behavior* 58 (3): 410-14. <https://doi.org/10.1016/j.yhbeh.2010.05.017>.
- Bensafi, Moustafa, Emilia Iannilli, Johan Poncelet, Han-Seok Seo, Johannes Gerber, Catherine Rouby, et Thomas Hummel. 2012. « Dissociated Representations of Pleasant and Unpleasant Olfacto-Trigeminal Mixtures: An fMRI Study ». *PloS One* 7 (6): e38358. <https://doi.org/10.1371/journal.pone.0038358>.

- Bensafi, Jessica Porter, Sandra Pouliot, Joel Mainland, Bradley Johnson, Christina Zelano, Natasha Young, et al. 2003. « Olfactomotor Activity during Imagery Mimics That during Perception ». *Nature Neuroscience* 6 (11): 1142-44. <https://doi.org/10.1038/nn1145>.
- Bensafi, C. Rouby, V. Farget, B. Bertrand, M. Vigouroux, et A. Holley. 2002. « Autonomic Nervous System Responses to Odours: The Role of Pleasantness and Arousal ». *Chemical Senses* 27 (8): 703-9. <https://doi.org/10.1093/chemse/27.8.703>.
- Bensafi, C. Rouby, V. Farget, M. Vigouroux, et A. Holley. 2002. « Asymmetry of Pleasant vs. Unpleasant Odor Processing during Affective Judgment in Humans ». *Neuroscience Letters* 328 (3): 309-13. [https://doi.org/10.1016/s0304-3940\(02\)00548-7](https://doi.org/10.1016/s0304-3940(02)00548-7).
- Bensafi, Noam Sobel, et Rehan M. Khan. 2007. « Hedonic-Specific Activity in Piriform Cortex during Odor Imagery Mimics That during Odor Perception ». *Journal of Neurophysiology* 98 (6): 3254-62. <https://doi.org/10.1152/jn.00349.2007>.
- Berglund, B., U. Berglund, T. Engen, et G. Ekman. 1973. « Multidimensional Analysis of Twenty-One Odors ». *Scandinavian Journal of Psychology* 14 (2): 131-37. <https://doi.org/10.1111/j.1467-9450.1973.tb00104.x>.
- Berke, Joshua D. 2018. « What Does Dopamine Mean? » *Nature Neuroscience* 21 (6): 787-93. <https://doi.org/10.1038/s41593-018-0152-y>.
- Bernabei, Mara, Romeo Beccherelli, Emiliano Zampetti, Simone Pantalei, et Krishna C. Persaud. 2013. « Mimicking Biological Olfaction with Very Large Chemical Arrays ». In *Neuromorphic Olfaction*, édité par Krishna C. Persaud, Santiago Marco, et Agustín Gutiérrez-Gálvez. Frontiers in Neuroengineering. Boca Raton (FL): CRC Press/Taylor & Francis. <http://www.ncbi.nlm.nih.gov/books/NBK298828/>.
- Berridge, et Morten L. Kringelbach. 2015. « Pleasure Systems in the Brain ». *Neuron* 86 (3): 646-64. <https://doi.org/10.1016/j.neuron.2015.02.018>.
- Berridge, et T. E. Robinson. 1998. « What Is the Role of Dopamine in Reward: Hedonic Impact, Reward Learning, or Incentive Saliency? » *Brain Research. Brain Research Reviews* 28 (3): 309-69. [https://doi.org/10.1016/s0165-0173\(98\)00019-8](https://doi.org/10.1016/s0165-0173(98)00019-8).
- Berridge, Terry E. Robinson, et J. Wayne Aldridge. 2009. « Dissecting Components of Reward: “Liking”, “Wanting”, and Learning ». *Current Opinion in Pharmacology* 9 (1): 65-73. <https://doi.org/10.1016/j.coph.2008.12.014>.
- Bhatnagar, K. P., R. C. Kennedy, G. Baron, et R. A. Greenberg. 1987. « Number of Mitral Cells and the Bulb Volume in the Aging Human Olfactory Bulb: A Quantitative Morphological Study ». *The Anatomical Record* 218 (1): 73-87. <https://doi.org/10.1002/ar.1092180112>.
- Bielajew, C. H., et T. Harris. 1991. « Self-Stimulation: A Rewarding Decade ». *Journal of Psychiatry & Neuroscience: JPN* 16 (3): 109-14.
- Bishop, M. P., S. T. Elder, et R. G. Heath. 1963. « Intracranial Self-Stimulation in Man ». *Science (New York, N.Y.)* 140 (3565): 394-96. <https://doi.org/10.1126/science.140.3565.394>.
- Bitter, Thomas, Hilmar Gudziol, Hartmut Peter Burmeister, Hans-Joachim Mentzel, Orlando Guntinas-Lichius, et Christian Gaser. 2010. « Anosmia Leads to a Loss of Gray Matter in Cortical Brain Areas ». *Chemical Senses* 35 (5): 407-15. <https://doi.org/10.1093/chemse/bjq028>.
- Bodyak, N., et B. Slotnick. 1999. « Performance of Mice in an Automated Olfactometer: Odor Detection, Discrimination and Odor Memory ». *Chemical Senses* 24 (6): 637-45. <https://doi.org/10.1093/chemse/24.6.637>.
- Bouchard, Viviane. 2013. *Les Déserts, Témoins de l'histoire du monde*. Québec Amérique.
- Bousefsaf, F., C. Maaoui, et A. Pruski. 2016. « Peripheral Vasomotor Activity Assessment Using a Continuous Wavelet Analysis on Webcam Photoplethysmographic Signals ».

- Bio-Medical Materials and Engineering* 27 (5): 527-38. <https://doi.org/10.3233/BME-161606>.
- Bragulat, Veronique, Mario Dzemidzic, Carolina Bruno, Cari A. Cox, Thomas Talavage, Robert V. Considine, et David A. Kareken. 2010. « Food-Related Odor Probes of Brain Reward Circuits during Hunger: A Pilot fMRI Study ». *Obesity (Silver Spring, Md.)* 18 (8): 1566-71. <https://doi.org/10.1038/oby.2010.57>.
- Bragulat, Veronique, Mario Dzemidzic, Thomas Talavage, Dena Davidson, Sean J. O'Connor, et David A. Kareken. 2008. « Alcohol Sensitizes Cerebral Responses to the Odors of Alcoholic Drinks: An fMRI Study ». *Alcoholism, Clinical and Experimental Research* 32 (7): 1124-34. <https://doi.org/10.1111/j.1530-0277.2008.00693.x>.
- Brauchli, P., P. B. Rüegg, F. Etzweiler, et H. Zeier. 1995. « Electrocutaneous and Autonomic Alteration by Administration of a Pleasant and an Unpleasant Odor ». *Chemical Senses* 20 (5): 505-15. <https://doi.org/10.1093/chemse/20.5.505>.
- Brechbühl, Julien, Magali Klaey, et Marie-Christine Broillet. 2008. « Grueneberg Ganglion Cells Mediate Alarm Pheromone Detection in Mice ». *Science (New York, N.Y.)* 321 (5892): 1092-95. <https://doi.org/10.1126/science.1160770>.
- Brechbühl, Julien, Fabian Moine, Magali Klaey, Monique Nenniger-Tosato, Nicolas Hurni, Frank Sporkert, Christian Giroud, et Marie-Christine Broillet. 2013. « Mouse Alarm Pheromone Shares Structural Similarity with Predator Scents ». *Proceedings of the National Academy of Sciences of the United States of America* 110 (12): 4762-67. <https://doi.org/10.1073/pnas.1214249110>.
- Breer, Heinz. 2003. « Olfactory Receptors: Molecular Basis for Recognition and Discrimination of Odors ». *Analytical and Bioanalytical Chemistry* 377 (3): 427-33. <https://doi.org/10.1007/s00216-003-2113-9>.
- Brennan, P., et E. B. Keverne. 2015. « Biological Complexity and Adaptability of Simple Mammalian Olfactory Memory Systems ». *Neuroscience and Biobehavioral Reviews* 50 (mars): 29-40. <https://doi.org/10.1016/j.neubiorev.2014.10.020>.
- Buck, L., et R. Axel. 1991. « A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Recognition ». *Cell* 65 (1): 175-87. [https://doi.org/10.1016/0092-8674\(91\)90418-x](https://doi.org/10.1016/0092-8674(91)90418-x).
- Buck, L. B. 2000. « The Molecular Architecture of Odor and Pheromone Sensing in Mammals ». *Cell* 100 (6): 611-18. [https://doi.org/10.1016/s0092-8674\(00\)80698-4](https://doi.org/10.1016/s0092-8674(00)80698-4).
- Budinger, E., P. Heil, A. Hess, et H. Scheich. 2006. « Multisensory Processing via Early Cortical Stages: Connections of the Primary Auditory Cortical Field with Other Sensory Systems ». *Neuroscience* 143 (4): 1065-83. <https://doi.org/10.1016/j.neuroscience.2006.08.035>.
- Buonviso, N., et M. A. Chaput. 1990. « Response Similarity to Odors in Olfactory Bulb Output Cells Presumed to Be Connected to the Same Glomerulus: Electrophysiological Study Using Simultaneous Single-Unit Recordings ». *Journal of Neurophysiology* 63 (3): 447-54. <https://doi.org/10.1152/jn.1990.63.3.447>.
- Bushdid, C., M. O. Magnasco, L. B. Vosshall, et A. Keller. 2014. « Humans Can Discriminate More than 1 Trillion Olfactory Stimuli ». *Science (New York, N.Y.)* 343 (6177): 1370-72. <https://doi.org/10.1126/science.1249168>.
- Busto, Germain U., Julie E. Elie, Florence Kermen, Samuel Garcia, Joëlle Sacquet, François Jourdan, Dominique Marcel, Nathalie Mandairon, et Anne Didier. 2009. « Expression of Zif268 in the Granule Cell Layer of the Adult Mouse Olfactory Bulb Is Modulated by Experience ». *The European Journal of Neuroscience* 29 (7): 1431-39. <https://doi.org/10.1111/j.1460-9568.2009.06689.x>.

- Cain, W. S., R. de Wijk, C. Lulejian, F. Schiet, et L. C. See. 1998. « Odor Identification: Perceptual and Semantic Dimensions ». *Chemical Senses* 23 (3): 309-26. <https://doi.org/10.1093/chemse/23.3.309>.
- Caissard, Jean-Claude, Sylvie Baudino, et Philippe Hugueney. 2009. « Le parfum des roses ». *Le parfum des roses*, n° 376: 56-61.
- Calcagnetti, D. J., L. A. Quatrella, et M. D. Schechter. 1996. « Olfactory Bulbectomy Disrupts the Expression of Cocaine-Induced Conditioned Place Preference ». *Physiology & Behavior* 59 (4-5): 597-604. [https://doi.org/10.1016/0031-9384\(95\)02119-1](https://doi.org/10.1016/0031-9384(95)02119-1).
- Calu, Donna J., Matthew R. Roesch, Thomas A. Stalnaker, et Geoffrey Schoenbaum. 2007. « Associative Encoding in Posterior Piriform Cortex during Odor Discrimination and Reversal Learning ». *Cerebral Cortex (New York, N.Y.: 1991)* 17 (6): 1342-49. <https://doi.org/10.1093/cercor/bhl045>.
- Cansler, Hillary L., Katherine N. Wright, Lucas A. Stetzik, et Daniel W. Wesson. 2020. « Neurochemical Organization of the Ventral Striatum's Olfactory Tubercle ». *Journal of Neurochemistry* 152 (4): 425-48. <https://doi.org/10.1111/jnc.14919>.
- Carlezon, William A., et Elena H. Chartoff. 2007. « Intracranial Self-Stimulation (ICSS) in Rodents to Study the Neurobiology of Motivation ». *Nature Protocols* 2 (11): 2987-95. <https://doi.org/10.1038/nprot.2007.441>.
- Carriero, Giovanni, Laura Uva, Vadym Gnatkovsky, et Marco de Curtis. 2009. « Distribution of the Olfactory Fiber Input into the Olfactory Tubercle of the in Vitro Isolated Guinea Pig Brain ». *Journal of Neurophysiology* 101 (3): 1613-19. <https://doi.org/10.1152/jn.90792.2008>.
- Castro, D. C., et K. C. Berridge. 2014. « Advances in the Neurobiological Bases for Food “liking” versus “Wanting” ». *Physiology & Behavior* 136 (septembre): 22-30. <https://doi.org/10.1016/j.physbeh.2014.05.022>.
- Cavallin, Melissa Ann, Katelyn Powell, K. C. Biju, et Debra Ann Fadool. 2010. « State-Dependent Sculpting of Olfactory Sensory Neurons Is Attributed to Sensory Enrichment, Odor Deprivation, and Aging ». *Neuroscience Letters* 483 (2): 90-95. <https://doi.org/10.1016/j.neulet.2010.07.059>.
- Chalke, H. D., J. R. Dewhurst, et C. W. Ward. 1958. « Loss of Sense of Smell in Old People: A Possible Contributory Factor in Accidental Poisoning from Town Gas ». *Public Health* 72 (6): 223-30. [https://doi.org/10.1016/s0033-3506\(58\)80053-0](https://doi.org/10.1016/s0033-3506(58)80053-0).
- Chastrette, M. 1997. « Trends in Structure-Odor Relationships ». *SAR and QSAR in Environmental Research* 6 (3-4): 215-54. <https://doi.org/10.1080/10629369708033253>.
- Childs, Emma, Robert S. Astur, et Harriet de Wit. 2017. « Virtual Reality Conditioned Place Preference Using Monetary Reward ». *Behavioural Brain Research* 322 (Pt A): 110-14. <https://doi.org/10.1016/j.bbr.2017.01.019>.
- Cho, Jin Hyung, Janet E. A. Prince, Tyler Cutforth, et Jean-François Cloutier. 2011. « The Pattern of Glomerular Map Formation Defines Responsiveness to Aversive Odorants in Mice ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31 (21): 7920-26. <https://doi.org/10.1523/JNEUROSCI.2460-10.2011>.
- Chrea, Christelle, Didier Grandjean, Sylvain Delplanque, Isabelle Cayeux, Bénédicte Le Calvé, Laurence Aymard, Maria Inés Velazco, David Sander, et Klaus R. Scherer. 2009. « Mapping the Semantic Space for the Subjective Experience of Emotional Responses to Odors ». *Chemical Senses* 34 (1): 49-62. <https://doi.org/10.1093/chemse/bjn052>.
- Chu, Simon, et John J. Downes. 2002. « Proust Nose Best: Odors Are Better Cues of Autobiographical Memory ». *Memory & Cognition* 30 (4): 511-18. <https://doi.org/10.3758/bf03194952>.

- Clavier, R. M., et C. R. Gerfen. 1982. « Intracranial Self-Stimulation in the Thalamus of the Rat ». *Brain Research Bulletin* 8 (4): 353-58. [https://doi.org/10.1016/0361-9230\(82\)90072-7](https://doi.org/10.1016/0361-9230(82)90072-7).
- Cleland, Thomas A., Brett A. Johnson, Michael Leon, et Christiane Linster. 2007. « Relational Representation in the Olfactory System ». *Proceedings of the National Academy of Sciences of the United States of America* 104 (6): 1953-58. <https://doi.org/10.1073/pnas.0608564104>.
- Cleland, Thomas A., et Praveen Sethupathy. 2006. « Non-Topographical Contrast Enhancement in the Olfactory Bulb ». *BMC Neuroscience* 7 (janvier): 7. <https://doi.org/10.1186/1471-2202-7-7>.
- Collier, Timothy J., Nicholas M. Kanaan, et Jeffrey H. Kordower. 2017. « Aging and Parkinson's Disease: Different Sides of the Same Coin? » *Movement Disorders: Official Journal of the Movement Disorder Society* 32 (7): 983-90. <https://doi.org/10.1002/mds.27037>.
- Conley, David B., Alan M. Robinson, Michael J. Shinnors, et Robert C. Kern. 2003. « Age-Related Olfactory Dysfunction: Cellular and Molecular Characterization in the Rat ». *American Journal of Rhinology* 17 (3): 169-75.
- Courtiol, Emmanuelle, Nathalie Buonviso, et Philippe Litaudon. 2019. « Odorant Features Differentially Modulate Beta/Gamma Oscillatory Patterns in Anterior versus Posterior Piriform Cortex ». *Neuroscience* 409: 26-34. <https://doi.org/10.1016/j.neuroscience.2019.04.025>.
- Croy, Ilona, Edda Drechsler, Paul Hamilton, Thomas Hummel, et Håkan Olausson. 2016. « Olfactory Modulation of Affective Touch Processing - A Neurophysiological Investigation ». *NeuroImage* 135: 135-41. <https://doi.org/10.1016/j.neuroimage.2016.04.046>.
- Cummings, Diana M., et Leonardo Belluscio. 2008. « Charting Plasticity in the Regenerating Maps of the Mammalian Olfactory Bulb ». *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry* 14 (3): 251-63. <https://doi.org/10.1177/1073858408315026>.
- Curcio, C. A., N. A. McNelly, et J. W. Hinds. 1985. « Aging in the Rat Olfactory System: Relative Stability of Piriform Cortex Contrasts with Changes in Olfactory Bulb and Olfactory Epithelium ». *The Journal of Comparative Neurology* 235 (4): 519-28. <https://doi.org/10.1002/cne.902350409>.
- Dardou, David, Frédérique Datiche, et Martine Cattarelli. 2008. « Memory Is Differently Impaired during Aging According to the Learning Tasks in the Rat ». *Behavioural Brain Research* 194 (2): 193-200. <https://doi.org/10.1016/j.bbr.2008.07.007>.
- David, Sophie. 2002. « Linguistic Expressions of Odors in French ». In *Olfaction, Taste and Cognition*, 82. Cambridge University Press. <https://halshs.archives-ouvertes.fr/halshs-00084167>.
- Demattè, M. Luisa, Isabella Endrizzi, et Flavia Gasperi. 2014. « Food Neophobia and Its Relation with Olfaction ». *Frontiers in Psychology* 5: 127. <https://doi.org/10.3389/fpsyg.2014.00127>.
- Desai, M., L. A. Pratt, H. Lentzner, et K. N. Robinson. 2001. « Trends in Vision and Hearing among Older Americans ». *Aging Trends (Hyattsville, Md.)*, n° 2 (mars): 1-8. <https://doi.org/10.1037/e620682007-001>.
- Devore, Sasha, Joshua Lee, et Christiane Linster. 2013. « Odor Preferences Shape Discrimination Learning in Rats ». *Behavioral Neuroscience* 127 (4): 498-504. <https://doi.org/10.1037/a0033329>.
- DiBenedictis, Brett T., Aadaeze O. Olugbemi, Michael J. Baum, et James A. Cherry. 2014. « 6-Hydroxydopamine Lesions of the Anteromedial Ventral Striatum Impair Opposite-Sex



- Urinary Odor Preference in Female Mice ». *Behavioural Brain Research* 274 (novembre): 243-47. <https://doi.org/10.1016/j.bbr.2014.08.024>.
- DiBenedictis, Olugbemi, Baum, Cherry. 2015. « DREADD-Induced Silencing of the Medial Olfactory Tubercle Disrupts the Preference of Female Mice for Opposite-Sex Chemosignals(1,2,3) ». *ENeuro* 2 (5). <https://doi.org/10.1523/ENEURO.0078-15.2015>.
- Dielenberg, R. A., G. E. Hunt, et I. S. McGregor. 2001. « “When a Rat Smells a Cat”: The Distribution of Fos Immunoreactivity in Rat Brain Following Exposure to a Predatory Odor ». *Neuroscience* 104 (4): 1085-97. [https://doi.org/10.1016/s0306-4522\(01\)00150-6](https://doi.org/10.1016/s0306-4522(01)00150-6).
- Distel, H., S. Ayabe-Kanamura, M. Martínez-Gómez, I. Schicker, T. Kobayakawa, S. Saito, et R. Hudson. 1999. « Perception of Everyday Odors--Correlation between Intensity, Familiarity and Strength of Hedonic Judgement ». *Chemical Senses* 24 (2): 191-99. <https://doi.org/10.1093/chemse/24.2.191>.
- Domínguez-Salazar, Emilio, Hishan F. Naser, et Javier Velázquez-Moctezuma. 2014. « D1-like Antagonist Blocks Conditioned Place Preference Induced by Ejaculation in Male Rats ». *Behavioural Brain Research* 269 (août): 15-19. <https://doi.org/10.1016/j.bbr.2014.04.026>.
- Dore, J.-C, G. Gordon, et J.-N. Jaubert. 1984. « Approche factorielle des relations entre structure chimique et note odorante ». *Approche factorielle des relations entre structure chimique et note odorante* 299 (7): 315-20.
- Doty, et Vidyulata Kamath. 2014. « The Influences of Age on Olfaction: A Review ». *Frontiers in Psychology* 5: 20. <https://doi.org/10.3389/fpsyg.2014.00020>.
- Doty, R. L. 1986. « Odor-Guided Behavior in Mammals ». *Experientia* 42 (3): 257-71. <https://doi.org/10.1007/BF01942506>.
- Doucette, Wilder, David H. Gire, Jennifer Whitesell, Vanessa Carmean, Mary T. Lucero, et Diego Restrepo. 2011. « Associative Cortex Features in the First Olfactory Brain Relay Station ». *Neuron* 69 (6): 1176-87. <https://doi.org/10.1016/j.neuron.2011.02.024>.
- Doucette, Wilder, et Diego Restrepo. 2008. « Profound Context-Dependent Plasticity of Mitral Cell Responses in Olfactory Bulb ». *PLoS Biology* 6 (10): e258. <https://doi.org/10.1371/journal.pbio.0060258>.
- Ehrlichman, H., S. Brown, J. Zhu, et S. Warrenburg. 1995. « Startle Reflex Modulation during Exposure to Pleasant and Unpleasant Odors ». *Psychophysiology* 32 (2): 150-54. <https://doi.org/10.1111/j.1469-8986.1995.tb03306.x>.
- Ehrlichman, H., et J. N. Halpern. 1988. « Affect and Memory: Effects of Pleasant and Unpleasant Odors on Retrieval of Happy and Unhappy Memories ». *Journal of Personality and Social Psychology* 55 (5): 769-79. <https://doi.org/10.1037//0022-3514.55.5.769>.
- Ehrlichman, H., S. B. Kuhl, J. Zhu, et S. Warrenburg. 1997. « Startle Reflex Modulation by Pleasant and Unpleasant Odors in a Between-Subjects Design ». *Psychophysiology* 34 (6): 726-29. <https://doi.org/10.1111/j.1469-8986.1997.tb02149.x>.
- Engen, Trygg. 1991. *Odor Sensation and Memory*. Greenwood Publishing Group.
- Enwere, Emeka, Tetsuro Shingo, Christopher Gregg, Hirokazu Fujikawa, Shigeki Ohta, et Samuel Weiss. 2004. « Aging Results in Reduced Epidermal Growth Factor Receptor Signaling, Diminished Olfactory Neurogenesis, and Deficits in Fine Olfactory Discrimination ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 24 (38): 8354-65. <https://doi.org/10.1523/JNEUROSCI.2751-04.2004>.
- Erman, Adolf, et Hermann Ranke. 1994. *La civilisation égyptienne*. Payot.
- Fallon, J. H., et R. Y. Moore. 1978. « Catecholamine Innervation of the Basal Forebrain. IV. Topography of the Dopamine Projection to the Basal Forebrain and Neostriatum ». *The*

- Ferdenzi, Camille, Arnaud Fournel, Marc Thévenet, Géraldine Coppin, et Moustafa Bensafi. 2015. « Viewing Olfactory Affective Responses Through the Sniff Prism: Effect of Perceptual Dimensions and Age on Olfactomotor Responses to Odors ». *Frontiers in Psychology* 6: 1776. <https://doi.org/10.3389/fpsyg.2015.01776>.
- Ferdenzi, Camille, Pauline Joussain, Bérengère Digard, Lucie Luneau, Jelena Djordjevic, et Moustafa Bensafi. 2017. « Individual Differences in Verbal and Non-Verbal Affective Responses to Smells: Influence of Odor Label Across Cultures ». *Chemical Senses* 42 (1): 37-46. <https://doi.org/10.1093/chemse/bjw098>.
- Ferdenzi, Camille, Harilanto Razafindrazaka, Nicolas Baldovini, Daphnée Poupon, Denis Pierron, et Moustafa Bensafi. 2019. « Influence of Gender and Culture on the Perception of Acidic Compounds of Human Body Odor ». *Physiology & Behavior* 210: 112561. <https://doi.org/10.1016/j.physbeh.2019.112561>.
- Ferreri, Laura, Ernest Mas-Herrero, Robert J. Zatorre, Pablo Ripollés, Alba Gomez-Andres, Helena Alicart, Guillem Olivé, et al. 2019. « Dopamine Modulates the Reward Experiences Elicited by Music ». *Proceedings of the National Academy of Sciences of the United States of America* 116 (9): 3793-98. <https://doi.org/10.1073/pnas.1811878116>.
- Ferrero, David M., Jamie K. Lemon, Daniela Fluegge, Stan L. Pashkovski, Wayne J. Korzan, Sandeep Robert Datta, Marc Spehr, Markus Fendt, et Stephen D. Liberles. 2011. « Detection and Avoidance of a Carnivore Odor by Prey ». *Proceedings of the National Academy of Sciences of the United States of America* 108 (27): 11235-40. <https://doi.org/10.1073/pnas.1103317108>.
- Fisher, Helen, Arthur Aron, et Lucy L. Brown. 2005. « Romantic Love: An fMRI Study of a Neural Mechanism for Mate Choice ». *The Journal of Comparative Neurology* 493 (1): 58-62. <https://doi.org/10.1002/cne.20772>.
- Fitzgerald, Brynn J., Kara Richardson, et Daniel W. Wesson. 2014. « Olfactory Tubercle Stimulation Alters Odor Preference Behavior and Recruits Forebrain Reward and Motivational Centers ». *Frontiers in Behavioral Neuroscience* 8: 81. <https://doi.org/10.3389/fnbeh.2014.00081>.
- Fletcher, Max L. 2012. « Olfactory Aversive Conditioning Alters Olfactory Bulb Mitral/Tufted Cell Glomerular Odor Responses ». *Frontiers in Systems Neuroscience* 6: 16. <https://doi.org/10.3389/fnsys.2012.00016>.
- Forest, Jérémy, Laura Chalénçon, Maëllie Midroit, Claire Terrier, Isabelle Caillé, Joëlle Sacquet, Claire Benetollo, et al. 2020. « Role of Adult-Born Versus Preexisting Neurons Born at P0 in Olfactory Perception in a Complex Olfactory Environment in Mice ». *Cerebral Cortex (New York, N.Y.: 1991)* 30 (2): 534-49. <https://doi.org/10.1093/cercor/bhz105>.
- Forest, Jérémy, Mélissa Moreno, Matthias Cavelius, Laura Chalénçon, Anne Ziessel, Joëlle Sacquet, Marion Richard, Anne Didier, et Nathalie Mandairon. 2019. « Short-Term Availability of Adult-Born Neurons for Memory Encoding ». *Nature Communications* 10 (1): 5609. <https://doi.org/10.1038/s41467-019-13521-7>.
- Fournel, A., E. Iannilli, C. Ferdenzi, A. Werner, T. Hummel, et M. Bensafi. 2020. « A Methodological Investigation of a Flexible Surface MRI Coil to Obtain Functional Signals from the Human Olfactory Bulb ». *Journal of Neuroscience Methods* 335 (avril): 108624. <https://doi.org/10.1016/j.jneumeth.2020.108624>.
- Frank, Thomas, Nila R. Mönig, Chie Satou, Shin-Ichi Higashijima, et Rainer W. Friedrich. 2019. « Associative Conditioning Remaps Odor Representations and Modifies

- Inhibition in a Higher Olfactory Brain Area ». *Nature Neuroscience* 22 (11): 1844-56. <https://doi.org/10.1038/s41593-019-0495-z>.
- Frederick, Donald E., Austin Brown, Elizabeth Brim, Nisarg Mehta, Mark Vujovic, et Leslie M. Kay. 2016. « Gamma and Beta Oscillations Define a Sequence of Neurocognitive Modes Present in Odor Processing ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 36 (29): 7750-67. <https://doi.org/10.1523/JNEUROSCI.0569-16.2016>.
- Frumin, Idan, Ofer Perl, Yaara Endevelt-Shapira, Ami Eisen, Neetai Eshel, Iris Heller, Maya Shemesh, et al. 2015. « A Social Chemosignaling Function for Human Handshaking ». *ELife* 4 (mars). <https://doi.org/10.7554/eLife.05154>.
- Gadziola, Marie A., Kate A. Tylicki, Diana L. Christian, et Daniel W. Wesson. 2015. « The Olfactory Tubercle Encodes Odor Valence in Behaving Mice ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 35 (11): 4515-27. <https://doi.org/10.1523/JNEUROSCI.4750-14.2015>.
- Gallagher, M., et A. A. Chiba. 1996. « The Amygdala and Emotion ». *Current Opinion in Neurobiology* 6 (2): 221-27. [https://doi.org/10.1016/s0959-4388\(96\)80076-6](https://doi.org/10.1016/s0959-4388(96)80076-6).
- Genva, Manon, Thierry Kenne Kemene, Magali Deleu, Laurence Lins, et Marie-Laure Fauconnier. 2019. « Is It Possible to Predict the Odor of a Molecule on the Basis of Its Structure? » *International Journal of Molecular Sciences* 20 (12). <https://doi.org/10.3390/ijms20123018>.
- Gervais, Rémi, Nathalie Buonviso, Claire Martin, et Nadine Ravel. 2007. « What Do Electrophysiological Studies Tell Us about Processing at the Olfactory Bulb Level? » *Journal of Physiology, Paris* 101 (1-3): 40-45. <https://doi.org/10.1016/j.jphysparis.2007.10.006>.
- Ghosh, Sulagna, Stephen D. Larson, Hooman Hefzi, Zachary Marnoy, Tyler Cutforth, Kartheek Dokka, et Kristin K. Baldwin. 2011. « Sensory Maps in the Olfactory Cortex Defined by Long-Range Viral Tracing of Single Neurons ». *Nature* 472 (7342): 217-20. <https://doi.org/10.1038/nature09945>.
- Gobert, E. G. 1961. *Tunis et les parfums*.
- Gocel, James, et John Larson. 2013. « Evidence for Loss of Synaptic AMPA Receptors in Anterior Piriform Cortex of Aged Mice ». *Frontiers in Aging Neuroscience* 5: 39. <https://doi.org/10.3389/fnagi.2013.00039>.
- Golden, Sam A., Mitra Heshmati, Meghan Flanigan, Daniel J. Christoffel, Kevin Guise, Madeline L. Pfau, Hossein Aleyasin, et al. 2016. « Basal Forebrain Projections to the Lateral Habenula Modulate Aggression Reward ». *Nature* 534 (7609): 688-92. <https://doi.org/10.1038/nature18601>.
- Gottfried, Jay A. 2010. « Central Mechanisms of Odour Object Perception ». *Nature Reviews. Neuroscience* 11 (9): 628-41. <https://doi.org/10.1038/nrn2883>.
- Gottfried, Jay A., Ralf Deichmann, Joel S. Winston, et Raymond J. Dolan. 2002. « Functional Heterogeneity in Human Olfactory Cortex: An Event-Related Functional Magnetic Resonance Imaging Study ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 22 (24): 10819-28.
- Gottfried, Jay A., John O'Doherty, et Raymond J. Dolan. 2002. « Appetitive and Aversive Olfactory Learning in Humans Studied Using Event-Related Functional Magnetic Resonance Imaging ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 22 (24): 10829-37.
- Grabenhorst, Fabian, et Edmund T. Rolls. 2009. « Different Representations of Relative and Absolute Subjective Value in the Human Brain ». *NeuroImage* 48 (1): 258-68. <https://doi.org/10.1016/j.neuroimage.2009.06.045>.

- Grabenhorst, Fabian, Edmund T. Rolls, Christian Margot, Maria A. A. P. da Silva, et Maria Ines Velazco. 2007. « How Pleasant and Unpleasant Stimuli Combine in Different Brain Regions: Odor Mixtures ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 27 (49): 13532-40. <https://doi.org/10.1523/JNEUROSCI.3337-07.2007>.
- Grelat, Anne, Laura Benoit, Sébastien Wagner, Carine Moigneu, Pierre-Marie Lledo, et Mariana Alonso. 2018. « Adult-Born Neurons Boost Odor-Reward Association ». *Proceedings of the National Academy of Sciences of the United States of America* 115 (10): 2514-19. <https://doi.org/10.1073/pnas.1716400115>.
- Guerrieri, Fernando, Harald Lachnit, Bertram Gerber, et Martin Giurfa. 2005. « Olfactory Blocking and Odorant Similarity in the Honeybee ». *Learning & Memory (Cold Spring Harbor, N.Y.)* 12 (2): 86-95. <https://doi.org/10.1101/lm.79305>.
- Guthrie, K. M., A. J. Anderson, M. Leon, et C. Gall. 1993. « Odor-Induced Increases in c-Fos MRNA Expression Reveal an Anatomical “Unit” for Odor Processing in Olfactory Bulb ». *Proceedings of the National Academy of Sciences of the United States of America* 90 (8): 3329-33. <https://doi.org/10.1073/pnas.90.8.3329>.
- Haddad, Rafi, Rehan Khan, Yuji K. Takahashi, Kensaku Mori, David Harel, et Noam Sobel. 2008. « A Metric for Odorant Comparison ». *Nature Methods* 5 (5): 425-29. <https://doi.org/10.1038/nmeth.1197>.
- Haehner, Antje, Clara Tosch, Martin Wolz, Lisa Klingelhofer, Mareike Fauser, Alexander Storch, Heinz Reichmann, et Thomas Hummel. 2013. « Olfactory Training in Patients with Parkinson’s Disease ». *PloS One* 8 (4): e61680. <https://doi.org/10.1371/journal.pone.0061680>.
- Hegoburu, Chloé, Yannick Sevelinges, Marc Thévenet, Rémi Gervais, Sandrine Parrot, et Anne-Marie Mouly. 2009. « Differential Dynamics of Amino Acid Release in the Amygdala and Olfactory Cortex during Odor Fear Acquisition as Revealed with Simultaneous High Temporal Resolution Microdialysis ». *Learning & Memory (Cold Spring Harbor, N.Y.)* 16 (11): 687-97. <https://doi.org/10.1101/lm.1584209>.
- Henion, K. E. 1971. « Odor Pleasantness and Intensity: A Single Dimension? » *Journal of Experimental Psychology* 90 (2): 275-79. <https://doi.org/10.1037/h0031549>.
- Herz, R. S. 1998. « Are Odors the Best Cues to Memory? A Cross-Modal Comparison of Associative Memory Stimuli ». *Annals of the New York Academy of Sciences* 855 (novembre): 670-74. <https://doi.org/10.1111/j.1749-6632.1998.tb10643.x>.
- Hinds, J. W., et N. A. McNelly. 1981. « Aging in the Rat Olfactory System: Correlation of Changes in the Olfactory Epithelium and Olfactory Bulb ». *The Journal of Comparative Neurology* 203 (3): 441-53. <https://doi.org/10.1002/cne.902030308>.
- Hu, Ji, Chun Zhong, Cheng Ding, Qiuyi Chi, Andreas Walz, Peter Mombaerts, Hiroaki Matsunami, et Minmin Luo. 2007. « Detection of Near-Atmospheric Concentrations of CO<sub>2</sub> by an Olfactory Subsystem in the Mouse ». *Science (New York, N.Y.)* 317 (5840): 953-57. <https://doi.org/10.1126/science.1144233>.
- Huang, Ping, Hanhua Huang, Qiuling Luo, et Lei Mo. 2016. « The Difference between Aesthetic Appreciation of Artistic and Popular Music: Evidence from an fMRI Study ». *PloS One* 11 (11): e0165377. <https://doi.org/10.1371/journal.pone.0165377>.
- Hummel, Thomas, Karo Rissom, Jens Reden, Aantje Hähner, Mark Weidenbecher, et Karl-Bernd Hüttenbrink. 2009. « Effects of Olfactory Training in Patients with Olfactory Loss ». *The Laryngoscope* 119 (3): 496-99. <https://doi.org/10.1002/lary.20101>.
- Hussain, Ashiq, Luis R. Saraiva, David M. Ferrero, Gaurav Ahuja, Venkatesh S. Krishna, Stephen D. Liberles, et Sigrun I. Korsching. 2013. « High-Affinity Olfactory Receptor for the Death-Associated Odor Cadaverine ». *Proceedings of the National Academy of*

- Sciences of the United States of America* 110 (48): 19579-84. <https://doi.org/10.1073/pnas.1318596110>.
- Igarashi, Kei M., Nao Ieki, Myungho An, Yukie Yamaguchi, Shin Nagayama, Ko Kobayakawa, Reiko Kobayakawa, et al. 2012. « Parallel Mitral and Tufted Cell Pathways Route Distinct Odor Information to Different Targets in the Olfactory Cortex ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32 (23): 7970-85. <https://doi.org/10.1523/JNEUROSCI.0154-12.2012>.
- Ikemoto, Satoshi. 2003. « Involvement of the Olfactory Tubercle in Cocaine Reward: Intracranial Self-Administration Studies ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 23 (28): 9305-11.
- Ikemoto. 2007. « Dopamine Reward Circuitry: Two Projection Systems from the Ventral Midbrain to the Nucleus Accumbens-Olfactory Tubercle Complex ». *Brain Research Reviews* 56 (1): 27-78. <https://doi.org/10.1016/j.brainresrev.2007.05.004>.
- Ikemoto, Satoshi, et Antonello Bonci. 2014. « Neurocircuitry of Drug Reward ». *Neuropharmacology* 76 Pt B (janvier): 329-41. <https://doi.org/10.1016/j.neuropharm.2013.04.031>.
- Ikemoto, Satoshi, et Kathleen M. Donahue. 2005. « A Five-Minute, but Not a Fifteen-Minute, Conditioning Trial Duration Induces Conditioned Place Preference for Cocaine Administration into the Olfactory Tubercle ». *Synapse (New York, N.Y.)* 56 (1): 57-59. <https://doi.org/10.1002/syn.20124>.
- Ikemoto, Satoshi, Mei Qin, et Zhong-Hua Liu. 2005. « The Functional Divide for Primary Reinforcement of D-Amphetamine Lies between the Medial and Lateral Ventral Striatum: Is the Division of the Accumbens Core, Shell, and Olfactory Tubercle Valid? ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 25 (20): 5061-65. <https://doi.org/10.1523/JNEUROSCI.0892-05.2005>.
- Imaizumi, M., M. Takeda, et T. Fushiki. 2000. « Effects of Oil Intake in the Conditioned Place Preference Test in Mice ». *Brain Research* 870 (1-2): 150-56. [https://doi.org/10.1016/s0006-8993\(00\)02416-1](https://doi.org/10.1016/s0006-8993(00)02416-1).
- Imamura, Albert E. Ayoub, Pasko Rakic, et Charles A. Greer. 2011. « Timing of Neurogenesis Is a Determinant of Olfactory Circuitry ». *Nature Neuroscience* 14 (3): 331-37. <https://doi.org/10.1038/nn.2754>.
- Irwin, I., L. E. DeLanney, T. McNeill, P. Chan, L. S. Forno, G. M. Murphy, D. A. Di Monte, M. S. Sandy, et J. W. Langston. 1994. « Aging and the Nigrostriatal Dopamine System: A Non-Human Primate Study ». *Neurodegeneration: A Journal for Neurodegenerative Disorders, Neuroprotection, and Neuroregeneration* 3 (4): 251-65.
- Jagetia, Sonum, Adrianna J. Milton, Lucas A. Stetzk, Samantha Liu, Kavya Pai, Keiko Arakawa, Nathalie Mandairon, et Daniel W. Wesson. 2018. « Inter- and Intra-Mouse Variability in Odor Preferences Revealed in an Olfactory Multiple-Choice Test ». *Behavioral Neuroscience* 132 (2): 88-98. <https://doi.org/10.1037/bne0000233>.
- Janak, Patricia H., et Kay M. Tye. 2015. « From Circuits to Behaviour in the Amygdala ». *Nature* 517 (7534): 284-92. <https://doi.org/10.1038/nature14188>.
- Jin, Jingwen, Christina Zelano, Jay A. Gottfried, et Aprajita Mohanty. 2015. « Human Amygdala Represents the Complete Spectrum of Subjective Valence ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 35 (45): 15145-56. <https://doi.org/10.1523/JNEUROSCI.2450-15.2015>.
- Johanson, I. B., et W. G. Hall. 1979. « Appetitive Learning in 1-Day-Old Rat Pups ». *Science (New York, N.Y.)* 205 (4404): 419-21. <https://doi.org/10.1126/science.451612>.
- Johnson, Brett A., et Michael Leon. 2007. « Chemotopic Odorant Coding in a Mammalian Olfactory System ». *The Journal of Comparative Neurology* 503 (1): 1-34. <https://doi.org/10.1002/cne.21396>.

- Johnson, D. M., K. R. Illig, M. Behan, et L. B. Haberly. 2000. « New Features of Connectivity in Piriform Cortex Visualized by Intracellular Injection of Pyramidal Cells Suggest That “Primary” Olfactory Cortex Functions like “Association” Cortex in Other Sensory Systems ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 20 (18): 6974-82.
- Johnson, Kari A., Lucas Voyvodic, Gabriel C. Loewinger, Yolanda Mateo, et David M. Lovinger. 2020. « Operant Self-Stimulation of Thalamic Terminals in the Dorsomedial Striatum Is Constrained by Metabotropic Glutamate Receptor 2 ». *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, janvier. <https://doi.org/10.1038/s41386-020-0626-y>.
- Johnson, Lulu Tsai, Dheeraj S. Roy, David H. Valenzuela, Colleen Mosley, Angeliki Magklara, Stavros Lomvardas, Stephen D. Liberles, et Gilad Barnea. 2012. « Neurons Expressing Trace Amine-Associated Receptors Project to Discrete Glomeruli and Constitute an Olfactory Subsystem ». *Proceedings of the National Academy of Sciences of the United States of America* 109 (33): 13410-15. <https://doi.org/10.1073/pnas.1206724109>.
- Jönsson, Fredrik U., Henrik Olsson, et Mats J. Olsson. 2005. « Odor Emotionality Affects the Confidence in Odor Naming ». *Chemical Senses* 30 (1): 29-35. <https://doi.org/10.1093/chemse/bjh254>.
- Joussain, Pauline, Marion Bessy, Arnaud Fournel, Camille Ferdenzi, Catherine Rouby, Floriane Delphin-Combe, Pierre Krolak-Salmon, et Moustafa Bensafi. 2016. « Altered Affective Evaluations of Smells in Alzheimer’s Disease ». *Journal of Alzheimer’s Disease: JAD* 49 (2): 433-41. <https://doi.org/10.3233/JAD-150332>.
- Joussain, Pauline, Amandine Chakirian, Florence Kermen, Catherine Rouby, et Moustafa Bensafi. 2011. « Physicochemical Influence on Odor Hedonics: Where Does It Occur First? ». *Communicative & Integrative Biology* 4 (5): 563-65. <https://doi.org/10.4161/cib.4.5.15811>.
- Joussain, Pauline, Camille Ferdenzi, Jelena Djordjevic, et Moustafa Bensafi. 2017. « Relationship Between Psychophysiological Responses to Aversive Odors and Nutritional Status During Normal Aging ». *Chemical Senses* 42 (6): 465-72. <https://doi.org/10.1093/chemse/bjx027>.
- Joussain, Pauline, Marc Thevenet, Catherine Rouby, et Moustafa Bensafi. 2013. « Effect of Aging on Hedonic Appreciation of Pleasant and Unpleasant Odors ». *PloS One* 8 (4): e61376. <https://doi.org/10.1371/journal.pone.0061376>.
- Kadohisa, Mikiko, et Wilson. 2006. « Separate Encoding of Identity and Similarity of Complex Familiar Odors in Piriform Cortex ». *Proceedings of the National Academy of Sciences of the United States of America* 103 (41): 15206-11. <https://doi.org/10.1073/pnas.0604313103>.
- Kang, Ningdong, Michael J. Baum, et James A. Cherry. 2009. « A Direct Main Olfactory Bulb Projection to the “vomeronasal” Amygdala in Female Mice Selectively Responds to Volatile Pheromones from Males ». *The European Journal of Neuroscience* 29 (3): 624-34. <https://doi.org/10.1111/j.1460-9568.2009.06638.x>.
- Kareken, David A., Eric D. Claus, Merav Sabri, Mario Dzemic, Ann E. K. Kosobud, Alexander J. Radnovich, Dwight Hector, et al. 2004. « Alcohol-Related Olfactory Cues Activate the Nucleus Accumbens and Ventral Tegmental Area in High-Risk Drinkers: Preliminary Findings ». *Alcoholism, Clinical and Experimental Research* 28 (4): 550-57. <https://doi.org/10.1097/01.alc.0000122764.60626.af>.
- Kay, Jennifer Beshel, Jorge Brea, Claire Martin, Daniel Rojas-Líbano, et Nancy Kopell. 2009. « Olfactory Oscillations: The What, How and What For ». *Trends in Neurosciences* 32 (4): 207-14. <https://doi.org/10.1016/j.tins.2008.11.008>.

- Kay, L. M., et G. Laurent. 1999. « Odor- and Context-Dependent Modulation of Mitral Cell Activity in Behaving Rats ». *Nature Neuroscience* 2 (11): 1003-9. <https://doi.org/10.1038/14801>.
- Keller, Andreas, et Leslie B. Vosshall. 2007. « Influence of Odorant Receptor Repertoire on Odor Perception in Humans and Fruit Flies ». *Proceedings of the National Academy of Sciences of the United States of America* 104 (13): 5614-19. <https://doi.org/10.1073/pnas.0605321104>.
- Keller, Andreas, Hanyi Zhuang, Qiuyi Chi, Leslie B. Vosshall, et Hiroaki Matsunami. 2007. « Genetic Variation in a Human Odorant Receptor Alters Odour Perception ». *Nature* 449 (7161): 468-72. <https://doi.org/10.1038/nature06162>.
- Kermen, A. Chakirian, C. Sezille, P. Joussain, G. Le Goff, A. Ziessel, M. Chastrette, et al. 2011. « Molecular Complexity Determines the Number of Olfactory Notes and the Pleasantness of Smells ». *Scientific Reports* 1: 206. <https://doi.org/10.1038/srep00206>.
- Kermen, Florence, Lea Darnet, Christoph Wiest, Fabrizio Palumbo, Jack Bechert, Ozge Uslu, et Emre Yaksi. 2020. « Stimulus-Specific Behavioral Responses of Zebrafish to a Large Range of Odors Exhibit Individual Variability ». *BMC Biology* 18 (1): 66. <https://doi.org/10.1186/s12915-020-00801-8>.
- Kermen, Maëllie Midroit, Nicola Kuczewski, Jérémy Forest, Marc Thévenet, Joëlle Sacquet, Claire Benetollo, Marion Richard, Anne Didier, et Nathalie Mandairon. 2016. « Topographical Representation of Odor Hedonics in the Olfactory Bulb ». *Nature Neuroscience* 19 (7): 876-78. <https://doi.org/10.1038/nn.4317>.
- Khan, Rehan M., Chung-Hay Luk, Adeen Flinker, Amit Aggarwal, Hadas Lapid, Rafi Haddad, et Noam Sobel. 2007. « Predicting Odor Pleasantness from Odorant Structure: Pleasantness as a Reflection of the Physical World ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 27 (37): 10015-23. <https://doi.org/10.1523/JNEUROSCI.1158-07.2007>.
- Khan, Evelien Vaes, et Peter Mombaerts. 2013. « Temporal Patterns of Odorant Receptor Gene Expression in Adult and Aged Mice ». *Molecular and Cellular Neurosciences* 57 (novembre): 120-29. <https://doi.org/10.1016/j.mcn.2013.08.001>.
- Kiselycznyk, Carly L., Steven Zhang, et Christiane Linster. 2006. « Role of Centrifugal Projections to the Olfactory Bulb in Olfactory Processing ». *Learning & Memory (Cold Spring Harbor, N.Y.)* 13 (5): 575-79. <https://doi.org/10.1101/lm.285706>.
- Kleene, Steven J. 2008. « The Electrochemical Basis of Odor Transduction in Vertebrate Olfactory Cilia ». *Chemical Senses* 33 (9): 839-59. <https://doi.org/10.1093/chemse/bjn048>.
- Kobayakawa, Ko, Reiko Kobayakawa, Hideyuki Matsumoto, Yuichiro Oka, Takeshi Imai, Masahito Ikawa, Masaru Okabe, et al. 2007. « Innate versus Learned Odour Processing in the Mouse Olfactory Bulb ». *Nature* 450 (7169): 503-8. <https://doi.org/10.1038/nature06281>.
- Konstantinidis, Iordanis, Evangelia Tsakiropoulou, Paschalia Bekiaridou, Chrysa Kazantzidou, et Jannis Constantinidis. 2013. « Use of Olfactory Training in Post-Traumatic and Postinfectious Olfactory Dysfunction ». *The Laryngoscope* 123 (12): E85-90. <https://doi.org/10.1002/lary.24390>.
- Kornetsky, C., D. Huston-Lyons, et L. J. Porrino. 1991. « The Role of the Olfactory Tubercle in the Effects of Cocaine, Morphine and Brain-Stimulation Reward ». *Brain Research* 541 (1): 75-81. [https://doi.org/10.1016/0006-8993\(91\)91076-d](https://doi.org/10.1016/0006-8993(91)91076-d).
- Kosaka, T., et K. Kosaka. 2009. « Olfactory Bulb Anatomy ». In *Encyclopedia of Neuroscience*, édité par Larry R. Squire, 59-69. Oxford: Academic Press. <https://doi.org/10.1016/B978-008045046-9.01686-7>.

- Kosaka, Toshio, et Katsuko Kosaka. 2004. « Neuronal Gap Junctions between Intraglomerular Mitral/Tufted Cell Dendrites in the Mouse Main Olfactory Bulb ». *Neuroscience Research* 49 (4): 373-78. <https://doi.org/10.1016/j.neures.2004.04.003>.
- Kovács, K. J. 2008. « Measurement of Immediate-Early Gene Activation- c-Fos and Beyond ». *Journal of Neuroendocrinology* 20 (6): 665-72. <https://doi.org/10.1111/j.1365-2826.2008.01734.x>.
- Kraemer, Simone, et Raimund Apfelbach. 2004. « Olfactory Sensitivity, Learning and Cognition in Young Adult and Aged Male Wistar Rats ». *Physiology & Behavior* 81 (3): 435-42. <https://doi.org/10.1016/j.physbeh.2004.01.012>.
- Lacey, Simon, Henrik Hagtvéd, Vanessa M. Patrick, Amy Anderson, Randall Stilla, Gopikrishna Deshpande, Xiaoping Hu, João R. Sato, Srinivas Reddy, et K. Sathian. 2011. « Art for Reward's Sake: Visual Art Recruits the Ventral Striatum ». *NeuroImage* 55 (1): 420-33. <https://doi.org/10.1016/j.neuroimage.2010.11.027>.
- Lafreniere, Denis, et Norman Mann. 2009. « Anosmia: Loss of Smell in the Elderly ». *Otolaryngologic Clinics of North America* 42 (1): 123-31, x. <https://doi.org/10.1016/j.otc.2008.09.001>.
- Lapid, Hadas, Sagit Shushan, Anton Plotkin, Hillary Voet, Yehudah Roth, Thomas Hummel, Elad Schneidman, et Noam Sobel. 2011. « Neural Activity at the Human Olfactory Epithelium Reflects Olfactory Perception ». *Nature Neuroscience* 14 (11): 1455-61. <https://doi.org/10.1038/nn.2926>.
- Largey, G. P., et D. R. Watson. 1972. « The Sociology of Odors ». *AJS; American Journal of Sociology* 77 (6): 1021-34. <https://doi.org/10.1086/225257>.
- Larsson, L. Farde, T. Hummel, M. Witt, N. Erixon Lindroth, et L. Bäckman. 2009. « Age-Related Loss of Olfactory Sensitivity: Association to Dopamine Transporter Binding in Putamen ». *Neuroscience* 161 (2): 422-26. <https://doi.org/10.1016/j.neuroscience.2009.03.074>.
- Larsson, Maria, et Johan Willander. 2009. « Autobiographical Odor Memory ». *Annals of the New York Academy of Sciences* 1170 (juillet): 318-23. <https://doi.org/10.1111/j.1749-6632.2009.03934.x>.
- LaSarge, Candi L., Karienn Souza Montgomery, Catherine Tucker, G. Simona Slaton, William H. Griffith, Barry Setlow, et Jennifer L. Bizon. 2007. « Deficits across Multiple Cognitive Domains in a Subset of Aged Fischer 344 Rats ». *Neurobiology of Aging* 28 (6): 928-36. <https://doi.org/10.1016/j.neurobiolaging.2006.04.010>.
- Laska, Matthias. 2002. « Olfactory Discrimination Ability for Aromatic Odorants as a Function of Oxygen Moiety ». *Chemical Senses* 27 (1): 23-29. <https://doi.org/10.1093/chemse/27.1.23>.
- Lawless, H., et T. Engen. 1977. « Associations to Odors: Interference, Mnemonics, and Verbal Labeling ». *Journal of Experimental Psychology. Human Learning and Memory* 3 (1): 52-59.
- Lazarini, Françoise, et Pierre-Marie Lledo. 2011. « Is Adult Neurogenesis Essential for Olfaction? ». *Trends in Neurosciences* 34 (1): 20-30. <https://doi.org/10.1016/j.tins.2010.09.006>.
- Le Guérer, Annick. 2002. *Les Pouvoirs de l'odeur*. Odile Jacob.
- LeDoux, Joseph. 2012. « Rethinking the Emotional Brain ». *Neuron* 73 (4): 653-76. <https://doi.org/10.1016/j.neuron.2012.02.004>.
- Lee, Anderson C., Huikai Tian, Xavier Grosmaître, et Minghong Ma. 2009. « Expression Patterns of Odorant Receptors and Response Properties of Olfactory Sensory Neurons in Aged Mice ». *Chemical Senses* 34 (8): 695-703. <https://doi.org/10.1093/chemse/bjp056>.



- Li, Jiafeng Jia, et Xiaochun Wang. 2020. « Unpleasant Food Odors Modulate the Processing of Facial Expressions: An Event-Related Potential Study ». *Frontiers in Neuroscience* 14: 686. <https://doi.org/10.3389/fnins.2020.00686>.
- Li, Qian, et Stephen D. Liberles. 2015. « Aversion and Attraction through Olfaction ». *Current Biology: CB* 25 (3): R120-29. <https://doi.org/10.1016/j.cub.2014.11.044>.
- Liberles, Stephen D. 2015. « Trace Amine-Associated Receptors: Ligands, Neural Circuits, and Behaviors ». *Current Opinion in Neurobiology* 34 (octobre): 1-7. <https://doi.org/10.1016/j.conb.2015.01.001>.
- Linster, Christiane, et Thomas A. Cleland. 2016. « Neuromodulation of Olfactory Transformations ». *Current Opinion in Neurobiology* 40: 170-77. <https://doi.org/10.1016/j.conb.2016.07.006>.
- Liu, Zhihui, Zijun Chen, Congping Shang, Fei Yan, Yingchao Shi, Jiajing Zhang, Baole Qu, et al. 2017. « IGF1-Dependent Synaptic Plasticity of Mitral Cells in Olfactory Memory during Social Learning ». *Neuron* 95 (1): 106-122.e5. <https://doi.org/10.1016/j.neuron.2017.06.015>.
- Lledo, Pierre-Marie, Gilles Gheusi, et Jean-Didier Vincent. 2005. « Information Processing in the Mammalian Olfactory System ». *Physiological Reviews* 85 (1): 281-317. <https://doi.org/10.1152/physrev.00008.2004>.
- Lledo, Pierre-Marie, et Matt Valley. 2016. « Adult Olfactory Bulb Neurogenesis ». *Cold Spring Harbor Perspectives in Biology* 8 (8). <https://doi.org/10.1101/cshperspect.a018945>.
- Lombion-Pouthier, Sandrine, Pierre Vandell, Sylvie Nezelof, Emmanuel Haffen, et Jean-Louis Millot. 2006. « Odor Perception in Patients with Mood Disorders ». *Journal of Affective Disorders* 90 (2-3): 187-91. <https://doi.org/10.1016/j.jad.2005.11.012>.
- Luisier, Anne-Claude, Geneviève Petitpierre, Annick Clerc Béro, Anne-Raphaëlle Richoz, Junpeng Lao, Roberto Caldara, et Moustafa Bensafi. 2019. « Visual and Hedonic Perception of Food Stimuli in Children with Autism Spectrum Disorders and Their Relationship to Food Neophobia ». *Perception* 48 (3): 197-213. <https://doi.org/10.1177/0301006619828300>.
- Luisier, Anne-Claude, Genevieve Petitpierre, Annick Clerc Béro, David Garcia-Burgos, et Moustafa Bensafi. 2019. « Effects of Familiarization on Odor Hedonic Responses and Food Choices in Children with Autism Spectrum Disorders ». *Autism: The International Journal of Research and Practice* 23 (6): 1460-71. <https://doi.org/10.1177/1362361318815252>.
- Luisier, Anne-Claude, Genevieve Petitpierre, Camille Ferdenzi, Annick Clerc Béro, Agnes Giboreau, Catherine Rouby, et Moustafa Bensafi. 2015. « Odor Perception in Children with Autism Spectrum Disorder and Its Relationship to Food Neophobia ». *Frontiers in Psychology* 6: 1830. <https://doi.org/10.3389/fpsyg.2015.01830>.
- Luskin, M. B., et J. L. Price. 1983. « The Topographic Organization of Associational Fibers of the Olfactory System in the Rat, Including Centrifugal Fibers to the Olfactory Bulb ». *The Journal of Comparative Neurology* 216 (3): 264-91. <https://doi.org/10.1002/cne.902160305>.
- Lynch, Gary, et Richard Granger. 1989. « Simulation and Analysis of a Simple Cortical Network ». Édité par Robert D. Hawkins et Gordon H. Bower. *Psychology of Learning and Motivation, Computational Models of Learning in Simple Neural Systems*, 23 (janvier): 205-41. [https://doi.org/10.1016/S0079-7421\(08\)60112-7](https://doi.org/10.1016/S0079-7421(08)60112-7).
- Mackay-Sim, A., et P. W. Kittel. 1991. « On the Life Span of Olfactory Receptor Neurons ». *The European Journal of Neuroscience* 3 (3): 209-15. <https://doi.org/10.1111/j.1460-9568.1991.tb00081.x>.
- Macrides, F., B. J. Davis, W. M. Youngs, N. S. Nadi, et F. L. Margolis. 1981. « Cholinergic and Catecholaminergic Afferents to the Olfactory Bulb in the Hamster: A

- Neuroanatomical, Biochemical, and Histochemical Investigation ». *The Journal of Comparative Neurology* 203 (3): 495-514. <https://doi.org/10.1002/cne.902030311>.
- Malnic, B., J. Hirono, T. Sato, et L. B. Buck. 1999. « Combinatorial Receptor Codes for Odors ». *Cell* 96 (5): 713-23. [https://doi.org/10.1016/s0092-8674\(00\)80581-4](https://doi.org/10.1016/s0092-8674(00)80581-4).
- Mandaïron, Nathalie, Nicola Kuczewski, Florence Kermen, Jérémy Forest, Maellie Midroit, Marion Richard, Marc Thevenet, Joelle Sacquet, Christiane Linster, et Anne Didier. 2018. « Opposite Regulation of Inhibition by Adult-Born Granule Cells during Implicit versus Explicit Olfactory Learning ». *ELife* 7. <https://doi.org/10.7554/eLife.34976>.
- Mandaïron, Nathalie, Shane T. Peace, Karim Boudadi, Christine E. Boxhorn, Venkata Anupama Narla, Sara D. Suffis, et Thomas A. Cleland. 2011. « Compensatory Responses to Age-Related Decline in Odor Quality Acuity: Cholinergic Neuromodulation and Olfactory Enrichment ». *Neurobiology of Aging* 32 (12): 2254-65. <https://doi.org/10.1016/j.neurobiolaging.2009.12.024>.
- Mandaïron, Nathalie, Johan Poncelet, Moustafa Bensafi, et Anne Didier. 2009. « Humans and Mice Express Similar Olfactory Preferences ». *PloS One* 4 (1): e4209. <https://doi.org/10.1371/journal.pone.0004209>.
- Mantel, Marylou, Camille Ferdenzi, Jean-Michel Roy, et Moustafa Bensafi. 2019. « Individual Differences as a Key Factor to Uncover the Neural Underpinnings of Hedonic and Social Functions of Human Olfaction: Current Findings from PET and FMRI Studies and Future Considerations ». *Brain Topography* 32 (6): 977-86. <https://doi.org/10.1007/s10548-019-00733-9>.
- Markovic, Katrin, Udo Reulbach, Agapi Vassiliadu, Jens Lunkenheimer, Birgit Lunkenheimer, Rita Spannenberger, et Norbert Thuerauf. 2007. « Good News for Elderly Persons: Olfactory Pleasure Increases at Later Stages of the Life Span ». *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 62 (11): 1287-93. <https://doi.org/10.1093/gerona/62.11.1287>.
- Marschner, A., T. Mell, I. Wartenburger, A. Villringer, F. M. Reischies, et H. R. Heekeren. 2005. « Reward-Based Decision-Making and Aging ». *Brain Research Bulletin*, 2nd Conference on NeuroEconomics - ConNEcs 2004, 67 (5): 382-90. <https://doi.org/10.1016/j.brainresbull.2005.06.010>.
- Martin, Claire, Rémi Gervais, Etienne Hugues, Belkacem Messaoudi, et Nadine Ravel. 2004. « Learning Modulation of Odor-Induced Oscillatory Responses in the Rat Olfactory Bulb: A Correlate of Odor Recognition? » *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 24 (2): 389-97. <https://doi.org/10.1523/JNEUROSCI.3433-03.2004>.
- Martínez-Ricós, Joana, Carmen Agustín-Pavón, Enrique Lanuza, et Fernando Martínez-García. 2007. « Intraspecific Communication through Chemical Signals in Female Mice: Reinforcing Properties of Involatile Male Sexual Pheromones ». *Chemical Senses* 32 (2): 139-48. <https://doi.org/10.1093/chemse/bjl039>.
- Mathuru, Ajay S., Caroline Kibat, Wei Fun Cheong, Guanghou Shui, Markus R. Wenk, Rainer W. Friedrich, et Suresh Jesuthasan. 2012. « Chondroitin Fragments Are Odorants That Trigger Fear Behavior in Fish ». *Current Biology: CB* 22 (6): 538-44. <https://doi.org/10.1016/j.cub.2012.01.061>.
- McGuigan, Sara, Shou-Han Zhou, Méadhbh B. Brosnan, Dominic Thyagarajan, Mark A. Bellgrove, et Trevor T.-J. Chong. 2019. « Dopamine Restores Cognitive Motivation in Parkinson's Disease ». *Brain: A Journal of Neurology* 142 (3): 719-32. <https://doi.org/10.1093/brain/awy341>.
- McReynolds, Jayme R., Analisa Taylor, Oliver Vranjkovic, Terra Ambrosius, Olivia Derricks, Brittany Nino, Beliz Kurtoglu, et al. 2017. « Corticosterone Potentiation of Cocaine-Induced Reinstatement of Conditioned Place Preference in Mice Is Mediated by

- Blockade of the Organic Cation Transporter 3 ». *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 42 (3): 757-65. <https://doi.org/10.1038/npp.2016.187>.
- Meierhenrich, Uwe J., Jérôme Golebiowski, Xavier Fernandez, et Daniel Cabrol-Bass. 2004. « The Molecular Basis of Olfactory Chemoreception ». *Angewandte Chemie (International Ed. in English)* 43 (47): 6410-12. <https://doi.org/10.1002/anie.200462322>.
- Meiners, Torsten, Felix Wäckers, et Wallace Joe Lewis. 2002. « The Effect of Molecular Structure on Olfactory Discrimination by the Parasitoid Microplitis Croceipes ». *Chemical Senses* 27 (9): 811-16. <https://doi.org/10.1093/chemse/27.9.811>.
- Mick, G., H. Cooper, et M. Magnin. 1993. « Retinal Projection to the Olfactory Tubercle and Basal Telencephalon in Primates ». *The Journal of Comparative Neurology* 327 (2): 205-19. <https://doi.org/10.1002/cne.903270204>.
- Miltner, W., M. Matjak, C. Braun, H. Diekmann, et S. Brody. 1994. « Emotional Qualities of Odors and Their Influence on the Startle Reflex in Humans ». *Psychophysiology* 31 (1): 107-10. <https://doi.org/10.1111/j.1469-8986.1994.tb01030.x>.
- Miyamichi, Kazunari, Fernando Amat, Farshid Moussavi, Chen Wang, Ian Wickersham, Nicholas R. Wall, Hiroki Taniguchi, et al. 2011. « Cortical Representations of Olfactory Input by Trans-Synaptic Tracing ». *Nature* 472 (7342): 191-96. <https://doi.org/10.1038/nature09714>.
- Moberg, Paul J., Steven E. Arnold, Richard L. Doty, Christian Kohler, Stephen Kanos, Steven Seigel, Raquel E. Gur, et Bruce I. Turetsky. 2003. « Impairment of Odor Hedonics in Men with Schizophrenia ». *The American Journal of Psychiatry* 160 (10): 1784-89. <https://doi.org/10.1176/appi.ajp.160.10.1784>.
- Mobley, Arie S., Diego J. Rodriguez-Gil, Fumiaki Imamura, et Charles A. Greer. 2014. « Aging in the Olfactory System ». *Trends in Neurosciences* 37 (2): 77-84. <https://doi.org/10.1016/j.tins.2013.11.004>.
- Moreno, Kevin Bath, Nicola Kuczewski, Joëlle Sacquet, Anne Didier, et Nathalie Mandairon. 2012. « Action of the Noradrenergic System on Adult-Born Cells Is Required for Olfactory Learning in Mice ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32 (11): 3748-58. <https://doi.org/10.1523/JNEUROSCI.6335-11.2012>.
- Moreno, Christiane Linster, Olga Escanilla, Joëlle Sacquet, Anne Didier, et Nathalie Mandairon. 2009. « Olfactory Perceptual Learning Requires Adult Neurogenesis ». *Proceedings of the National Academy of Sciences of the United States of America* 106 (42): 17980-85. <https://doi.org/10.1073/pnas.0907063106>.
- Moreno, Marion Richard, Benoit Landrein, Joëlle Sacquet, Anne Didier, et Nathalie Mandairon. 2014. « Alteration of Olfactory Perceptual Learning and Its Cellular Basis in Aged Mice ». *Neurobiology of Aging* 35 (3): 680-91. <https://doi.org/10.1016/j.neurobiolaging.2013.08.034>.
- Mori, K., et Y. Yoshihara. 1995. « Molecular Recognition and Olfactory Processing in the Mammalian Olfactory System ». *Progress in Neurobiology* 45 (6): 585-619. [https://doi.org/10.1016/0301-0082\(94\)00058-p](https://doi.org/10.1016/0301-0082(94)00058-p).
- Mori, Kensaku, et Hitoshi Sakano. 2011. « How Is the Olfactory Map Formed and Interpreted in the Mammalian Brain? » *Annual Review of Neuroscience* 34: 467-99. <https://doi.org/10.1146/annurev-neuro-112210-112917>.
- Morrison, E. E., et R. M. Costanzo. 1990. « Morphology of the Human Olfactory Epithelium ». *The Journal of Comparative Neurology* 297 (1): 1-13. <https://doi.org/10.1002/cne.902970102>.

- Moskowitz, H. R., et C. D. Barbe. 1977. « Profiling of Odor Components and Their Mixtures ». *Sensory Processes* 1 (3): 212-26.
- Mouly, A. M., A. Fort, N. Ben-Boutayab, et R. Gervais. 2001. « Olfactory Learning Induces Differential Long-Lasting Changes in Rat Central Olfactory Pathways ». *Neuroscience* 102 (1): 11-21. [https://doi.org/10.1016/s0306-4522\(00\)00476-0](https://doi.org/10.1016/s0306-4522(00)00476-0).
- Mrochen, A., F. Marxreiter, Z. Kohl, J. Schlachetzki, B. Renner, T. Schenk, J. Winkler, et J. Klucken. 2016. « From Sweet to Sweat: Hedonic Olfactory Range Is Impaired in Parkinson's Disease ». *Parkinsonism & Related Disorders* 22 (janvier): 9-14. <https://doi.org/10.1016/j.parkreldis.2015.09.035>.
- Murata, Koshi, Michiko Kanno, Nao Ieki, Kensaku Mori, et Masahiro Yamaguchi. 2015. « Mapping of Learned Odor-Induced Motivated Behaviors in the Mouse Olfactory Tubercle ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 35 (29): 10581-99. <https://doi.org/10.1523/JNEUROSCI.0073-15.2015>.
- Murphy. 2008. « The Chemical Senses and Nutrition in Older Adults ». *Journal of Nutrition For the Elderly* 27 (3-4): 247-65. <https://doi.org/10.1080/01639360802261862>.
- Murphy, C., W. S. Cain, et L. M. Bartoshuk. 1977. « Mutual Action of Taste and Olfaction ». *Sensory Processes* 1 (3): 204-11.
- Nadal, Marcos, et Martin Skov. 2018. « The Pleasure of Art as a Matter of Fact ». *Proceedings. Biological Sciences* 285 (1875). <https://doi.org/10.1098/rspb.2017.2252>.
- Naessen, R. 1971. « An Enquiry on the Morphological Characteristics and Possible Changes with Age in the Olfactory Region of Man ». *Acta Oto-Laryngologica* 71 (1): 49-62. <https://doi.org/10.3109/00016487109125332>.
- Nagayama, Shin, Ryota Homma, et Fumiaki Imamura. 2014. « Neuronal Organization of Olfactory Bulb Circuits ». *Frontiers in Neural Circuits* 8: 98. <https://doi.org/10.3389/fncir.2014.00098>.
- Nakashima, T., C. P. Kimmelman, et J. B. Snow. 1984. « Structure of Human Fetal and Adult Olfactory Neuroepithelium ». *Archives of Otolaryngology (Chicago, Ill.: 1960)* 110 (10): 641-46. <https://doi.org/10.1001/archotol.1984.00800360013003>.
- Naudin, Marine, Tatiana Carl, Simon Surguladze, Catherine Guillen, Philippe Gaillard, Catherine Belzung, Wissam El-Hage, et Borianna Atanasova. 2014. « Perceptive Biases in Major Depressive Episode ». *PloS One* 9 (2): e86832. <https://doi.org/10.1371/journal.pone.0086832>.
- Nemitz, J. W., et S. J. Goldberg. 1983. « Neuronal Responses of Rat Pyriform Cortex to Odor Stimulation: An Extracellular and Intracellular Study ». *Journal of Neurophysiology* 49 (1): 188-203. <https://doi.org/10.1152/jn.1983.49.1.188>.
- Nieh, Edward H., Sung-Yon Kim, Praneeth Namburi, et Kay M. Tye. 2013. « Optogenetic Dissection of Neural Circuits Underlying Emotional Valence and Motivated Behaviors ». *Brain Research* 1511 (mai): 73-92. <https://doi.org/10.1016/j.brainres.2012.11.001>.
- Norrara, Bianca, Felipe P. Fiuza, Ana Cristina Arrais, Ianara Mendonça Costa, Jose Ronaldo Santos, Rovenia Clara G. J. Engelberth, Jeferson Souza Cavalcante, Fausto Pierdoná Guzen, Jose Rodolfo Lopes P. Cavalcanti, et Marco Aurelio M. Freire. 2018. « Pattern of Tyrosine Hydroxylase Expression during Aging of Mesolimbic Pathway of the Rat ». *Journal of Chemical Neuroanatomy* 92: 83-91. <https://doi.org/10.1016/j.jchemneu.2018.05.004>.
- Olds, et Fobes. 1981. « The Central Basis of Motivation: Intracranial Self-Stimulation Studies ». *Annual Review of Psychology* 32: 523-74. <https://doi.org/10.1146/annurev.ps.32.020181.002515>.

- Olds, et Milner. 1954. « Positive Reinforcement Produced by Electrical Stimulation of Septal Area and Other Regions of Rat Brain ». *Journal of Comparative and Physiological Psychology* 47 (6): 419-27. <https://doi.org/10.1037/h0058775>.
- Olofsson, Jonas K., Emily Rogalski, Theresa Harrison, M.-Marsel Mesulam, et Jay A. Gottfried. 2013. « A Cortical Pathway to Olfactory Naming: Evidence from Primary Progressive Aphasia ». *Brain: A Journal of Neurology* 136 (Pt 4): 1245-59. <https://doi.org/10.1093/brain/awt019>.
- Paik, S. I., M. N. Lehman, A. M. Seiden, H. J. Duncan, et D. V. Smith. 1992. « Human Olfactory Biopsy. The Influence of Age and Receptor Distribution ». *Archives of Otolaryngology--Head & Neck Surgery* 118 (7): 731-38. <https://doi.org/10.1001/archotol.1992.01880070061012>.
- Patel, Roseanne C., et John Larson. 2009. « Impaired Olfactory Discrimination Learning and Decreased Olfactory Sensitivity in Aged C57Bl/6 Mice ». *Neurobiology of Aging* 30 (5): 829-37. <https://doi.org/10.1016/j.neurobiolaging.2007.08.007>.
- Patin, Alexandra, et Bettina M. Pause. 2015. « Human Amygdala Activations during Nasal Chemoreception ». *Neuropsychologia* 78 (novembre): 171-94. <https://doi.org/10.1016/j.neuropsychologia.2015.10.009>.
- Perry, Rosemarie E., Syrina Al Aïn, Charlis Raineiki, Regina M. Sullivan, et Donald A. Wilson. 2016. « Development of Odor Hedonics: Experience-Dependent Ontogeny of Circuits Supporting Maternal and Predator Odor Responses in Rats ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 36 (25): 6634-50. <https://doi.org/10.1523/JNEUROSCI.0632-16.2016>.
- Pessiglione, Mathias, Liane Schmidt, Bogdan Draganski, Raffael Kalisch, Hakwan Lau, Ray J. Dolan, et Chris D. Frith. 2007. « How the Brain Translates Money into Force: A Neuroimaging Study of Subliminal Motivation ». *Science (New York, N.Y.)* 316 (5826): 904-6. <https://doi.org/10.1126/science.1140459>.
- Petit-Skinner, Solange. 1981. *The Nauruans: Nature and Supernature in an Island of the Central Pacific*. MacDuff Press.
- Phillips, A. G., et G. J. Mogenson. 1969. « Self-Stimulation of the Olfactory Bulb ». *Physiology and Behavior* 4: 195-97.
- Pinching, A. J., et T. P. Powell. 1971. « The Neuron Types of the Glomerular Layer of the Olfactory Bulb ». *Journal of Cell Science* 9 (2): 305-45.
- Pinching and Powel. 1972. « Experimental Studies on the Axons Intrinsic to the Glomerular Layer of the Olfactory Bulb ». *Journal of Cell Science* 10 (3): 637-55.
- Poncelet, Johan, Fanny Rinck, Fanny Bourgeat, Benoist Schaal, Catherine Rouby, Moustafa Bensafi, et Thomas Hummel. 2010. « The Effect of Early Experience on Odor Perception in Humans: Psychological and Physiological Correlates ». *Behavioural Brain Research* 208 (2): 458-65. <https://doi.org/10.1016/j.bbr.2009.12.011>.
- Porter, Jess, Brent Craven, Rehan M. Khan, Shao-Ju Chang, Irene Kang, Benjamin Judkewitz, Benjamin Judkewicz, Jason Volpe, Gary Settles, et Noam Sobel. 2007. « Mechanisms of Scent-Tracking in Humans ». *Nature Neuroscience* 10 (1): 27-29. <https://doi.org/10.1038/nn1819>.
- Porter, R. H., J. M. Cernoch, et F. J. McLaughlin. 1983. « Maternal Recognition of Neonates through Olfactory Cues ». *Physiology & Behavior* 30 (1): 151-54. [https://doi.org/10.1016/0031-9384\(83\)90051-3](https://doi.org/10.1016/0031-9384(83)90051-3).
- Porter, R. H., et F. Etscorn. 1974. « Olfactory Imprinting Resulting from Brief Exposure in *Acomys Cahirinus* ». *Nature* 250 (5469): 732-33. <https://doi.org/10.1038/250732a0>.
- Price, J. L. 1973. « An Autoradiographic Study of Complementary Laminar Patterns of Termination of Afferent Fibers to the Olfactory Cortex ». *The Journal of Comparative Neurology* 150 (1): 87-108. <https://doi.org/10.1002/cne.901500105>.

- Proserpio, Cristina, Cees de Graaf, Monica Laureati, Ella Pagliarini, et Sanne Boesveldt. 2017. « Impact of Ambient Odors on Food Intake, Saliva Production and Appetite Ratings ». *Physiology & Behavior* 174: 35-41. <https://doi.org/10.1016/j.physbeh.2017.02.042>.
- Raineki, Charlis, Stephanie Moriceau, et Regina M. Sullivan. 2010. « Developing a Neurobehavioral Animal Model of Infant Attachment to an Abusive Caregiver ». *Biological Psychiatry* 67 (12): 1137-45. <https://doi.org/10.1016/j.biopsych.2009.12.019>.
- Ressler, K. J., S. L. Sullivan, et L. B. Buck. 1993. « A Zonal Organization of Odorant Receptor Gene Expression in the Olfactory Epithelium ». *Cell* 73 (3): 597-609. [https://doi.org/10.1016/0092-8674\(93\)90145-g](https://doi.org/10.1016/0092-8674(93)90145-g).
- Rétiveau, A. N., E. Chambers Iv, et G. A. Milliken. 2004. « Common and Specific Effects of Fine Fragrances on the Mood of Women ». *Journal of Sensory Studies* 19 (5): 373-94. <https://doi.org/10.1111/j.1745-459x.2004.102803.x>.
- Rey, Nolwen L., Joëlle Sacquet, Alexandra Veyrac, François Jourdan, et Anne Didier. 2012. « Behavioral and Cellular Markers of Olfactory Aging and Their Response to Enrichment ». *Neurobiology of Aging* 33 (3): 626.e9-626.e23. <https://doi.org/10.1016/j.neurobiolaging.2011.03.026>.
- Richard, Marion B., Seth R. Taylor, et Charles A. Greer. 2010. « Age-Induced Disruption of Selective Olfactory Bulb Synaptic Circuits ». *Proceedings of the National Academy of Sciences of the United States of America* 107 (35): 15613-18. <https://doi.org/10.1073/pnas.1007931107>.
- Richardson, J. T., et G. M. Zucco. 1989. « Cognition and Olfaction: A Review ». *Psychological Bulletin* 105 (3): 352-60. <https://doi.org/10.1037/0033-2909.105.3.352>.
- Roberts, Sarah A., Amanda J. Davidson, Lynn McLean, Robert J. Beynon, et Jane L. Hurst. 2012. « Pheromonal Induction of Spatial Learning in Mice ». *Science (New York, N.Y.)* 338 (6113): 1462-65. <https://doi.org/10.1126/science.1225638>.
- Robinson, Terry E., et Kent C. Berridge. 2003. « Addiction ». *Annual Review of Psychology* 54: 25-53. <https://doi.org/10.1146/annurev.psych.54.101601.145237>.
- Rodden, Frank, et Brigitte Stemmer. 2008. « A Brief Introduction to Common Neuroimaging Techniques ». *Handbook of the Neuroscience of Language*, décembre, 57-68. <https://doi.org/10.1016/B978-0-08-045352-1.00006-9>.
- Roesch, Matthew R., Thomas A. Stalnaker, et Geoffrey Schoenbaum. 2007. « Associative Encoding in Anterior Piriform Cortex versus Orbitofrontal Cortex during Odor Discrimination and Reversal Learning ». *Cerebral Cortex (New York, N.Y.: 1991)* 17 (3): 643-52. <https://doi.org/10.1093/cercor/bhk009>.
- Rolls. 2005. « Taste, Olfactory, and Food Texture Processing in the Brain, and the Control of Food Intake ». *Physiology & Behavior* 85 (1): 45-56. <https://doi.org/10.1016/j.physbeh.2005.04.012>.
- Rolls, Edmund T., Morten L. Kringelbach, et Ivan E. T. de Araujo. 2003. « Different Representations of Pleasant and Unpleasant Odours in the Human Brain ». *The European Journal of Neuroscience* 18 (3): 695-703. <https://doi.org/10.1046/j.1460-9568.2003.02779.x>.
- Rolls, et J. H. Rolls. 1997. « Olfactory Sensory-Specific Satiety in Humans ». *Physiology & Behavior* 61 (3): 461-73. [https://doi.org/10.1016/s0031-9384\(96\)00464-7](https://doi.org/10.1016/s0031-9384(96)00464-7).
- Root, Cory M., Christine A. Denny, René Hen, et Richard Axel. 2014. « The Participation of Cortical Amygdala in Innate, Odour-Driven Behaviour ». *Nature* 515 (7526): 269-73. <https://doi.org/10.1038/nature13897>.
- Rosen, Jeffrey B., Arun Asok, et Trisha Chakraborty. 2015. « The Smell of Fear: Innate Threat of 2,5-Dihydro-2,4,5-Trimethylthiazoline, a Single Molecule Component of a Predator Odor ». *Frontiers in Neuroscience* 9: 292. <https://doi.org/10.3389/fnins.2015.00292>.

- Ross, Jordan M., et Max L. Fletcher. 2019. « Aversive Learning-Induced Plasticity throughout the Adult Mammalian Olfactory System: Insights across Development ». *Journal of Bioenergetics and Biomembranes* 51 (1): 15-27. <https://doi.org/10.1007/s10863-018-9770-z>.
- Roth, Tania L., et Regina M. Sullivan. 2005. « Memory of Early Maltreatment: Neonatal Behavioral and Neural Correlates of Maternal Maltreatment within the Context of Classical Conditioning ». *Biological Psychiatry* 57 (8): 823-31. <https://doi.org/10.1016/j.biopsych.2005.01.032>.
- Royet, Jean-P., Jane Plailly, Chantal Delon-Martin, David A. Kareken, et Christoph Segebarth. 2003. « fMRI of Emotional Responses to Odors: Influence of Hedonic Valence and Judgment, Handedness, and Gender ». *NeuroImage* 20 (2): 713-28. [https://doi.org/10.1016/S1053-8119\(03\)00388-4](https://doi.org/10.1016/S1053-8119(03)00388-4).
- Rudy, J. W., et M. D. Cheate. 1977. « Odor-Aversion Learning in Neonatal Rats ». *Science (New York, N.Y.)* 198 (4319): 845-46. <https://doi.org/10.1126/science.918668>.
- Saito, Harumi, Qiuyi Chi, Hanyi Zhuang, Hiroaki Matsunami, et Joel D. Mainland. 2009. « Odor Coding by a Mammalian Receptor Repertoire ». *Science Signaling* 2 (60): ra9. <https://doi.org/10.1126/scisignal.2000016>.
- Saito, Harumi, Hirofumi Nishizumi, Satoshi Suzuki, Hideyuki Matsumoto, Nao Ieki, Takaya Abe, Hiroshi Kiyonari, et al. 2017. « Immobility Responses Are Induced by Photoactivation of Single Glomerular Species Responsive to Fox Odour TMT ». *Nature Communications* 8: 16011. <https://doi.org/10.1038/ncomms16011>.
- Salcedo, Ernesto, Chunbo Zhang, Eugene Kronberg, et Diego Restrepo. 2005. « Analysis of Training-Induced Changes in Ethyl Acetate Odor Maps Using a New Computational Tool to Map the Glomerular Layer of the Olfactory Bulb ». *Chemical Senses* 30 (7): 615-26. <https://doi.org/10.1093/chemse/bji055>.
- Salimpoor, Valorie N., Mitchel Benovoy, Kevin Larcher, Alain Dagher, et Robert J. Zatorre. 2011. « Anatomically Distinct Dopamine Release during Anticipation and Experience of Peak Emotion to Music ». *Nature Neuroscience* 14 (2): 257-62. <https://doi.org/10.1038/nn.2726>.
- Sama-ul-Haq, null, Mohammad Tahir, et Khalid P. Lone. 2008. « Age and Gender-Related Differences in Mitral Cells of Olfactory Bulb ». *Journal of the College of Physicians and Surgeons--Pakistan: JCPSP* 18 (11): 669-73. <https://doi.org/10.2008/JCPSP.669973>.
- Santos, Daniel V., Evan R. Reiter, Laurence J. DiNardo, et Richard M. Costanzo. 2004. « Hazardous Events Associated with Impaired Olfactory Function ». *Archives of Otolaryngology--Head & Neck Surgery* 130 (3): 317-19. <https://doi.org/10.1001/archotol.130.3.317>.
- Saucier, D., et L. Astic. 1986. « Analysis of the Topographical Organization of Olfactory Epithelium Projections in the Rat ». *Brain Research Bulletin* 16 (4): 455-62. [https://doi.org/10.1016/0361-9230\(86\)90173-5](https://doi.org/10.1016/0361-9230(86)90173-5).
- Schaal, B., L. Marlier, et R. Soussignan. 2000. « Human Foetuses Learn Odours from Their Pregnant Mother's Diet ». *Chemical Senses* 25 (6): 729-37. <https://doi.org/10.1093/chemse/25.6.729>.
- Schiffman, David E. Robinson, et Robert P. Erickson. 1977. « Multidimensional Scaling of Odorants: Examination of Psychological and Physicochemical Dimensions ». *Chemical Senses* 2 (3): 375-90. <https://doi.org/10.1093/chemse/2.3.375>.
- Schiffman, E. A. Sattely-Miller, M. S. Suggs, et B. G. Graham. 1995. « The Effect of Pleasant Odors and Hormone Status on Mood of Women at Midlife ». *Brain Research Bulletin* 36 (1): 19-29. [https://doi.org/10.1016/0361-9230\(94\)00133-1](https://doi.org/10.1016/0361-9230(94)00133-1).

- Schiffman, M. S. Suggs, et E. A. Sattely-Miller. 1995. « Effect of Pleasant Odors on Mood of Males at Midlife: Comparison of African-American and European-American Men ». *Brain Research Bulletin* 36 (1): 31-37. [https://doi.org/10.1016/0361-9230\(94\)00134-m](https://doi.org/10.1016/0361-9230(94)00134-m).
- Schiffman, et Jennifer Zervakis. 2002. « Taste and Smell Perception in the Elderly: Effect of Medications and Disease ». *Advances in Food and Nutrition Research* 44: 247-346. [https://doi.org/10.1016/s1043-4526\(02\)44006-5](https://doi.org/10.1016/s1043-4526(02)44006-5).
- Schneider, Nanette Y., Coralie Piccin, Frédérique Datiche, et Gérard Coureaud. 2016. « Spontaneous Brain Processing of the Mammary Pheromone in Rabbit Neonates Prior to Milk Intake ». *Behavioural Brain Research* 313: 191-200. <https://doi.org/10.1016/j.bbr.2016.07.014>.
- Schneider, S. P., et F. Macrides. 1978. « Laminar Distributions of Internuerons in the Main Olfactory Bulb of the Adult Hamster ». *Brain Research Bulletin* 3 (1): 73-82. [https://doi.org/10.1016/0361-9230\(78\)90063-1](https://doi.org/10.1016/0361-9230(78)90063-1).
- Schoppa, N. E., J. M. Kinzie, Y. Sahara, T. P. Segerson, et G. L. Westbrook. 1998. « Dendrodendritic Inhibition in the Olfactory Bulb Is Driven by NMDA Receptors ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 18 (17): 6790-6802.
- Schoppa, N. E., et G. L. Westbrook. 2001. « Glomerulus-Specific Synchronization of Mitral Cells in the Olfactory Bulb ». *Neuron* 31 (4): 639-51. [https://doi.org/10.1016/s0896-6273\(01\)00389-0](https://doi.org/10.1016/s0896-6273(01)00389-0).
- Schuligoj, R., J. Fernandez, R. P. Heavens, et D. J. Sirinathsinghji. 1993. « Decreased Tyrosine Hydroxylase mRNA but Not Cholecystokinin mRNA in the Pars Compacta of the Substantia Nigra and Ventral Tegmental Area of Aged Rats ». *Brain Research. Molecular Brain Research* 19 (4): 333-38. [https://doi.org/10.1016/0169-328x\(93\)90135-c](https://doi.org/10.1016/0169-328x(93)90135-c).
- Schultz, W. 1998. « Predictive Reward Signal of Dopamine Neurons ». *Journal of Neurophysiology* 80 (1): 1-27. <https://doi.org/10.1152/jn.1998.80.1.1>.
- Schultz, W., et R. Romo. 1990. « Dopamine Neurons of the Monkey Midbrain: Contingencies of Responses to Stimuli Eliciting Immediate Behavioral Reactions ». *Journal of Neurophysiology* 63 (3): 607-24. <https://doi.org/10.1152/jn.1990.63.3.607>.
- Schwob, J. E., et J. L. Price. 1978. « The Cortical Projection of the Olfactory Bulb: Development in Fetal and Neonatal Rats Correlated with Quantitative Variations in Adult Rats ». *Brain Research* 151 (2): 369-74. [https://doi.org/10.1016/0006-8993\(78\)90891-0](https://doi.org/10.1016/0006-8993(78)90891-0).
- Schwob and Price. 1984. « The Development of Axonal Connections in the Central Olfactory System of Rats ». *The Journal of Comparative Neurology* 223 (2): 177-202. <https://doi.org/10.1002/cne.902230204>.
- Scott, J. W., R. L. McBride, et S. P. Schneider. 1980. « The Organization of Projections from the Olfactory Bulb to the Piriform Cortex and Olfactory Tubercle in the Rat ». *The Journal of Comparative Neurology* 194 (3): 519-34. <https://doi.org/10.1002/cne.901940304>.
- Segura, Bàrbara, Hugo César Baggio, Elisabeth Solana, Eva M. Palacios, Pere Vendrell, Núria Bargalló, et Carme Junqué. 2013. « Neuroanatomical Correlates of Olfactory Loss in Normal Aged Subjects ». *Behavioural Brain Research* 246 (juin): 148-53. <https://doi.org/10.1016/j.bbr.2013.02.025>.
- Sellings, L. E. McQuade, et P. B. S. Clarke. 2006a. « Characterization of Dopamine-Dependent Rewarding and Locomotor Stimulant Effects of Intravenously-Administered Methylphenidate in Rats ». *Neuroscience* 141 (3): 1457-68. <https://doi.org/10.1016/j.neuroscience.2006.04.040>.



- Sellings, Lindsey E. McQuade, et Paul B. S. Clarke. 2006b. « Evidence for Multiple Sites within Rat Ventral Striatum Mediating Cocaine-Conditioned Place Preference and Locomotor Activation ». *The Journal of Pharmacology and Experimental Therapeutics* 317 (3): 1178-87. <https://doi.org/10.1124/jpet.105.100339>.
- Seo, Han-Seok, Marco Guarneros, Robyn Hudson, Hans Distel, Byung-Chan Min, Jin-Kyu Kang, Ilona Croy, Jan Vodicka, et Thomas Hummel. 2011. « Attitudes toward Olfaction: A Cross-Regional Study ». *Chemical Senses* 36 (2): 177-87. <https://doi.org/10.1093/chemse/bjq112>.
- Seubert, Janina, Erika J. Laukka, Debora Rizzuto, Thomas Hummel, Laura Fratiglioni, Lars Bäckman, et Maria Larsson. 2017. « Prevalence and Correlates of Olfactory Dysfunction in Old Age: A Population-Based Study ». *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 72 (8): 1072-79. <https://doi.org/10.1093/gerona/glx054>.
- Seubert, Janina, Amy F. Rea, James Loughead, et Ute Habel. 2009. « Mood Induction with Olfactory Stimuli Reveals Differential Affective Responses in Males and Females ». *Chemical Senses* 34 (1): 77-84. <https://doi.org/10.1093/chemse/bjn054>.
- Sevelinges, Yannick, Rémi Gervais, Belkacem Messaoudi, Lionel Granjon, et Anne-Marie Mouly. 2004. « Olfactory Fear Conditioning Induces Field Potential Potentiation in Rat Olfactory Cortex and Amygdala ». *Learning & Memory (Cold Spring Harbor, N.Y.)* 11 (6): 761-69. <https://doi.org/10.1101/lm.83604>.
- Shepherd, Gordon M. 2004. « The Human Sense of Smell: Are We Better than We Think? » *PLoS Biology* 2 (5): E146. <https://doi.org/10.1371/journal.pbio.0020146>.
- Shin, Rick, Mei Qin, Zhong-Hua Liu, et Satoshi Ikemoto. 2008. « Intracranial Self-Administration of MDMA into the Ventral Striatum of the Rat: Differential Roles of the Nucleus Accumbens Shell, Core, and Olfactory Tubercle ». *Psychopharmacology* 198 (2): 261-70. <https://doi.org/10.1007/s00213-008-1131-x>.
- Shipley, M. T. 1985. « Transport of Molecules from Nose to Brain: Transneuronal Anterograde and Retrograde Labeling in the Rat Olfactory System by Wheat Germ Agglutinin-Horseradish Peroxidase Applied to the Nasal Epithelium ». *Brain Research Bulletin* 15 (2): 129-42. [https://doi.org/10.1016/0361-9230\(85\)90129-7](https://doi.org/10.1016/0361-9230(85)90129-7).
- Sojitra, Ravi B., Itamar Lerner, Jessica R. Petok, et Mark A. Gluck. 2018. « Age Affects Reinforcement Learning through Dopamine-Based Learning Imbalance and High Decision Noise-Not through Parkinsonian Mechanisms ». *Neurobiology of Aging* 68: 102-13. <https://doi.org/10.1016/j.neurobiolaging.2018.04.006>.
- Sosulski, Dara L., Maria Lissitsyna Bloom, Tyler Cutforth, Richard Axel, et Sandeep Robert Datta. 2011. « Distinct Representations of Olfactory Information in Different Cortical Centres ». *Nature* 472 (7342): 213-16. <https://doi.org/10.1038/nature09868>.
- Soussignan, R., B. Schaal, L. Marlier, et T. Jiang. 1997. « Facial and Autonomic Responses to Biological and Artificial Olfactory Stimuli in Human Neonates: Re-Examining Early Hedonic Discrimination of Odors ». *Physiology & Behavior* 62 (4): 745-58. [https://doi.org/10.1016/s0031-9384\(97\)00187-x](https://doi.org/10.1016/s0031-9384(97)00187-x).
- Steinberg, Elizabeth E., Josiah R. Boivin, Benjamin T. Saunders, Ilana B. Witten, Karl Deisseroth, et Patricia H. Janak. 2014. « Positive Reinforcement Mediated by Midbrain Dopamine Neurons Requires D1 and D2 Receptor Activation in the Nucleus Accumbens ». *PLoS One* 9 (4): e94771. <https://doi.org/10.1371/journal.pone.0094771>.
- Stettler, Dan D., et Richard Axel. 2009. « Representations of Odor in the Piriform Cortex ». *Neuron* 63 (6): 854-64. <https://doi.org/10.1016/j.neuron.2009.09.005>.
- Stevens. 1958. « Problems and Methods of Psychophysics ». *Psychological Bulletin* 55 (4): 177-96. <https://doi.org/10.1037/h0044251>.

- Stevens, Joseph C., William S. Cain, David E. Weinstein, et John B. Pierce. 1987. « Aging Impairs the Ability to Detect Gas Odor ». *Fire Technology* 23 (3): 198-204. <https://doi.org/10.1007/BF01036936>.
- Stevenson, Richard J. 2010. « An Initial Evaluation of the Functions of Human Olfaction ». *Chemical Senses* 35 (1): 3-20. <https://doi.org/10.1093/chemse/bjp083>.
- Strotmann, J., I. Wanner, T. Helfrich, A. Beck, C. Meinken, S. Kubick, et H. Breer. 1994. « Olfactory Neurones Expressing Distinct Odorant Receptor Subtypes Are Spatially Segregated in the Nasal Neuroepithelium ». *Cell and Tissue Research* 276 (3): 429-38. <https://doi.org/10.1007/BF00343941>.
- Sucker, Kirsten, Ralf Both, Michael Bischoff, Rainer Guski, et Gerhard Winneke. 2008. « Odor Frequency and Odor Annoyance. Part I: Assessment of Frequency, Intensity and Hedonic Tone of Environmental Odors in the Field ». *International Archives of Occupational and Environmental Health* 81 (6): 671-82. <https://doi.org/10.1007/s00420-007-0259-z>.
- Sullivan, R. M., et D. A. Wilson. 1993. « Role of the Amygdala Complex in Early Olfactory Associative Learning ». *Behavioral Neuroscience* 107 (2): 254-63. <https://doi.org/10.1037//0735-7044.107.2.254>.
- Sultan, Sébastien, Nolwen Rey, Joelle Sacquet, Nathalie Mandairon, et Anne Didier. 2011. « Newborn Neurons in the Olfactory Bulb Selected for Long-Term Survival through Olfactory Learning Are Prematurely Suppressed When the Olfactory Memory Is Erased ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31 (42): 14893-98. <https://doi.org/10.1523/JNEUROSCI.3677-11.2011>.
- Suzuki, Y., H. D. Critchley, J. Suckling, R. Fukuda, S. C. Williams, C. Andrew, R. Howard, et al. 2001. « Functional Magnetic Resonance Imaging of Odor Identification: The Effect of Aging ». *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 56 (12): M756-760. <https://doi.org/10.1093/gerona/56.12.m756>.
- Swanson, L. W., et G. D. Petrovich. 1998. « What Is the Amygdala? » *Trends in Neurosciences* 21 (8): 323-31. [https://doi.org/10.1016/s0166-2236\(98\)01265-x](https://doi.org/10.1016/s0166-2236(98)01265-x).
- Tahsili-Fahadan, Pouya, Noushin Yahyavi-Firouz-Abadi, Mohammad Ali Khoshnoodi, Rouzbeh Motiei-Langroudi, Seyed Amir Tahaei, Mohammad Hossein Ghahremani, et Ahmad Reza Dehpour. 2006. « Agmatine Potentiates Morphine-Induced Conditioned Place Preference in Mice: Modulation by Alpha2-Adrenoceptors ». *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 31 (8): 1722-32. <https://doi.org/10.1038/sj.npp.1300929>.
- Takahashi. 2014. « Olfactory Systems and Neural Circuits That Modulate Predator Odor Fear ». *Frontiers in Behavioral Neuroscience* 8: 72. <https://doi.org/10.3389/fnbeh.2014.00072>.
- Takahashi, Shin Nagayama, et Kensaku Mori. 2004. « Detection and Masking of Spoiled Food Smells by Odor Maps in the Olfactory Bulb ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 24 (40): 8690-94. <https://doi.org/10.1523/JNEUROSCI.2510-04.2004>.
- Tiedemann, Lena J., Arjen Alink, Judith Beck, Christian Büchel, et Stefanie Brassens. 2020. « Valence Encoding Signals in the Human Amygdala and the Willingness to Eat ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 40 (27): 5264-72. <https://doi.org/10.1523/JNEUROSCI.2382-19.2020>.
- Toepper, Max. 2017. « Dissociating Normal Aging from Alzheimer's Disease: A View from Cognitive Neuroscience ». *Journal of Alzheimer's Disease* 57 (2): 331-52. <https://doi.org/10.3233/JAD-161099>.

- Tong, Michelle T., Shane T. Peace, et Thomas A. Cleland. 2014. « Properties and Mechanisms of Olfactory Learning and Memory ». *Frontiers in Behavioral Neuroscience* 8: 238. <https://doi.org/10.3389/fnbeh.2014.00238>.
- Touhara, K., S. Sengoku, K. Inaki, A. Tsuboi, J. Hirono, T. Sato, H. Sakano, et T. Haga. 1999. « Functional Identification and Reconstitution of an Odorant Receptor in Single Olfactory Neurons ». *Proceedings of the National Academy of Sciences of the United States of America* 96 (7): 4040-45. <https://doi.org/10.1073/pnas.96.7.4040>.
- Troccaz, Myriam, Gerrit Borchard, Christine Vuilleumier, Sophie Raviot-Derrien, Yvan Niclass, Sabine Beccucci, et Christian Starckenmann. 2009. « Gender-Specific Differences between the Concentrations of Nonvolatile (R)/(S)-3-Methyl-3-Sulfanylhexan-1-ol and (R)/(S)-3-Hydroxy-3-Methyl-Hexanoic Acid Odor Precursors in Axillary Secretions ». *Chemical Senses* 34 (3): 203-10. <https://doi.org/10.1093/chemse/bjn076>.
- Tye, Kay M., Julie J. Mirzabekov, Melissa R. Warden, Emily A. Ferenczi, Hsing-Chen Tsai, Joel Finkelstein, Sung-Yon Kim, et al. 2013. « Dopamine Neurons Modulate Neural Encoding and Expression of Depression-Related Behaviour ». *Nature* 493 (7433): 537-41. <https://doi.org/10.1038/nature11740>.
- Ueno, Hiroshi, Atsumi Shimada, Shunsuke Suemitsu, Shinji Murakami, Naoya Kitamura, Kenta Wani, Yu Takahashi, et al. 2019. « Comprehensive Behavioral Study of the Effects of Vanillin Inhalation in Mice ». *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* 115 (juillet): 108879. <https://doi.org/10.1016/j.biopha.2019.108879>.
- Vassar, R., J. Ngai, et R. Axel. 1993. « Spatial Segregation of Odorant Receptor Expression in the Mammalian Olfactory Epithelium ». *Cell* 74 (2): 309-18. [https://doi.org/10.1016/0092-8674\(93\)90422-m](https://doi.org/10.1016/0092-8674(93)90422-m).
- Vieillard, Sandrine, Lucas Ronat, Alessia Baccarani, Benoist Schaal, Jean-Yves Baudouin, et Renaud Brochard. 2020. « Age Differences in Olfactory Affective Responses: Evidence for a Positivity Effect and an Emotional Dedifferentiation ». *Neuropsychology, Development, and Cognition. Section B, Aging, Neuropsychology and Cognition*, août, 1-14. <https://doi.org/10.1080/13825585.2020.1799926>.
- Voorn, Pieter, Louk J. M. J. Vanderschuren, Henk J. Groenewegen, Trevor W. Robbins, et Cyriel M. A. Pennartz. 2004. « Putting a Spin on the Dorsal-Ventral Divide of the Striatum ». *Trends in Neurosciences* 27 (8): 468-74. <https://doi.org/10.1016/j.tins.2004.06.006>.
- Vrontou, Sophia, Allan M. Wong, Kristofer K. Rau, H. Richard Koerber, et David J. Anderson. 2013. « Genetic Identification of C Fibres That Detect Massage-like Stroking of Hairy Skin in Vivo ». *Nature* 493 (7434): 669-73. <https://doi.org/10.1038/nature11810>.
- Walliczek-Dworschak, Ute, et Thomas Hummel. 2017. « The Human Sense of Olfaction ». *Facial Plastic Surgery: FPS* 33 (4): 396-404. <https://doi.org/10.1055/s-0037-1603828>.
- Walsh-Messinger, Julie, Philip S. Wong, Daniel Antonius, Kevin McMahon, Lewis A. Opler, Paul Michael Ramirez, et Dolores Malaspina. 2018. « Sex Differences in Hedonic Judgement of Odors in Schizophrenia Cases and Healthy Controls ». *Psychiatry Research* 269: 345-53. <https://doi.org/10.1016/j.psychres.2018.08.058>.
- Wang, Paul J. Eslinger, Michael B. Smith, et Qing X. Yang. 2005. « Functional Magnetic Resonance Imaging Study of Human Olfaction and Normal Aging ». *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 60 (4): 510-14. <https://doi.org/10.1093/gerona/60.4.510>.
- Wang, Jia Qi, Shiliang Zhang, Huikun Wang, et Marisela Morales. 2015. « Rewarding Effects of Optical Stimulation of Ventral Tegmental Area Glutamatergic Neurons ». *The*

- Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 35 (48): 15948-54. <https://doi.org/10.1523/JNEUROSCI.3428-15.2015>.
- Wassiliwizky, Eugen, Stefan Koelsch, Valentin Wagner, Thomas Jacobsen, et Winfried Menninghaus. 2017. « The Emotional Power of Poetry: Neural Circuitry, Psychophysiology and Compositional Principles ». *Social Cognitive and Affective Neuroscience* 12 (8): 1229-40. <https://doi.org/10.1093/scan/nsx069>.
- Watanabe, H. 1987. « Differential Decrease in the Rate of Dopamine Synthesis in Several Dopaminergic Neurons of Aged Rat Brain ». *Experimental Gerontology* 22 (1): 17-25. [https://doi.org/10.1016/0531-5565\(87\)90011-8](https://doi.org/10.1016/0531-5565(87)90011-8).
- Weiler, E., et A. I. Farbman. 1997. « Proliferation in the Rat Olfactory Epithelium: Age-Dependent Changes ». *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 17 (10): 3610-22.
- Weisfeld, Glenn E., Tiffany Czilli, Krista A. Phillips, James A. Gall, et Cary M. Lichtman. 2003. « Possible Olfaction-Based Mechanisms in Human Kin Recognition and Inbreeding Avoidance ». *Journal of Experimental Child Psychology* 85 (3): 279-95. [https://doi.org/10.1016/s0022-0965\(03\)00061-4](https://doi.org/10.1016/s0022-0965(03)00061-4).
- Werner, J S, D H Peterzell, et A J Scheetz. 1990. « Light, vision, and aging ». *Optometry and vision science : official publication of the American Academy of Optometry* 67 (3): 214-29.
- Wesson, Daniel W., et Donald A. Wilson. 2011. « Sniffing out the Contributions of the Olfactory Tubercle to the Sense of Smell: Hedonics, Sensory Integration, and More? ». *Neuroscience and Biobehavioral Reviews* 35 (3): 655-68. <https://doi.org/10.1016/j.neubiorev.2010.08.004>.
- Westberry, Jenne M., et Michael Meredith. 2016. « GABAergic Mechanisms Contributing to Categorical Amygdala Responses to Chemosensory Signals ». *Neuroscience* 331: 186-96. <https://doi.org/10.1016/j.neuroscience.2016.06.020>.
- White, Lowell E. 1965. « Olfactory Bulb Projections of the Rat ». *The Anatomical Record* 152 (4): 465-79. <https://doi.org/10.1002/ar.1091520406>.
- Wiegert, J. Simon, Mathias Mahn, Matthias Prigge, Yoav Printz, et Ofer Yizhar. 2017. « Silencing Neurons: Tools, Applications, and Experimental Constraints ». *Neuron* 95 (3): 504-29. <https://doi.org/10.1016/j.neuron.2017.06.050>.
- Willhite, David C., Katherine T. Nguyen, Arjun V. Masurkar, Charles A. Greer, Gordon M. Shepherd, et Wei R. Chen. 2006. « Viral Tracing Identifies Distributed Columnar Organization in the Olfactory Bulb ». *Proceedings of the National Academy of Sciences of the United States of America* 103 (33): 12592-97. <https://doi.org/10.1073/pnas.0602032103>.
- Wilson. 1998. « Synaptic Correlates of Odor Habituation in the Rat Anterior Piriform Cortex ». *Journal of Neurophysiology* 80 (2): 998-1001. <https://doi.org/10.1152/jn.1998.80.2.998>.
- Wilson, Donald A., et Regina M. Sullivan. 2011. « Cortical Processing of Odor Objects ». *Neuron* 72 (4): 506-19. <https://doi.org/10.1016/j.neuron.2011.10.027>.
- Wilson, et Christiane Linster. 2008. « Neurobiology of a Simple Memory ». *Journal of Neurophysiology* 100 (1): 2-7. <https://doi.org/10.1152/jn.90479.2008>.
- Wilson, et Richard J. Stevenson. 2003. « The Fundamental Role of Memory in Olfactory Perception ». *Trends in Neurosciences* 26 (5): 243-47. [https://doi.org/10.1016/S0166-2236\(03\)00076-6](https://doi.org/10.1016/S0166-2236(03)00076-6).
- Wilson, et R. M. Sullivan. 1994. « Neurobiology of Associative Learning in the Neonate: Early Olfactory Learning ». *Behavioral and Neural Biology* 61 (1): 1-18. [https://doi.org/10.1016/s0163-1047\(05\)80039-1](https://doi.org/10.1016/s0163-1047(05)80039-1).

- Wilson, Lei Yu, et David A. Bennett. 2011. « Odor Identification and Mortality in Old Age ». *Chemical Senses* 36 (1): 63-67. <https://doi.org/10.1093/chemse/bjq098>.
- Wisman, Arnaud, et Ilan Shrira. 2015. « The Smell of Death: Evidence That Putrescine Elicits Threat Management Mechanisms ». *Frontiers in Psychology* 6: 1274. <https://doi.org/10.3389/fpsyg.2015.01274>.
- Xiong, Angeline, et Daniel W. Wesson. 2016. « Illustrated Review of the Ventral Striatum's Olfactory Tubercle ». *Chemical Senses* 41 (7): 549-55. <https://doi.org/10.1093/chemse/bjw069>.
- Xu, Fuqiang, Michele Schaefer, Ikuhiro Kida, James Schafer, Nian Liu, Douglas L. Rothman, Fahmeed Hyder, Diego Restrepo, et Gordon M. Shepherd. 2005. « Simultaneous Activation of Mouse Main and Accessory Olfactory Bulbs by Odors or Pheromones ». *The Journal of Comparative Neurology* 489 (4): 491-500. <https://doi.org/10.1002/cne.20652>.
- Yabuki, Yoichi, Tetsuya Koide, Nobuhiko Miyasaka, Noriko Wakisaka, Miwa Masuda, Masamichi Ohkura, Junichi Nakai, et al. 2016. « Olfactory Receptor for Prostaglandin F2 $\alpha$  Mediates Male Fish Courtship Behavior ». *Nature Neuroscience* 19 (7): 897-904. <https://doi.org/10.1038/nn.4314>.
- Yamaguchi, Masahiro. 2017. « Functional Sub-Circuits of the Olfactory System Viewed from the Olfactory Bulb and the Olfactory Tubercle ». *Frontiers in Neuroanatomy* 11: 33. <https://doi.org/10.3389/fnana.2017.00033>.
- Yeomans, Martin R., Sirous Mobini, Toby D. Elliman, Helen C. Walker, et Richard J. Stevenson. 2006. « Hedonic and Sensory Characteristics of Odors Conditioned by Pairing with Tastants in Humans ». *Journal of Experimental Psychology. Animal Behavior Processes* 32 (3): 215-28. <https://doi.org/10.1037/0097-7403.32.3.215>.
- Yeshurun, Yaara, et Noam Sobel. 2010. « An Odor Is Not Worth a Thousand Words: From Multidimensional Odors to Unidimensional Odor Objects ». *Annual Review of Psychology* 61: 219-41, C1-5. <https://doi.org/10.1146/annurev.psych.60.110707.163639>.
- Yoder, Wendy M., Leslie S. Gaynor, Sara N. Burke, Barry Setlow, David W. Smith, et Jennifer L. Bizon. 2017. « Interaction between Age and Perceptual Similarity in Olfactory Discrimination Learning in F344 Rats: Relationships with Spatial Learning ». *Neurobiology of Aging* 53: 122-37. <https://doi.org/10.1016/j.neurobiolaging.2017.01.023>.
- Yousem, D. M., R. J. Geckle, W. B. Bilker, et R. L. Doty. 1998. « Olfactory Bulb and Tract and Temporal Lobe Volumes. Normative Data across Decades ». *Annals of the New York Academy of Sciences* 855 (novembre): 546-55. <https://doi.org/10.1111/j.1749-6632.1998.tb10624.x>.
- Yu, C. Ron, et Yunming Wu. 2017. « Regeneration and Rewiring of Rodent Olfactory Sensory Neurons ». *Experimental Neurology* 287 (Pt 3): 395-408. <https://doi.org/10.1016/j.expneurol.2016.06.001>.
- Yuan, Qi, Carolyn W. Harley, John H. McLean, et Thomas Knöpfel. 2002. « Optical Imaging of Odor Preference Memory in the Rat Olfactory Bulb ». *Journal of Neurophysiology* 87 (6): 3156-59. <https://doi.org/10.1152/jn.00917.2001>.
- Zald, D. H., et J. V. Pardo. 1997. « Emotion, Olfaction, and the Human Amygdala: Amygdala Activation during Aversive Olfactory Stimulation ». *Proceedings of the National Academy of Sciences of the United States of America* 94 (8): 4119-24. <https://doi.org/10.1073/pnas.94.8.4119>.
- Zarzo, Manuel. 2008. « Psychologic Dimensions in the Perception of Everyday Odors: Pleasantness and Edibility ». *Journal of Sensory Studies* 23 (3): 354-76. <https://doi.org/10.1111/j.1745-459X.2008.00160.x>.

- Zarzo. 2011. « Hedonic Judgments of Chemical Compounds Are Correlated with Molecular Size ». *Sensors (Basel, Switzerland)* 11 (4): 3667-86. <https://doi.org/10.3390/s110403667>.
- Zatorre, Robert J. 2015. « Musical Pleasure and Reward: Mechanisms and Dysfunction ». *Annals of the New York Academy of Sciences* 1337 (mars): 202-11. <https://doi.org/10.1111/nyas.12677>.
- Zeki, S. 2007. « The Neurobiology of Love ». *FEBS Letters* 581 (14): 2575-79. <https://doi.org/10.1016/j.febslet.2007.03.094>.
- Zelano, Moustafa Bensafi, Jess Porter, Joel Mainland, Brad Johnson, Elizabeth Bremner, Christina Telles, Rehan Khan, et Noam Sobel. 2005. « Attentional Modulation in Human Primary Olfactory Cortex ». *Nature Neuroscience* 8 (1). <https://doi.org/10.1038/nn1368>.
- Zelano, C., J. Montag, B. Johnson, R. Khan, et N. Sobel. 2007. « Dissociated Representations of Irritation and Valence in Human Primary Olfactory Cortex ». *Journal of Neurophysiology* 97 (3): 1969-76. <https://doi.org/10.1152/jn.01122.2006>.
- Zelano, Christina, et Noam Sobel. 2005. « Humans as an Animal Model for Systems-Level Organization of Olfaction ». *Neuron* 48 (3): 431-54. <https://doi.org/10.1016/j.neuron.2005.10.009>.
- Zhang, Zhijian, Qing Liu, Pengjie Wen, Jiaozhen Zhang, Xiaoping Rao, Ziming Zhou, Hongruo Zhang, et al. 2017. « Activation of the Dopaminergic Pathway from VTA to the Medial Olfactory Tubercle Generates Odor-Preference and Reward ». *ELife* 6. <https://doi.org/10.7554/eLife.25423>.
- Zhang, Zhijian, Hongruo Zhang, Pengjie Wen, Xutao Zhu, Li Wang, Qing Liu, Jie Wang, Xiaobin He, Huadong Wang, et Fuqiang Xu. 2017. « Whole-Brain Mapping of the Inputs and Outputs of the Medial Part of the Olfactory Tubercle ». *Frontiers in Neural Circuits* 11: 52. <https://doi.org/10.3389/fncir.2017.00052>.
- Zucco, Gesualdo M., Lara Aiello, Laura Turuani, et Egon Köster. 2012. « Odor-Evoked Autobiographical Memories: Age and Gender Differences along the Life Span ». *Chemical Senses* 37 (2): 179-89. <https://doi.org/10.1093/chemse/bjr089>.

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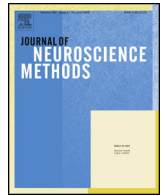
**ANNEXE 1 : "Méthode de recalage de cerveaux dans un atlas pour l'imagerie cellulaire." (publiée)**





Contents lists available at ScienceDirect

## Journal of Neuroscience Methods

journal homepage: [www.elsevier.com/locate/jneumeth](http://www.elsevier.com/locate/jneumeth)

## Non-imaged based method for matching brains in a common anatomical space for cellular imagery

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### HIGHLIGHTS

- A vectors field method for matching experimental brain sections in a reference atlas.
- Registration method based on plotted objects.
- Method applicable to any species and adapted for all cellular labeling.
- Method requiring equipment routinely used in laboratories and free software.

### ARTICLE INFO

#### Article history:

Received 12 January 2018

Received in revised form 6 April 2018

Accepted 7 April 2018

Available online 22 April 2018

#### Keywords:

Cellular brain mapping

Elastic registration

Vectors field

Brain atlas

### ABSTRACT

**Background:** Cellular imagery using histology sections is one of the most common techniques used in Neuroscience. However, this inescapable technique has severe limitations due to the need to delineate regions of interest on each brain, which is time consuming and variable across experimenters.

**New method:** We developed algorithms based on a vectors field elastic registration allowing fast, automatic realignment of experimental brain sections and associated labeling in a brain atlas with high accuracy and in a streamlined way. Thereby, brain areas of interest can be finely identified without outlining them and different experimental groups can be easily analyzed using conventional tools. This method directly readjusts labeling in the brain atlas without any intermediate manipulation of images.

**Results:** We mapped the expression of cFos, in the mouse brain (C57Bl/6J) after olfactory stimulation or a non-stimulated control condition and found an increased density of cFos-positive cells in the primary olfactory cortex but not in non-olfactory areas of the odor-stimulated animals compared to the controls.

**Comparison with existing method(s):** Existing methods of matching are based on image registration which often requires expensive material (two-photon tomography mapping or imaging with iDISCO) or are less accurate since they are based on mutual information contained in the images. Our new method is non-imaged based and relies only on the positions of detected labeling and the external contours of sections.

**Conclusions:** We thus provide a new method that permits automated matching of histology sections of experimental brains with a brain reference atlas.

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## 1. Introduction

Being able to precisely assign neural labeling to brain structures of interest is required for identifying the networks involved in processing neural information and more broadly for the understanding of the neural basis of physiological functions and how these are altered by aging or pathology.

The study of labeled histology slices is a powerful, widely-used technique with a high level of spatial resolution (cellular level) (Lesburgueres et al., 2011; Kermen et al., 2016) used to analyze brain function. This technique usually consists in delineating regions of interest (ROIs), then analyzing the labeling inside the ROIs to extract a labeling density. These densities can then be compared between ROIs and experimental conditions.

However, this inescapable technique has several limitations. The major issue relates to the necessity of visually delimiting the ROIs on each histological section for each subject in a reproducible and reliable manner. This can be difficult or impossible to implement due to the lack of anatomical landmarks, the inter-individual anatomical variability of the brain and possible deformations caused by the histological preparation of the slides (fixation, sectioning. . .). The lack of accuracy and consensus in identifying the ROIs can lead to contradictory scientific results (Radulovic et al., 1998; Franklin and Druhan, 2000; Miller and Marshall, 2004, 2005). A way to circumvent these issues is to use other techniques such as fMRI or TEP but these cannot attain similar cellular resolution (Kornblum et al., 2000; Houston et al., 2001; Herschman, 2003; Tsurugizawa et al., 2011; Lu et al., 2012; Jang, 2013; Zimmer et al., 2014). All the methods developed so far to match brain sections with an atlas are based on image collection and registration (Kopeck et al., 2011; Papp et al., 2016; Vandenberghe et al., 2016). The limitations of such image-based methods are dependent on the way the images are acquired, the cost (two-photon tomography mapping or imaging with iDISCO) (Ragan et al., 2012; Renier et al., 2016) or the accuracy of the matching method. In addition, after a block matching procedure, these methods use elastic deformation (Free Form Deformation model or similar) based on mutual information contained in the images, which is limiting for the analysis of low-level labeling (Kim et al., 1997; Rueckert et al., 1999; Ourselin et al., 2001).

To address these issues, we have developed a new method that is non-image based but that relies only on plotted objects directly extracted from histological sections. The method enables automated matching of histological sections from experimental brains on a digitized brain reference atlas. The proposed method allows high sensitivity matching using vectors field elastic registration. More precisely, we have developed a procedure (1) to allow fast, automatic realignment of labeling in brain structures or sub-regions of interest identified in the atlas without having to delineate them and (2) that limit variability due to inter-individual anatomical differences or tissue processing distortions. After matching the brains on the atlas, they become spatially comparable across all structures and sub-regions listed in the atlas and all brains can be easily and quickly analyzed using conventional mathematical or statistical tools. In addition to being more accurate, the method is simpler than previous image-based methods since it requires only the positions of labeled cells detected under the microscope acquired using mapping software, and the external contour of the sections. It thus minimizes the number of processing stages and reduces the risk of bias at each stage.

We have highlighted the efficiency of the method using neural activity assessment in response to olfactory stimuli. More precisely, we mapped the expression of an immediate early gene, cFos, which is commonly used to map neuronal activation (Maviel et al., 2004; Cruz et al., 2013; Redondo et al., 2014; Okuyama et al., 2016; Renier et al., 2016) in the mouse brain (C57Bl/6J) after olfactory stimulation or a non-stimulated control condition. Analysis of ROIs indicated an increased density of cFos-positive cells in the primary olfactory cortex but not in non-olfactory areas of the odor-stimulated animals compared to the controls which is consistent with previous studies in both animals and humans (Cattarelli et al., 1988; Savic et al., 2002; Illig and Haberly, 2003; Bensafi et al., 2007;

Rennaker et al., 2007; Stettler and Axel, 2009, Bensafi et al., 2012; Wu et al., 2012).

## 2. Materials and methods

### 2.1. Histology and cellular mapping on experimental brain sections

Our method consists in a high-accuracy matching of experimental brain sections with a reference brain atlas. It allows precise, automatic assignment of the labeled cells to a structure and permits between-group comparisons.

To validate the different steps of the matching method, we used the mouse brain and analyzed the expression of the immediate early gene *cfos* as an index of neural activity evoked by odor stimulation. More precisely, eight C57Bl6J mice aged 3–4 months (Charles River laboratory) were sacrificed after odor stimulation or not (Kermen et al., 2016). 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)). Every effort was made to minimize suffering. Frozen serial coronal sections (thickness: 14  $\mu\text{m}$ ; interval: 210  $\mu\text{m}$ ; 48 sections per animal) of the brain were processed for cFos immunohistochemistry (Fig. 1a). Briefly, a rabbit anti-cFos primary antibody (1: 5000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by a biotinylated goat anti-rabbit (1:200; Vector Laboratories, Burlingame, CA, USA) were used. Under the microscope, the external contour of each section was then manually outlined (in our example, we outlined the right hemisphere) and positive cells were automatically counted using mapping software (Mercator, Exploranova, La Rochelle, France) coupled to a Zeiss microscope. We also manually drew the inter-hemispheric fissure in order to later align the brains along the dorso-ventral axis (Figs. 1b and 2). It is worth noting that any mapping software is suitable as long as the coordinates of each labeled cell and contour point can be exported. A high labeled cell density is not a limitation factor since several cells can be separately identified even though they have close coordinates.

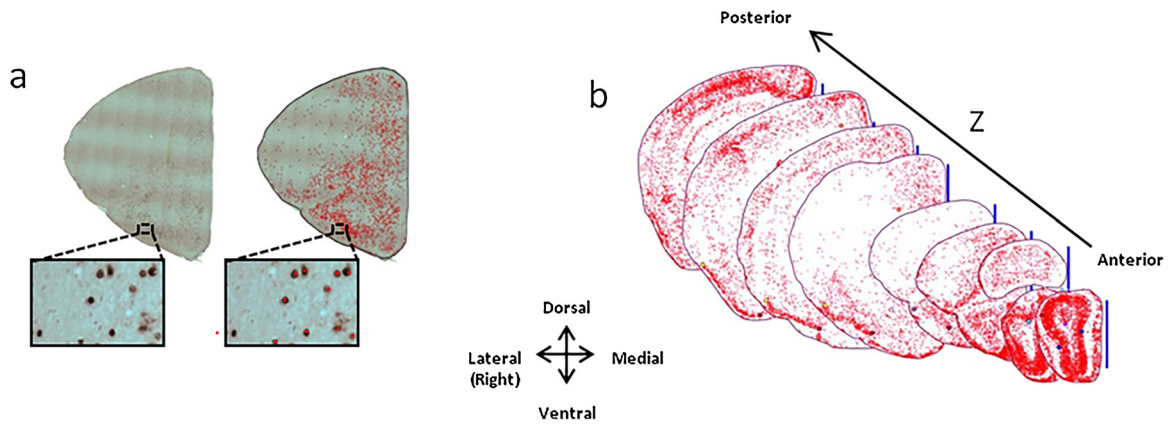
### 2.2. Paxinos Atlas as a common anatomical space

Brain sections from the mice were matched with the widely used Paxinos Atlas (« The mouse brain », Paxinos and Franklin's, 4th edition) which presents a large number of identified brain structures. We first digitalized the external contour and internal brain structures of this reference Atlas in the form of polylines from 9.84 mm to  $-0.36$  mm along the antero-posterior axis (0 at inter aural lines) with an interval of 120  $\mu\text{m}$  (86 sections) (Fig. 3). Since the Atlas does not completely cover the anterior part of the olfactory bulb, we extrapolated this missing part based on our olfactory bulb sections.

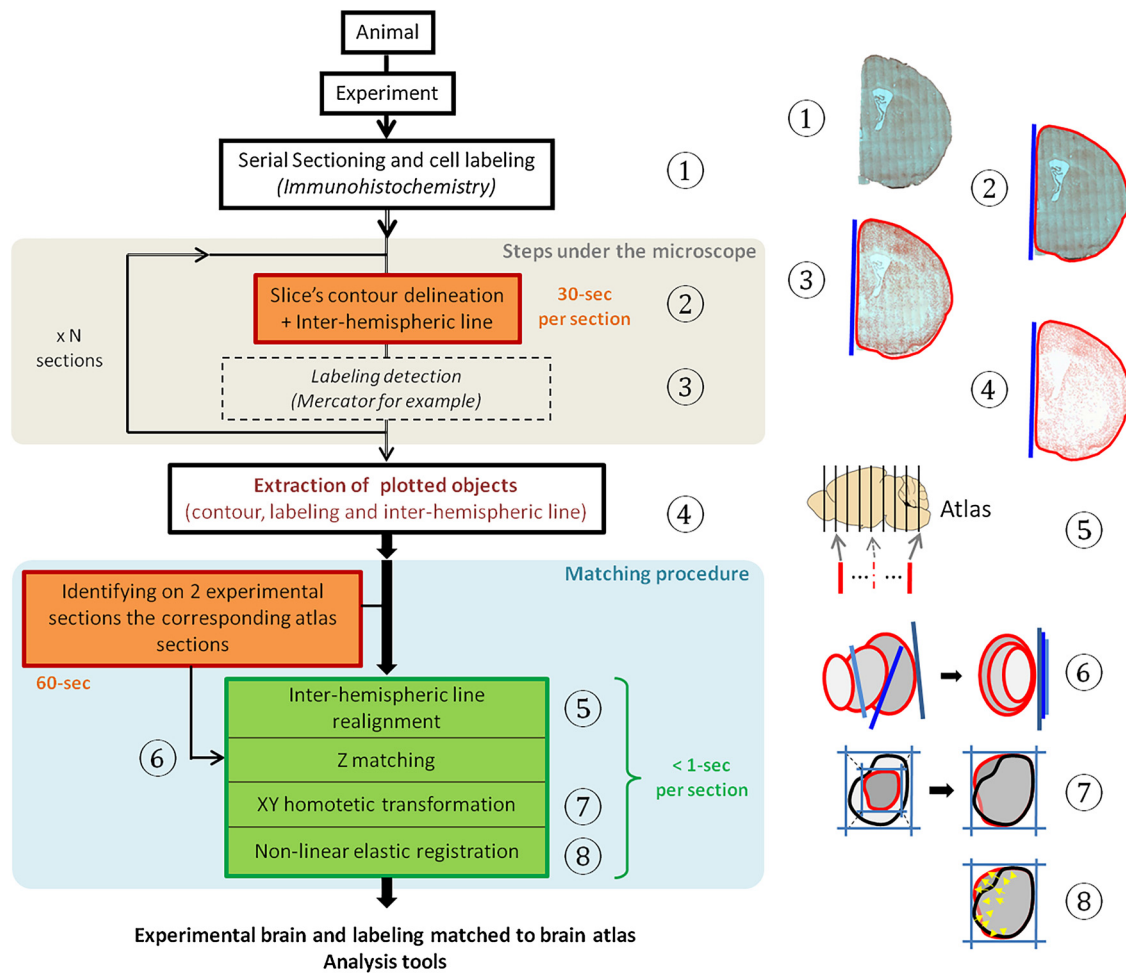
### 2.3. Matching experimental sections with Atlas sections

The matching procedure uses only the drawn contours and the inter-hemispheric lines (plotted objects) previously obtained from the histological sections. The first step of the procedure consists in automatically realigning and reorienting the external contours between them and to do this, all the sections were first grossly stacked on the XY plan. The external contours were then rotated so that they were parallel and translated to be realigned (X axis) using for both steps the interhemispheric axis as the reference line (Fig. 1b). This realignment and reorientation was also applied to the labeling (Fig. 2).

The second step consists in positioning all external contours along the Z axis. At least two histological sections (one in the anterior part and the other in the posterior part of the brain) need to



**Fig. 1.** Histological sections of experimental brains. **a.** Example of an histological section (interaural coordinate: 6.12 mm) extracted from an experimental brain after immunohistochemistry of cFos. cFos-positive cells are revealed in black (left) and can be automatically detected by a mapping software coupled to a microscope (right). **b.** Rotation and translation of the histological sections and cell labeling using the interhemispheric fissure as the common axis (blue line).

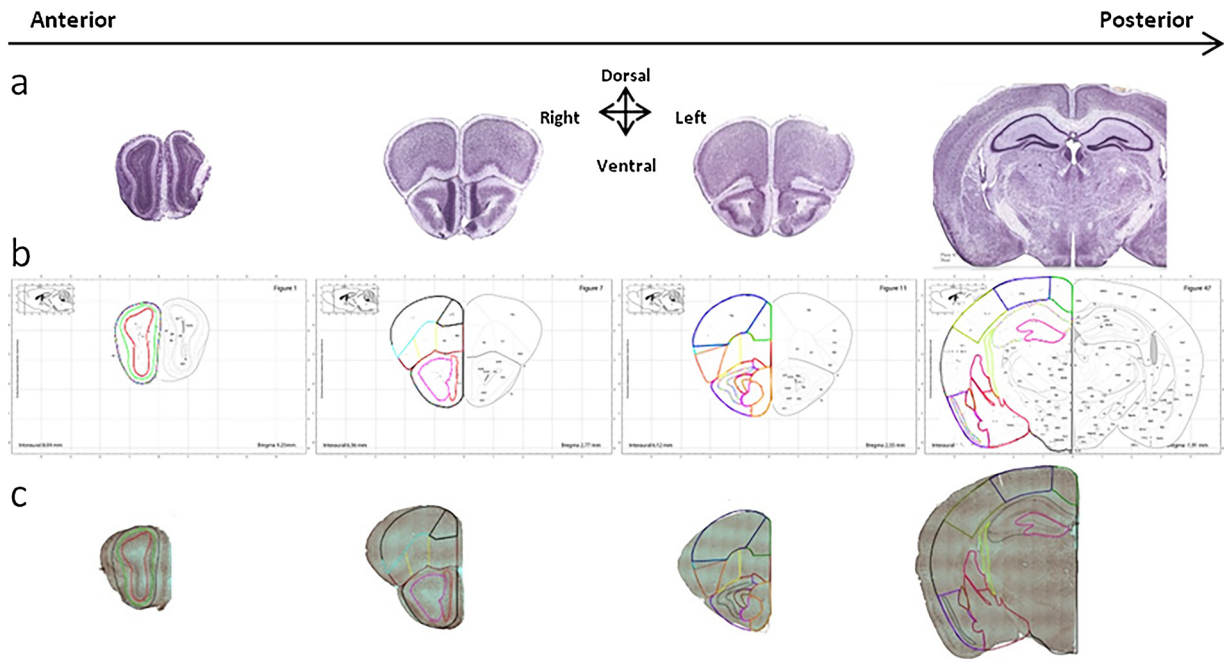


**Fig. 2.** Workflow of the entire procedure. This method includes two steps: one under the microscope (gray frame) and the matching procedure (blue frame). Manual stages are in orange boxes while automatic procedures are in green box. The plotted objects are the only data required for the matching procedure and no image are needed.

be manually associated with the two closest corresponding sections of the Atlas by visual inspection. The other sections are then automatically associated with the Atlas sections using a linear transformation (interpolation for the sections inside and extrapolation for the sections outside the interval depicted by the 2 reference sections) (Figs. 2 and 4). Matching along the Z axis can be improved

if additional experimental sections are associated with the Atlas because linear transformation is performed for each interval.

The third step consists in the readjustment of the external contours size in the XY plan. For each histological section to which a section of the Atlas has been associated, a homotetic transformation is applied along first the X and then the Y axis by the use of



**Fig. 3.** Paxinos Atlas as a common anatomical space.

**a.** Example of histological sections extracted from the Paxinos Atlas. From left to right, the interaural coordinates are 8.04; 6.56; 6.12; 1.88 mm **b.** Example of the corresponding plates of the digitized Atlas in which the different brain structures are delimited **c.** Plates of the digitized Atlas have been superposed onto histological sections after isotropic 2D rescaling. We can notice that due to the inter individual variability, the plates do not match the sections.

bounding boxes. This homothetic transformation is also applied to the labeling.

The fourth step consists in precisely matching the external contours of the histological sections with the corresponding external contours of the digitized Atlas using a method of nonlinear elastic registration. This registration is based on a computed vectors field that is applied to the labeling of the experimental sections. To do this and obtain optimal registration, three stages were required:

- (i) the inter-point distances of the external contours of both the Atlas and the experimental sections need to be equalized and intermediate points are added if necessary.
- (ii) a second order polynomial filter (window size: 4; between 3 and 5 are the optimal values for this kind of contour discretization) is applied to the histological contours to eliminate local irregularities and slightly smooth the contour. This step is essential so that the normals that are calculated on the contours are representative of the local curvature.
- (iii) vectors field is computed and thus:

- for each point of the experimental external contour, the normal to the point is computed as well as the intersection between the normal and the contour of the Atlas, generating a vector  $(u, v)$  which connects the two points. The set of these vectors forms the vectors field (Fig. 5a).
- we applied the vectors field  $(u_n, v_n)$  to the labeling of the histological section using the formulae given below (with  $n = 1$  to  $N$  the total number of vectors). The coordinates of the points of the contours were noted  $(X_n, Y_n)$  as were the labeling points  $(X'_m, Y'_m)$  (with  $m = 1$  to  $M$  the total number of labeling points in the section).

$$S_m = \sum_{n=1}^N \exp(-\sigma d_{nm})$$

$$d_{nm} = \sqrt{(X_n - X'_m)^2 + (Y_n - Y'_m)^2}$$

The new coordinates of  $m$  become:  $(X''_m, Y''_m)$  and are given by:

$$X''_m{}^{e;e} = X'_m + \frac{1}{S_m} \sum_{n=1}^N u_n \exp(-\sigma d_{nm})$$

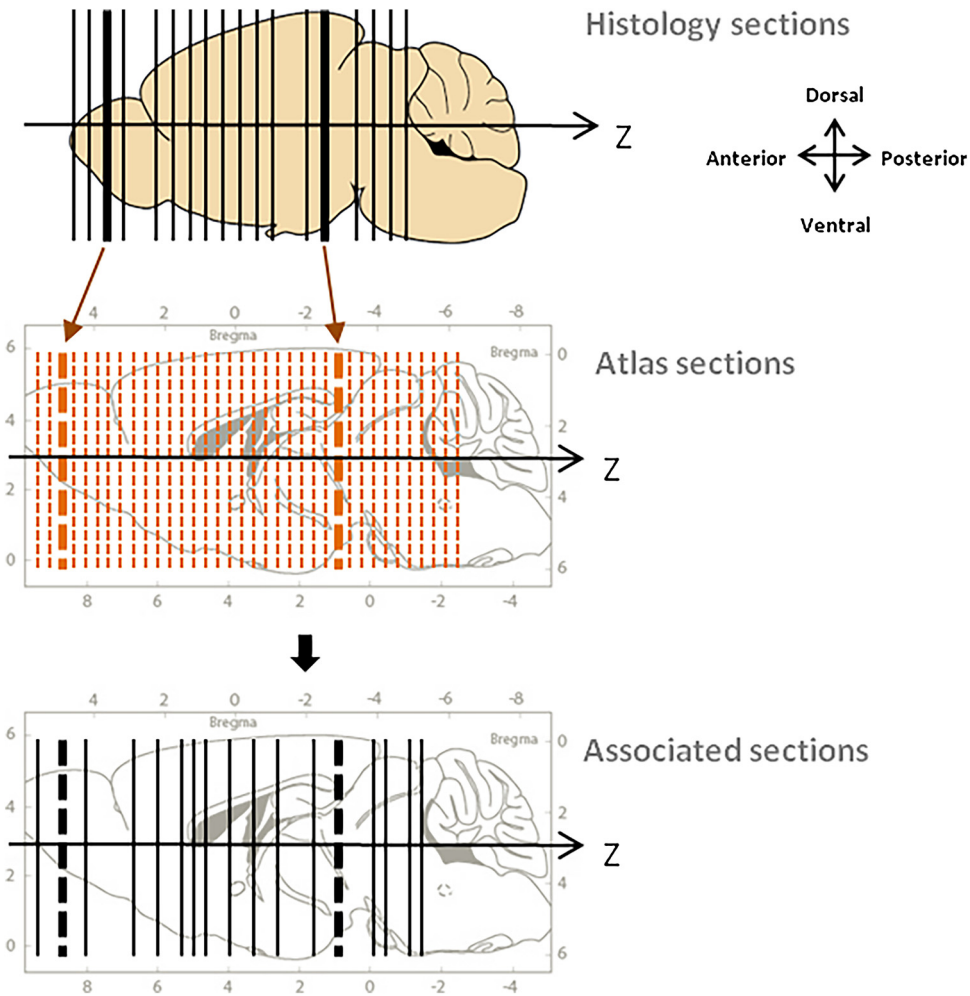
$$Y''_m{}^{e;e} = Y'_m + \frac{1}{S_m} \sum_{n=1}^N v_n \exp(-\sigma d_{nm})$$

The coefficient  $\sigma$  has been empirically established to be adapted to all cases and we choose  $\sigma = 0.01$  because in our case a vector positioned  $100 \mu\text{m}$  away from a point of the contour has twice less influence than a very close vector.

A pretreatment step can be introduced to restore the negentropy in the vectors field. When brain fissures are very deep or the tissue displays imperfections such as rips, the correspondence to the Atlas becomes aberrant and the transformation vectors false. The objective here is to modify the contour of the histological section before computing the vectors field. Indeed, points inducing a strong curvature can be iteratively eliminated and this process is stopped when the curvature is lower than a threshold (angle  $<60^\circ$ ). This procedure leads to locally 'filled' imperfections, something that cannot be done using the polynomial filter (Fig. 5b). Again, inter points distances are equalized and the vectors field computed.

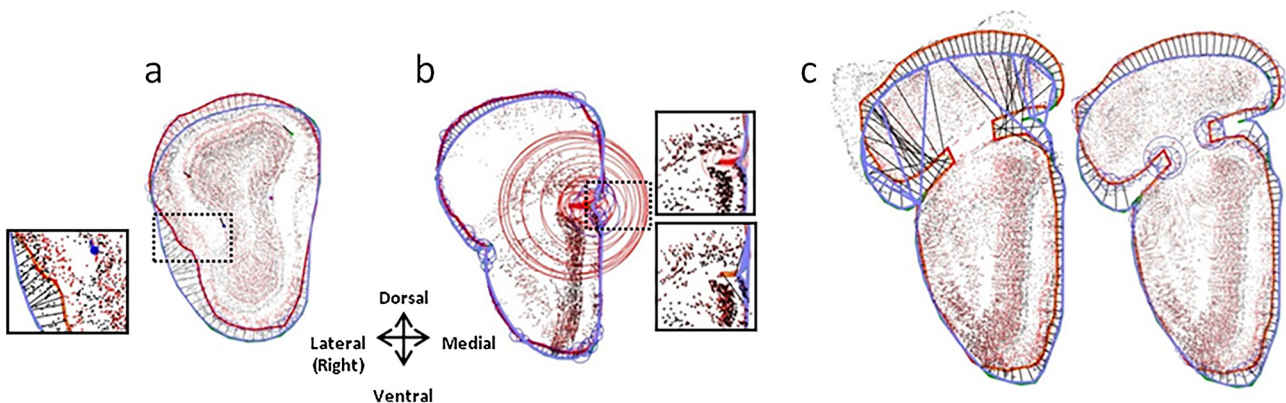
The last step consists in suppressing any vectors considered to be aberrant because of a gap between the histological contour and the Atlas contour (when fissures are deep and large). In this context, estimation of some vectors can be incorrect since the intersection points are too far apart. When the norm of the vector exceeds a given threshold ( $400 \mu\text{m}$  in our case), the vector is suppressed from the field in order to maintain the quality of the registration (Fig. 5c). The aim of these two final parts of the process is to increase





**Fig. 4.** Alignment of an experimental brain to the Paxinos Atlas.

(Up) Alignment of the histological sections along the antero-posterior axis (Z). (Middle) At least two histological sections are visually associated to the two closest corresponding sections of the Paxinos Atlas. (Down) The other sections are automatically associated to the Atlas sections thanks to a linear transformation (interpolation for the sections inside and extrapolation for the sections outside the interval depicted by the 2 reference sections).



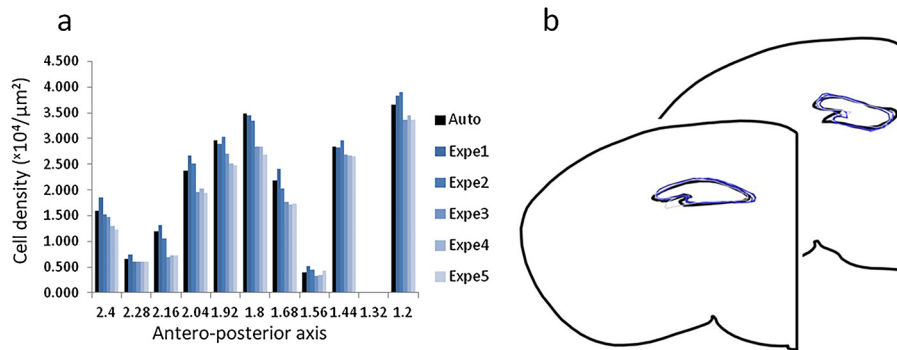
**Fig. 5.** Elastic registration.

**a.** Elastic registration by vectors field (black lines) is computed based on the external histological contours (red lines) and Atlas contours (blue lines) and applied to the labeling (red spot). This registration allows a precise positioning of the experimental labeling into the Atlas (black spots correspond to labeling after registration). Each labeled cell is submitted to the vector field and its shifting depends on its distance to the vectors. To improve registration, several algorithms have been added: **b.** To restore the negentropy in the vectors field, the contours presenting strong curvature can be iteratively eliminated (i.e. the curvature can be visualized with the diameter of the circle which is red when too strong and blue when acceptable). **c.** To suppress some vectors that are aberrant due to a too large distance between histological outline and the Atlas outline.

the robustness of the method even if the histological sections have imperfections.

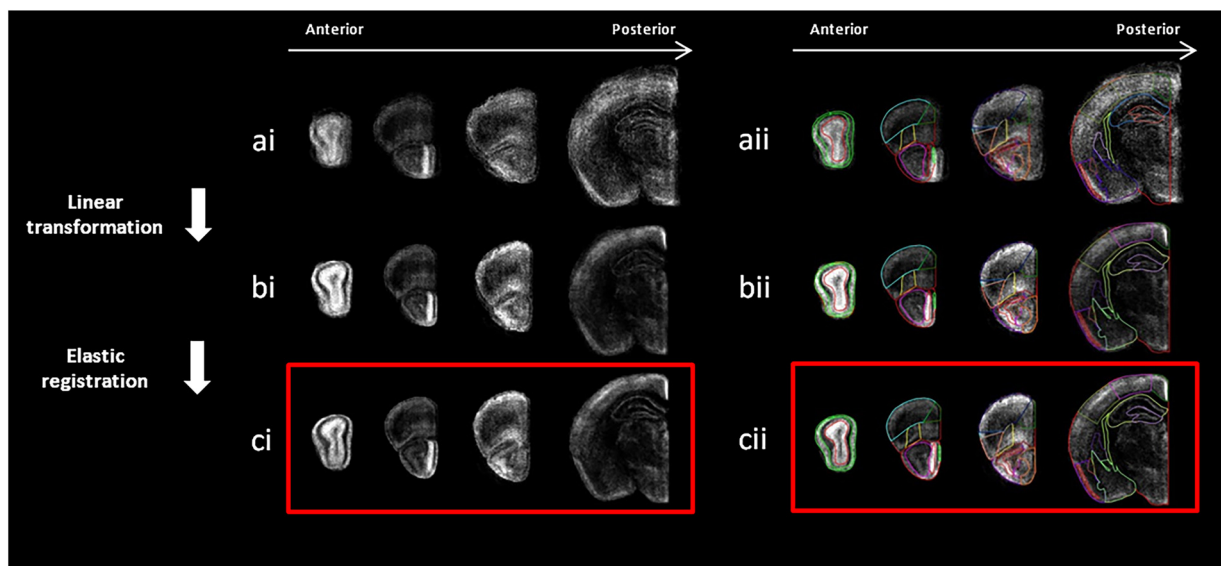
The entire matching process is summarized in the workflow (Fig. 2) and for information, processing one animal takes about 45 s

on a 2.3 GHz Intel Core i7, 4 Go computer. At the end of this computation, each labeled cell is correctly localized in the common Atlas and is thus spatially assigned to one region or sub-region. Different factors (cell density, number, mean. . .) can be automatically, easily



**Fig. 6.** Comparison between manual and automatic procedures.

a. Dorsal dentate gyrus (3–5 sections per animal from 1.2 to 2.4 interaural coordinate;  $n=6$ ) has been manually delineated by 5 experimenters. Cell density has been calculated for the 5 experimenters and for the automatic procedure. No group effect was found (Kruskal-Wallis Anova,  $H(5|156)=3.97$   $p=.553$ ). b. The outlines of the section and of the dorsal dental gyrus of the atlas (black lines) can be visualized and compared to the outlines drawn by the 5 experimenters and registered by the automatic procedure (colored lines) (example of two sections from two different animals at 1.44 and 2.4 interaural coordinate).



**Fig. 7.** Accuracy of Elastic registration: Validation in mice.

ai. Images generated after realignment (translation and rotation; see Fig. 2). aii. Same images with the superposed Atlas, a low level of accuracy of the superposition is observed. bi. Images after linear transformation (homothetic transformations classically used). bii. Same images with superposed Atlas, a better level of superposition is observed. ci. Images after elastic registration. cii. Same images with superposed Atlas, a high level of accuracy of the superposition with the Atlas is observed identifiable by the sharpness of the images especially at the level of the contours and small regions. (interaural coordinates of the images: 8.28, 6.60, 6.12 et 1.80).

and almost instantaneously extracted after registration from raw data and saved in a Table (Supp Fig. 1). Since the experimental sections have been matched to the Atlas, selecting any ROIs present in the Atlas yields the number or density of labeled cells in this particular ROI.

#### 2.4. Improvement of the method with a simple option

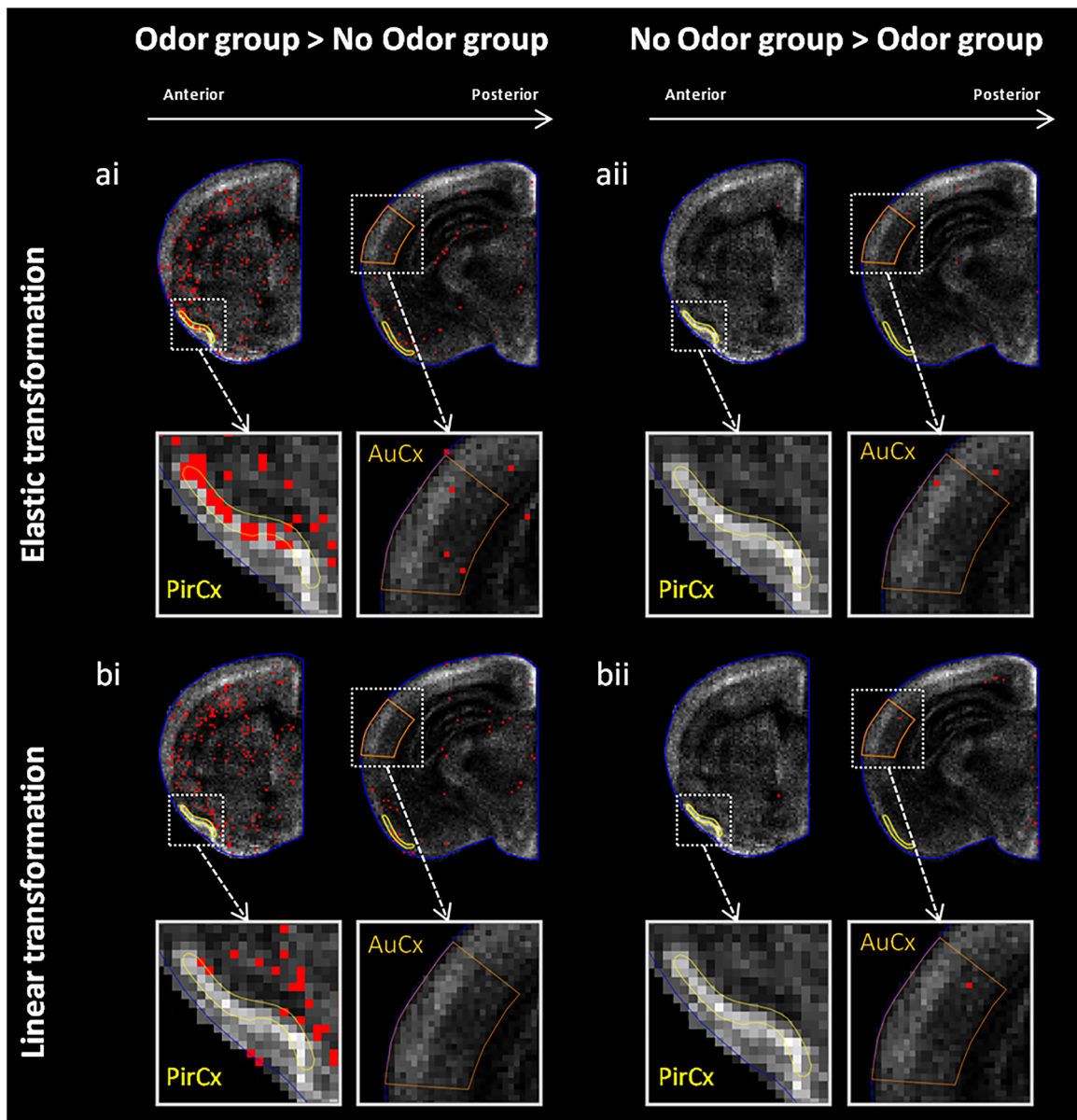
Histological sections with a lower quality (due to rips or deformations happening during the slicing process for example) or local deformations can lead to a less precise co-registration of some structures (in particular internal small ones). In this case, we can improve co-registration by adding some vectors into the vectors field, which will be automatically taken into account during the matching procedure. These vectors are built by defining anatomical landmarks in the atlas based on the problematic and manually pointing the corresponding landmarks on the histological sections (Fig. 5a).

The use of landmarks can also be pertinent to compare groups with anatomical deformations of specific structures due to genetic

or environmental manipulations. The number of landmarks used is unlimited but will increase the phase 2 of the workflow (Fig. 2) without changing the computation time.

#### 2.5. Additional tool for labeling analyses

Once all sections have been registered in the Atlas, a stack of those sections that include labeling along the Z axis is obtained. The 3D space in which the stack is placed can be discretized in isotropic sub-volumes ( $60 \times 60 \times 60 \mu\text{m}$ ). We thus get a matrix of labeling density that can be visualized as images similar to those obtained from MRI but in our case each sub-volume (or by analogy “voxel”) contains the exact number of counted labeled cells. The size of the voxel ( $60 \mu\text{m}$  side) was chosen in mice to allow the discretization of small regions of interest within the section (XY plan) without excessive interpolation along the Z axis (knowing that the interval between sections in the Atlas is  $120 \mu\text{m}$  and that some experimental sections can be missing). It should be noted that the size of the voxel can be adapted to the need of the experimental question or the species used for the study.



**Fig. 8.** Elastic registration versus linear transformation: Uncorrected Mann-Whitney analysis.

**a.** Uncorrected Mann-Whitney analysis of density labeling images after elastic registration. The images of density are processed as fMRI images. **ai.** Unilateral uncorrected Mann-Whitney (red spot  $p < 0.02$ ) is performed after elastic registration to compare mice stimulated with an odorant ( $n = 4$ ) to non-stimulated mice ( $n = 4$ ). Voxels with cell density significantly higher in the odor condition are represented with red spots. As expected, odor stimulation induces a significant increase of cFos labeling in an olfactory structure which is known to be activated by an odorant such as the piriform cortex (PirCx, interaural coordinate: 4.26; left) and has no effect on the auditory cortex (AuCx, interaural coordinate: 1.08; right). **aii.** Red spots represent here a significantly ( $p < 0.02$ ) higher level of cell density in the non-odor stimulated group compared to the odor stimulated animals. None or very few red spots are observed in this condition. **b.** Same uncorrected Mann-Whitney analysis has been performed on the same animals after classical linear transformation. **bi.** The lower precision of the transformation induces a lower accuracy of the position of the labeling in the piriform cortex and thus the difference between the two conditions is less evident (lower level of red spot). **bii.** Reverse comparison (non-odor > odor condition).

Interpolation is generally achieved using conventional methods (values of missing voxels are directly estimated by linear interpolation of neighbors) (Gao and Yin, 1999). These methods are not efficient when the anatomy between two neighboring sections is too dissimilar or if several sections are missing. To resolve this issue, we applied an object-based method (Grevera and Udupa, 1996) whose use is facilitated here thanks to the external contours of the Atlas serving as a bounding box. The object-based method allows estimation of both the value and position of missing voxels thus avoiding ghost and blurred images and preserving the coherency of the anatomical structures (Supp Fig. 2).

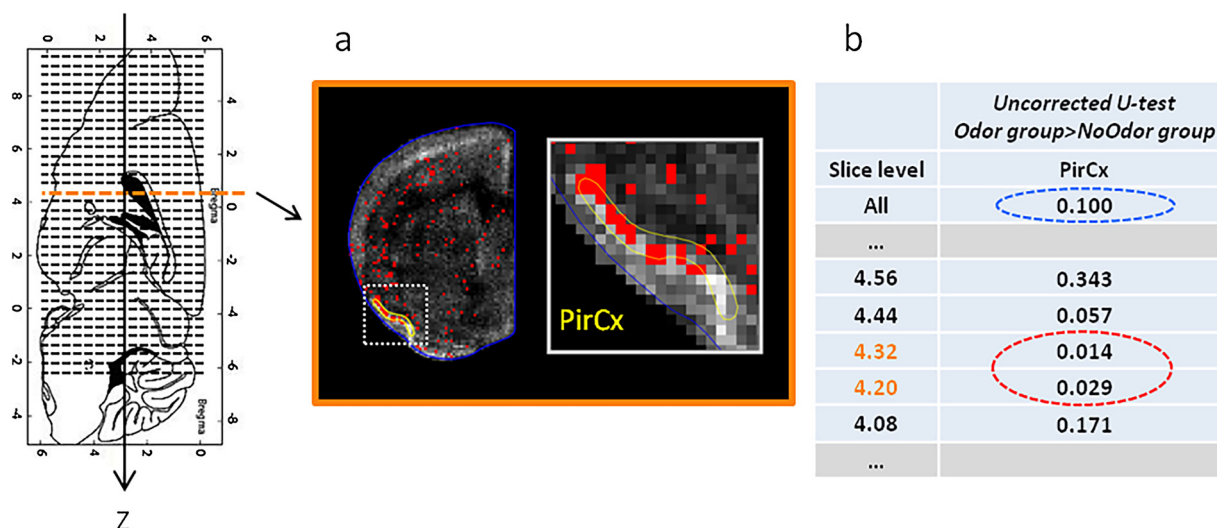
Based on this, we generated images of labeling density ( $150 \times 150 \times 204$  voxels) for each brain. The quality of the density

images generated by the method we propose is equivalent to that obtained using classic anatomic MRI (aMRI) and even superior to that obtained from classic functional MRI (fMRI) (Supp Fig. 3).

### 3. Results

A first way to validate the precision of the registration is to compare our automatic method to a manual procedure performed by 5 different experimenters. More precisely, the 5 experimenters assessed the density of labeled cells present in the dorsal dentate gyrus after manually outlining the structure on each section. These results were compared to the density of labeled cells in the same structure obtained after the automatic matching procedure. We





**Fig. 9.** Associated software.

Developed software allows automatic assessment of labeling quantity for all brains with high accuracy of labeling position in a structure without outlining it. **a.** Images of labeling density (interaural coordinate 4.26) with superposed uncorrected Mann-Whitney test results (red spot;  $p < 0.02$  for Odor > No odor condition;  $n = 4$  per group) are presented. **b.** Table showing the exact values of uncorrected Mann-Whitney test in the entire piriform cortex (first line: All) or at the chosen antero-posterior section level (from 4.08 to 4.56).

Our software allows 3D analysis of the brain and thus reveals differences of labeling in sub-structures (red dot) which are not observed when taking into account the entire structure for example (blue dot).

found no difference between the 5 experimenters and the automatic procedure on the cell density in the dentate gyrus (Fig. 6a). The accuracy of the automatic procedure is illustrated by the superposition of the outlines of the dentate gyrus from the Atlas and of the ones drawn by 5 experimenters registered by the procedure (Fig. 6b).

To assess the impact of the first manual step of our procedure (delineation of the external contour of the section) on the results, we compared the density of labeled cells obtained by three different experimenters that drew the external contours of the sections on four control mice. Then, the automatic realignment has been applied and the number of labeled cells in two different structures (dentate gyrus and the piriform cortex) was extracted. We found that the relative difference between experimenters for the dentate gyrus (3.67%; 1.91%, 3.76%) and piriform cortex (1.37%, 1.45%, 1.94%) remained inferior to 5%. Thus, manual contour delineation does not introduce variability on the results.

In addition, to visually estimate the accuracy of the method, we computed the images of density at different stages of the procedure. First, we performed the two first steps of the transformation: brain sections matched in Z axis and aligned and rotated in XY plan. These procedures were applied to the 8 brains generating 8 volumes of images of density that were then averaged. The resulting averaged volume shows blurry images with a large mismatch of spatial localizations (Fig. 7a). We then added the classical linear transformation. Again, the volumes of density were calculated for each mouse and averaged across the group of 8. The resulting images show an improvement of quality although some clear mismatches can still be observed on the contours and small internal structures (Fig. 7b).

Finally, we added to all previous steps the elastic registration on each animal. As previously, we then computed the volumes of density for each mouse and averaged them across the entire group. Results show a high level of accuracy of the matches between average brains with low levels of value dispersion (higher sharpness of external contours and structure edges) (Fig. 7c). The superposition of the Atlas images on the averaged images of experimental brains indicates high sensitivity matching (Fig. 7c).

Then, we analyzed the effect of odor stimulation on the activity of the primary olfactory cortex (piriform cortex). More specifically, we compared a first group of mice stimulated with an odorant (*cis*-3-Hexen-1-ol, 60  $\mu$ l at 1 Pa one hour before sacrifice;  $n = 4$ ) to a second, non-stimulated control group. cFos labeling was counted as an index of neural activity, elastic registration was applied to the individual brains and labeling was then averaged per group. We found higher cell density in the odor-stimulated group compared to the control group in the piriform cortex (uncorrected Mann-Whitney test,  $p < 0.02$ ) (Fig. 8a). The analysis of the auditory cortex, which is not directly related to olfactory processing, showed no modulation of activity after odor stimulation (Fig. 8b).

Statistical tests can also be performed at the level of a single section, structure or sub-structures and presented in tables (Fig. 9). Similar investigations can be performed using threshold difference analysis leading to the same results (Supp Fig. 4). It should be noted that while the piriform cortex is a narrow, winding structure located on the border of the section, its anatomical factors increasing the difficulty of matching, we obtained very precise results in terms of spatial localization.

All data (raw data, mean, statistical results...) can be visualized in 3D after the ROIs of the Atlas brain have been meshed (Activis software) (Thevenet and Cheylus, 2003; Martin et al., 2007) (Fig. 10).

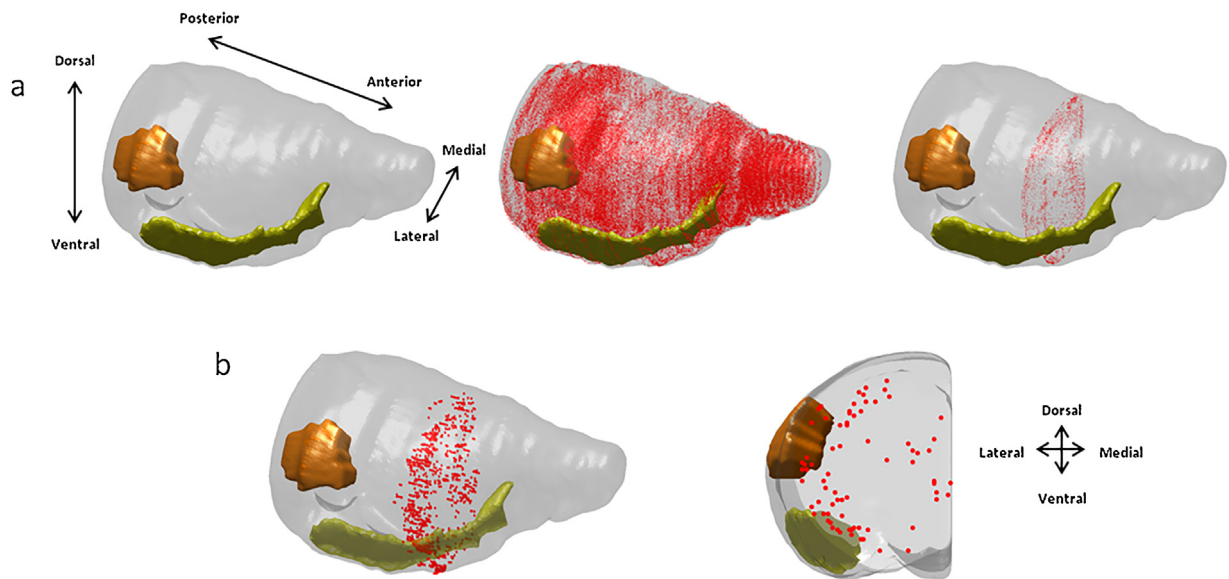
#### 4. Discussion

Imagery of cell labeling is a valuable tool to explore brain function but low-accuracy, slowness or cost of the available techniques are major hurdles.

Here, we present a method allowing high-accuracy matching of experimental brain sections in a common anatomical space for cellular analysis and inter group comparisons in a streamlined way developed under Matlab with open format files. Using this method will enable optimization of data analysis and lead to reliable reproducibility of the findings in cellular imaging.

Compared to the classical methods of histology analysis, in which the experimenter has to outline the ROIs (a step that is time consuming, often imprecise due to the lack of anatomical land-





**Fig. 10.** 3D Visualization after Elastic registration.

**a.** (Left) 3D mesh of the brain Atlas (grey) and ROIs (yellow: Piriform Cortex, orange: Auditory Cortex). (Middle) Visualization of the labeling (cFos in response to odor stimulation) for one mouse after elastic registration. (Right) Example of one single registered section with its labeling (coordinate: 4.32). **b.** (Left) 3D visualization of the statistical results after inter-group comparison on 13 sections (unilateral *t*-test (Odor > No Odor condition,  $n = 4$  mice/group, red spot  $p < 0.01$ , the size of the spots has been extended for a better visualization). (Right) Same for 1 section (interaural coordinate: 4.32, as previously shown in Fig. 8ai) after clipping the anterior part of the 3D mesh of the brain.

marks and subject to large heterogeneity from one experimenter to another) and assess the labeling in the ROIs on each histological section, this method is more reliable and increases productivity. No outlining of ROIs is required here, reducing errors and imprecisions and the regions are identified automatically and quasi instantaneously thanks to the matching to the Atlas. The analysis of labeling present in those regions is automatically and rapidly calculated. Our method is highly flexible since data from any ROI can be analyzed at a later stage without the necessity of going back to the sections and mapping the new structures since supplementary brain regions can be easily and quickly added or refined into the Atlas. This method could be used with species other than mice, for any cellular labeling and with open source mapping software coupled to a microscope.

Several techniques have been developed to match experimental brain sections to an Atlas. The major difference between the other techniques and ours is that they are based on image analysis and not directly on section analysis requiring more processing steps and/or more expensive equipment. For instance, two-photon tomography mapping or imaging with iDISCO (Vousden et al., 2015; Renier et al., 2016) are expensive and thus cannot be used routinely. In other studies, experimental sections were first digitized which transforms labeling into levels of gray, leading to a loss of spatial resolution and the absolute value of labeled cells (Lebenberg et al., 2010). In some other cases, an intermediate step of a blockface method is required to allow registration of histological sections into an Atlas (Dauguet et al., 2007; Lebenberg et al., 2010; Lebenberg et al., 2011; Vandenberghe et al., 2016). The registration of histological sections on the blockface photographs in this method assumes that sectioning does not induce spatial distortions, irregularities (rips, folds, holes...).

Our technique allows the matching of experimental brain sections and labeling in an Atlas in a streamlined way (i.e. without using different software or toolboxes for each step such as Anatomist, BrainVisa, BrainRat, Clearmap, SPMmouse, Elastix...). Indeed, after recovery of histological data (contour and labeling of each section), the software allows all the consecutive steps from the registration stages to data analysis (density, number, mean, statistical comparisons...) and visualization (density maps, 3D views...)

to be processed automatically and quickly. It is based on the original vectors field algorithm we developed that ensures accuracy and reproducibility.

We have shown that the method delivers reliable results with the olfactory system since an increase of labeling (cFos) in the olfactory cortex is observed after olfactory stimulation. The magnitude of this increase was higher after registration compared to classical transformation. In summary, our method permits the matching of experimental brain sections to an Atlas in a fast, robust and precise manner. We believe that it should be applicable to many questions in Neuroscience.

## Acknowledgments

This work was supported by CNRS, Inserm, Lyon 1 University and Fondation Roudnitska.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jneumeth.2018.04.004>.

## References

- Bensafi, M., Sobel, N., Khan, R.M., 2007. Hedonic-specific activity in piriform cortex during odor imagery mimics that during odor perception. *J. Neurophysiol.* 98, 3254–3262.
- Bensafi, M., Iannilli, E., Poncelet, J., Seo, H.S., Gerber, J., Rouby, C., Hummel, T., 2012. Dissociated representations of pleasant and unpleasant olfactory-trigeminal mixtures: an fMRI study. *PLoS One* 7, e38358.
- Cattarelli, M., Astic, L., Kauer, J.S., 1988. Metabolic mapping of 2-deoxyglucose uptake in the rat piriform cortex using computerized image processing. *Brain Res.* 442, 180–184.
- Cruz, F.C., Koya, E., Guez-Barber, D.H., Bossert, J.M., Lupica, C.R., Shaham, Y., Hope, B.T., 2013. New technologies for examining the role of neuronal ensembles in drug addiction and fear. *Nat. Rev. Neurosci.* 14, 743–754.
- Dauguet, J., Delzescaux, T., Conde, F., Mangin, J.F., Ayache, N., Hantraye, P., Frouin, V., 2007. Three-dimensional reconstruction of stained histological slices and 3D non-linear registration with in-vivo MRI for whole baboon brain. *J. Neurosci. Methods* 164, 191–204.

- Franklin, T.R., Druhan, J.P., 2000. Expression of Fos-related antigens in the nucleus accumbens and associated regions following exposure to a cocaine-paired environment. *Eur. J. Neurosci.* 12, 2097–2106.
- Gao, Q.H., Yin, F.F., 1999. Two-dimensional direction-based interpolation with local centered moments. *Graph. Model Image Process.* 61, 323–339.
- Grevera, G.J., Udupa, J.K., 1996. Shape-based interpolation of multidimensional grey-level images. *IEEE Trans. Med. Imaging* 15, 881–892.
- Herschman, H.R., 2003. Micro-PET imaging and small animal models of disease. *Curr. Opin. Immunol.* 15, 378–384.
- Houston, G.C., Papadakis, N.G., Carpenter, T.A., Hall, L.D., Mukherjee, B., James, M.F., Huang, C.L., 2001. Mapping of brain activation in response to pharmacological agents using fMRI in the rat. *Magn. Reson. Imaging* 19, 905–919.
- Illig, K.R., Haberly, L.B., 2003. Odor-evoked activity is spatially distributed in piriform cortex. *J. Comp. Neurol.* 457, 361–373.
- Jang, B.S., 2013. MicroSPECT and MicroPET imaging of small animals for drug development. *Toxicol. Res.* 29, 1–6.
- Kermen, F., Midroit, M., Kuczewski, N., Forest, J., Thevenet, M., Sacquet, J., Benetollo, C., Richard, M., Didier, A., Mandairon, N., 2016. Topographical representation of odor hedonics in the olfactory bulb. *Nat. Neurosci.* 19, 876–878.
- Kim, B., Boes, J.L., Frey, K.A., Meyer, C.R., 1997. Mutual information for automated unwarping of rat brain autoradiographs. *Neuroimage* 5, 31–40.
- Kopec, C.D., Bowers, A.C., Pai, S., Brody, C.D., 2011. Semi-automated atlas-based analysis of brain histological sections. *J. Neurosci. Methods* 196, 12–19.
- Kornblum, H.I., Araujo, D.M., Annala, A.J., Tatsukawa, K.J., Phelps, M.E., Cherry, S.R., 2000. In vivo imaging of neuronal activation and plasticity in the rat brain by high resolution positron emission tomography (microPET). *Nat. Biotechnol.* 18, 655–660.
- Lebenberg, J., Herard, A.S., Dubois, A., Daguette, J., Frouin, V., Dhenain, M., Hantraye, P., Delzescaux, T., 2010. Validation of MRI-based 3D digital atlas registration with histological and autoradiographic volumes: an anatomofunctional transgenic mouse brain imaging study. *Neuroimage* 51, 1037–1046.
- Lebenberg, J., Herard, A.S., Dubois, A., Dhenain, M., Hantraye, P., Delzescaux, T., 2011. A combination of atlas-based and voxel-wise approaches to analyze metabolic changes in autoradiographic data from Alzheimer's mice. *Neuroimage* 57, 1447–1457.
- Lesburgueres, E., Gobbo, O.L., Alaux-Cantin, S., Hambucken, A., Trifilieff, P., Bontempi, B., 2011. Early tagging of cortical networks is required for the formation of enduring associative memory. *Science* 331, 924–928.
- Lu, H., Chefer, S., Kurup, P.K., Guillem, K., Vaupel, D.B., Ross, T.J., Moore, A., Yang, Y., Peoples, L.L., Stein, E.A., 2012. fMRI response in the medial prefrontal cortex predicts cocaine but not sucrose self-administration history. *Neuroimage* 62, 1857–1866.
- Martin, C., Grenier, D., Thevenet, M., Vigouroux, M., Bertrand, B., Janier, M., Ravel, N., Litaudon, P., 2007. fMRI visualization of transient activations in the rat olfactory bulb using short odor stimulations. *Neuroimage* 36, 1288–1293.
- Maviel, T., Durkin, T.P., Menzaghi, F., Bontempi, B., 2004. Sites of neocortical reorganization critical for remote spatial memory. *Science* 305, 96–99.
- Miller, C.A., Marshall, J.F., 2004. Altered prelimbic cortex output during cue-elicited drug seeking. *J. Neurosci.* 24, 6889–6897.
- Miller, C.A., Marshall, J.F., 2005. Altered Fos expression in neural pathways underlying cue-elicited drug seeking in the rat. *Eur. J. Neurosci.* 21, 1385–1393.
- Okuyama, T., Kitamura, T., Roy, D.S., Itoharu, S., Tonegawa, S., 2016. Ventral CA1 neurons store social memory. *Science* 353, 1536–1541.
- Ourselin, S., Roche, A., Subsol, G., Pennec, X., Ayache, N., 2001. Reconstructing a 3D structure from serial histological sections. *Image Vision Comput.* 19, 25–31.
- Papp, E.A., Leergaard, T.B., Csucs, G., Bjaalie, J.G., 2016. Brain-wide mapping of axonal connections: workflow for automated detection and spatial analysis of labeling in microscopic sections. *Front. Neuroinf.* 10, 1–11.
- Radulovic, J., Kammermeier, J., Spiess, J., 1998. Relationship between fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. *J. Neurosci.* 18, 7452–7461.
- Ragan, T., Kadiri, L.R., Venkataraju, K.U., Bahlmann, K., Sutin, J., Taranda, J., Arganda-Carreras, I., Kim, Y., Seung, H.S., Osten, P., 2012. Serial two-photon tomography for automated ex vivo mouse brain imaging. *Nat. Methods* 9, 255–258.
- Redondo, R.L., Kim, J., Arons, A.L., Ramirez, S., Liu, X., Tonegawa, S., 2014. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. *Nature* 513, 426–430.
- Renier, N., Adams, E.L., Kirst, C., Wu, Z., Azevedo, R., Kohl, J., Autry, A.E., Kadiri, L., Umadevi Venkataraju, K., Zhou, Y., Wang, V.X., Tang, C.Y., Olsen, O., Dulac, C., Osten, P., Tessier-Lavigne, M., 2016. Mapping of brain activity by automated volume analysis of immediate early genes. *Cell* 165, 1789–1802.
- Rennaker, R.L., Chen, C.F., Ruyle, A.M., Sloan, A.M., Wilson, D.A., 2007. Spatial and temporal distribution of odorant-evoked activity in the piriform cortex. *J. Neurosci.* 27, 1534–1542.
- Rueckert, D., Sonoda, L.I., Hayes, C., Hill, D.L., Leach, M.O., Hawkes, D.J., 1999. Nonrigid registration using free-form deformations: application to breast MR images. *IEEE Trans. Med. Imaging* 18, 712–721.
- Savic, I., Gulyas, B., Berglund, H., 2002. Odorant differentiated pattern of cerebral activation: comparison of acetone and vanillin. *Hum. Brain Mapp.* 17, 17–27.
- Stettler, D.D., Axel, R., 2009. Representations of odor in the piriform cortex. *Neuron* 63, 854–864.
- Thevenet, M., Cheylus, A., 2003. Le logiciel ACTIVIS. Traitement et analyse d'images médicales 3D. *Santé et Systémique*, 191–199.
- Tsurugizawa, T., Uematsu, A., Uneyama, H., Torii, K., 2011. Different BOLD responses to intragastric load of L-glutamate and inosine monophosphate in conscious rats. *Chem. Senses* 36, 169–176.
- Vandenbergh, M.E., Herard, A.S., Souedet, N., Sadouni, E., Santin, M.D., Briet, D., Carre, D., Schulz, J., Hantraye, P., Chabrier, P.E., Rooney, T., Debeir, T., Blanchard, V., Pradier, L., Dhenain, M., Delzescaux, T., 2016. High-throughput 3D whole-brain quantitative histopathology in rodents. *Sci. Rep.* 6, 20958.
- Vousden, D.A., Epp, J., Okuno, H., Nieman, B.J., van Eede, M., Dazai, J., Ragan, T., Bito, H., Frankland, P.W., Lerch, J.P., Henkelman, R.M., 2015. Whole-brain mapping of behaviourally induced neural activation in mice. *Brain Struct. Funct.* 220, 2043–2057.
- Wu, K.N., Tan, B.K., Howard, J.D., Conley, D.B., Gottfried, J.A., 2012. Olfactory input is critical for sustaining odor quality codes in human orbitofrontal cortex. *Nat. Neurosci.* 15, 1313–1319.
- Zimmer, E.R., Parent, M.J., Cuellar, A.C., Gauthier, S., Rosa-Neto, P., 2014. MicroPET imaging and transgenic models: a blueprint for Alzheimer's disease clinical research. *Trends Neurosci.* 37, 629–641.


**ANNEXE 2 :** "Disponibilité à court terme des neurones  
issus de la neurogenèse adulte pour l'encodage de la  
mémoire." (publiée)

ARTICLE

<https://doi.org/10.1038/s41467-019-13521-7>

OPEN

# Short-term availability of adult-born neurons for memory encoding

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Adult olfactory neurogenesis provides waves of new neurons involved in memory encoding. However, how the olfactory bulb deals with neuronal renewal to ensure the persistence of pertinent memories and the flexibility to integrate new events remains unanswered. To address this issue, mice performed two successive olfactory discrimination learning tasks with varying times between tasks. We show that with a short time between tasks, adult-born neurons supporting the first learning task appear to be highly sensitive to interference. Furthermore, targeting these neurons using selective light-induced inhibition altered memory of this first task without affecting that of the second, suggesting that neurons in their critical period of integration may only support one memory trace. A longer period between the two tasks allowed for an increased resilience to interference. Hence, newly formed adult-born neurons regulate the transience or persistence of a memory as a function of information relevance and retrograde interference.

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All animals are exposed to a changing olfactory environment requiring constant adjustments of odor perception and memory, in order for the olfactory system to efficiently guide the animal's behavior. Adult-born neurons provided by neuroblasts formed in the subventricular zone<sup>1–4</sup>, constantly integrate the olfactory bulb (OB) and underlie olfactory learning<sup>5–14</sup>. However it has been proposed that, in the hippocampus, adult neurogenesis promotes forgetting. Indeed, an increase in hippocampal neurogenesis tends to enhance memory clearance thereby reducing proactive interference while decreased neurogenesis prevents forgetting and reduces behavioral flexibility<sup>15–17</sup>. Thus, the neuronal turnover provided by adult neurogenesis could sub-serve memory formation and regulate the transience of the memory trace. This raises the issue of how this 'remembering/forgetting' balance is regulated as a function of environmental demand, allowing the animal to keep relevant information in memory for optimal behavioral adaptation. However, the parameters determining memory persistence or forgetting such as information relevance or the time between successive inputs and to what extent they depend on adult-born neurons are unknown. More specifically, adult-born neurons show a critical period during which they can be recruited by learning<sup>6</sup> but whether they can support successive memory traces within this critical period and whether they suffer from memory interference are unknown.

Here, using the olfactory system in mice, we tackle the issue of how the acquisition of new memories influences the information already stored in the network and we identify the role of adult-born neurons in these processes. We used perceptual olfactory discrimination learning, which depends on newly formed neurons in the OB<sup>8</sup> to show that (1) adult-born neurons saved by learning are present in the OB as long as the task is remembered; (2) with a second learning task occurring soon after the first, introducing retrograde interference, the new memory overwrites the older one and alters survival of previously recruited adult-born neurons unless (3) the first learned odorants are maintained in the environment. Finally, using sequential labeling of adult-born neurons and selective optogenetic inactivation, we showed (4) that each successive learning is supported by a specific population of adult-born neurons.

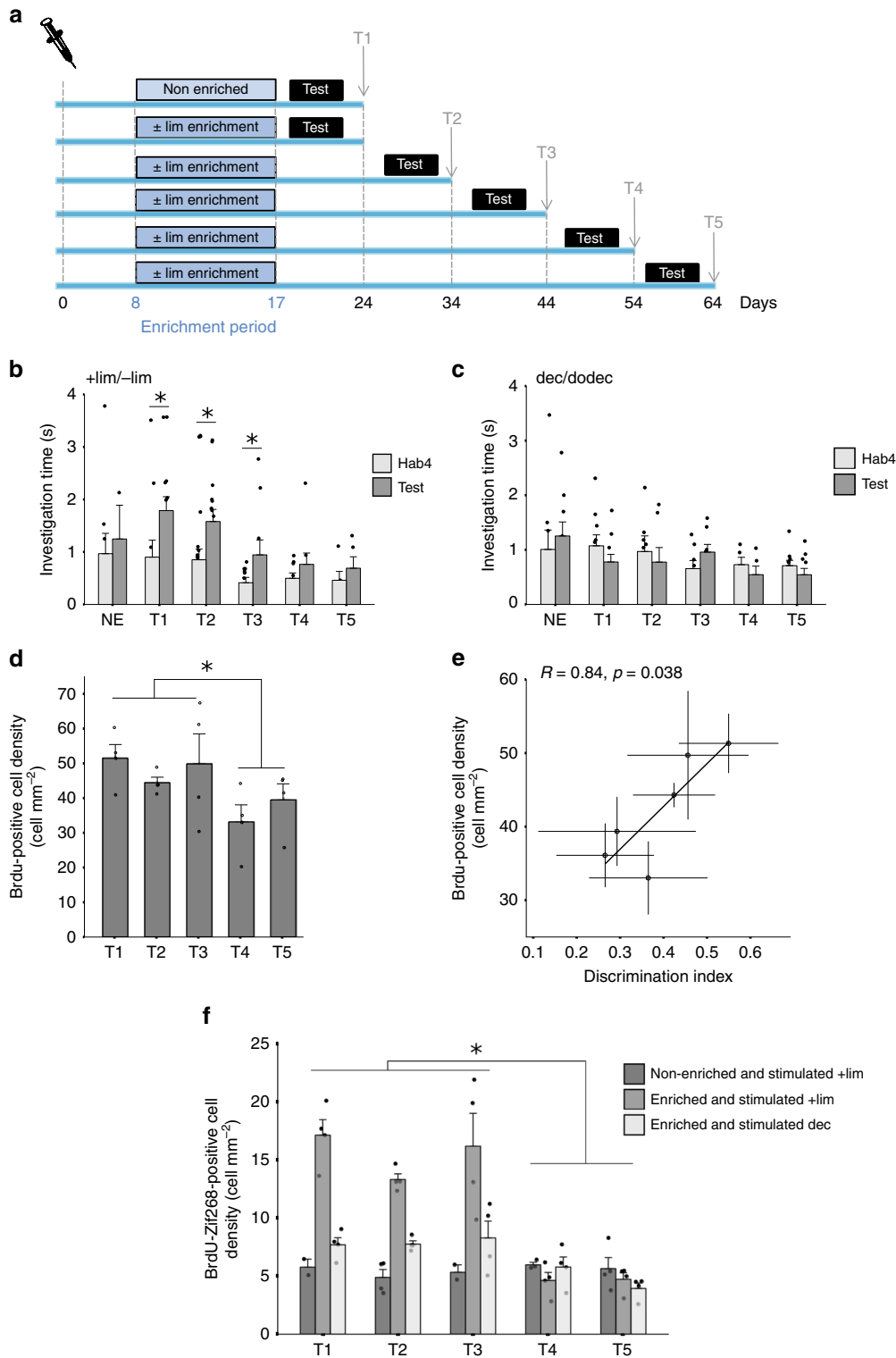
## Results

**Performances are linked to adult-born neurons fate.** Previous studies have reported increased survival of adult-born granule cells after perceptual discrimination learning<sup>8</sup>. To better understand the potential role of these neurons in long-term memory, we first asked whether increased bulbar neurogenesis persists for as long as the learned information is remembered. Thus, different groups of mice underwent perceptual discrimination learning consisting in a 10-day enrichment period with (+)limonene and (–)limonene (+lim and –lim), odorants which are not spontaneously discriminated by mice. Their performance in discriminating these two odorants was then analyzed from 1 to 5 weeks post learning (T1, T2, T3, T4, and T5, Fig. 1a) using a habituation/dishabituation test. In this test, mice were exposed to the first odorant of the pair 4 times (Hab1 to Hab4; inter-trial interval 5 min) followed by exposure to the second odorant of the pair (Test, 5 min between Hab4 and Test). The time spent by the mouse investigating the odorant was recorded for all trials. Decreased investigation time across habituation trials reflects habituation to the odorant used and a longer time spent investigating Test than Hab4 shows discrimination of the two odorants of the pair. At all time-points analyzed, mice exhibited habituation behavior (Supplementary Table 1, Supplementary Fig. 1). Regarding discrimination, using a two-way ANOVA, we observed significant differences between groups (NE: Non Enriched, T1, T2, T3, T4, T5:  $F_{(5,106)} = 2.39$ ;  $p = 0.043$ ) and effect of the trial (Hab4 vs Test,

$F_{(1,106)} = 9.89$ ;  $p = 0.002$ ). Enriched animals were able to discriminate the two enantiomers of limonene from T1 to T3 (Paired *t*-test,  $p < 0.05$  between Hab4 and Test) but not after the longer time periods T4 and T5 (Paired *t*-test,  $p > 0.05$  between Hab4 and Test; Supplementary Table 1, Fig. 1b). To assess the specificity of learning, discrimination of another pair of similar odorants, decanal/dodecanone (dec/dodec), to which the animals had not been enriched was also tested. No discrimination of dec and dodec was seen after enrichment with +lim and –lim, (ANOVA group effect:  $F_{(5,90)} = 1.18$ ,  $p = 0.32$ ; trial effect:  $F_{(1,90)} = 0.05$ ;  $p = 0.83$ ,  $p > 0.05$  between Hab4 and Test) (Supplementary Table 1; Fig. 1c). NE control animals did not discriminate any of the two odorant pairs (Paired *t*-test, T1:  $p > 0.05$  between Hab4 and Test; Supplementary Table 1; Fig. 1b, c).

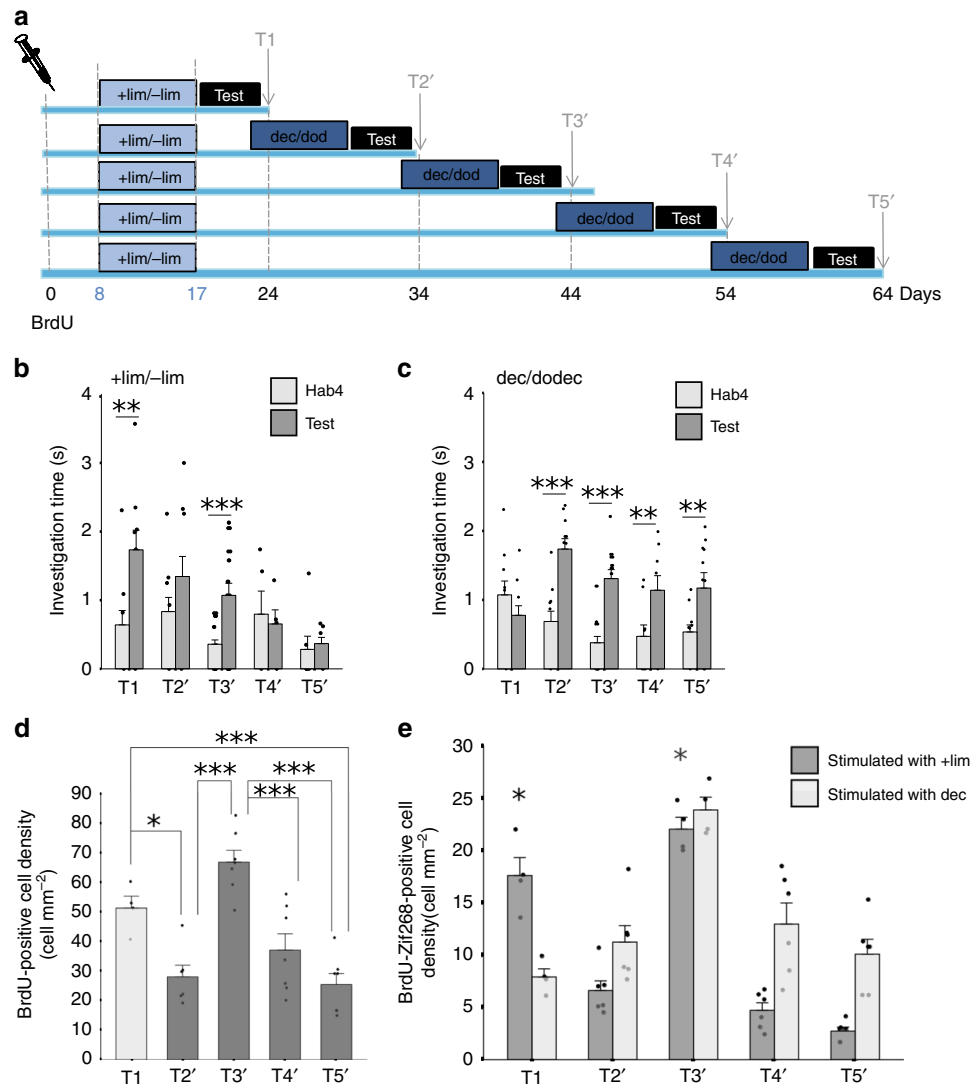
To assess adult-born neuron survival, a cohort of adult-born neurons present in the OB at the beginning of learning was labeled by injecting the DNA synthesis marker 5'-bromo-2'-deoxyuridine (BrdU) 8 days before learning<sup>8,9</sup>. BrdU-positive cells were counted in the granule cell layer for each time period post-learning (T1 to T5). We did not assess neurogenesis in the glomerular cell layer of the OB since we had previously found no modulation of the rate of neurogenesis in this layer after perceptual learning<sup>8</sup>. Analysis was performed based on learning performance, by comparing animals who discriminated after learning (T1, T2, and T3) to those who did not (T4 and T5) and to their corresponding NE groups (ANOVA,  $F_{(3,32)} = 4.1$ ,  $p = 0.014$ ; Fig. 1d and Supplementary Fig. 2). We observed a higher density of BrdU-positive cells in enriched groups able to discriminate the two odorants used for enrichment compared to enriched groups unable to do so (T1-T2-T3 versus T4-T5, *t*-test,  $p = 0.015$ , Fig. 1d) and compared to NE groups (enriched T1-T2-T3 vs non enriched T1-T2-T3, *t*-test,  $p = 0.028$ , Fig. 1d and Supplementary Fig. 2). Accordingly, the density of BrdU-positive cells was highly correlated to the index of discrimination (Pearson correlation:  $R = 0.84$ ;  $p = 0.038$ ) (Fig. 1e). The density of BrdU-positive cells showed no decline over the time points in NE animals (ANOVA,  $F_{(4,11)} = 0.8$ ,  $p = 0.5$ , Supplementary Fig. 2).

To determine whether the involvement of adult-born cell population in processing of the learned odorants relates to the discrimination ability, we assessed the density of cells co-expressing BrdU and Zif268 as an index of adult-born cell activation at the different time points post learning in three experimental groups. More precisely, we assessed the responsiveness of adult-born cells to +lim (odorant used for the enrichment) in enriched and NE animals. In addition, we assessed the response to dec (odorant not used for enrichment) of adult-born cells in +lim/–lim enriched animals (Fig. 1f). A 2-way ANOVA showed a group effect ( $F_{(2,40)} = 33.68$ ,  $p < 0.0001$ ), a time effect ( $F_{(2,40)} = 14.08$ ,  $p < 0.0001$ ) and an interaction ( $F_{(8,40)} = 8.13$ ,  $p < 0.0001$ ) indicating that the differences in BrdU/Zif268 positive cell density observed between groups depend on the time post learning. Interestingly, the density of Zif268 expression in adult-born neurons was significantly higher in enriched animals in response to +limonene at T1 to T3 compared to the other groups (Tukey tests, T1 versus T4 and T5,  $p = 0.0003$ ; T2 versus T4 and T5,  $p = 0.006$ ; T3 versus T4,  $p = 0.0005$  and T3 versus T5,  $p = 0.0006$ ). This result is in accordance with the performance of discrimination (Fig. 1f). Regarding the NE group, no time effect was observed (ANOVA,  $F_{(4,15)} = 4.66$ ,  $p = 0.84$ ). These changes were underlined not only by changes in the density of BrdU positive cells but also by changes in percentage of BrdU/Zif268 positive cells (Supplementary Fig. 3). Notably, a higher percentage of BrdU/Zif268 was found in enriched animals at T1-T3 compared to T4-T5 (*t*-test,  $p = 0.00022$ ) in response to +lim but not dec, indicating of a higher involvement of BrdU positive cells in processing the learned



**Fig. 1 The presence of adult-born neurons correlates with mnemonic performance.** **a** Experimental design. **b-f** Behavioral results. **b** Discrimination between +lim and -lim is assessed at different time points after the enrichment period. Non-enriched control (NE;  $n = 9$ ) animals do not discriminate the +lim from -lim at T1. After enrichment, the two odorants are discriminated at T1 ( $n = 11$ ), T2 ( $n = 5$ ), and T3 ( $n = 10$ ) but not at T4 ( $n = 9$ ) and T5 ( $n = 5$ ). **c** Dec and dodec are not discriminated at any time post-learning (NE  $n = 9$ ; T1  $n = 10$ ; T2  $n = 7$ ; T3  $n = 8$ ; T4  $n = 5$ ; T5  $n = 10$ ) (for clearer representation, 1 data point belonging to the Test trial of the NE group is not shown (investigation time = 6 s)). *T*-test,  $*p < 0.05$ . **d** Adult-born neuron density in the OB in enriched experimental groups. Groups that discriminated +lim/-lim (T1-T3) have a higher BrdU-positive cell density compared to groups that do not discriminate (T4-T5) ( $n = 4$ /group), *t*-test  $*p < 0.05$ . **e** Positive correlation between the discrimination index and adult-born neuron density in the OB. **f** Density of activated adult-born neurons (BrdU + /Zif268 + cells) in +lim/-lim enriched groups in response to +lim ( $n = 4$ /group) or dec ( $n = 5$ /group) and in control non-enriched in response to +lim (T1  $n = 2$ ; T2  $n = 4$ ; T3  $n = 2$ ; T4  $n = 3$ ; T5  $n = 4$ ). A significant increase of double-labeled cells is observed in enriched groups in response to learned odorant (+lim) in T1-T3 compared to T4-T5. Tukey tests,  $*p < 0.05$  for comparisons between enriched animals responding to +lim. Data are represented as data points and mean  $\pm$  sem. Source data are provided as a Source Data file.





**Fig. 2** Time between learning sessions has a critical impact on memory. **a** Experimental design. The groups differed by the interval between the two enrichment periods: 4, 14, 24 or 34 days separating the two enrichments. **b, c** Behavioral results. **b** Discrimination between +lim/−lim. Groups T1 and T3' discriminate. No discrimination is observed for the other groups (T2', T4', T5') (T1  $n = 11$ ; T2'  $n = 11$ ; T3'  $n = 11$ ; T4'  $n = 6$ ; T5'  $n = 7$ )  $t$ -test,  $^{**}p < 0.005$ ;  $^{***}p < 0.001$ . **c** Dec is discriminated from dodec in Group T2' to T5' (T1  $n = 10$ ; T2'  $n = 11$ ; T3'  $n = 11$ ; T4'  $n = 10$ ; T5'  $n = 11$ )  $t$ -test  $^{**}p < 0.005$ ;  $^{***}p < 0.001$ . **d** Adult-born neuron density. Higher density of adult-born neurons is observed in groups that are able to discriminate +lim from −lim (T1 and T3') ( $n = 6$ –7/group), Tukey corrected  $t$ -tests  $^{*}p < 0.05$ ;  $^{***}p < 0.001$ . **e** Density of activated adult-born neurons (BrdU+/Zif268+ cells) in response to +lim (T1  $n = 4$ ; T2'  $n = 6$ ; T3'  $n = 4$ ; T4'  $n = 6$ ; T5'  $n = 6$ ) or dec (T1  $n = 4$ ; T2'  $n = 6$ ; T3'  $n = 4$ ; T4'  $n = 6$ ; T5'  $n = 6$ ) after successive enrichments. Density of double-labeled cells is higher in response to +lim at T1 and T3' compared to T2', T4', and T5'. In response to dec, the density of BrdU/Zif268 cells is higher at T3' compared to the other groups.  $T$ -test  $^{*}p < 0.05$  for enriched animals responding to +lim at T1 and T3' different from all other groups. Data are represented as data points and mean  $\pm$  sem. Source data are provided as a Source Data file.

odorant in groups showing the ability to discriminate +lim from −lim.

In summary, perceptual memory showed a natural memory decay between T3 and T4. This memory alteration is accompanied by a decrease in the density of adult-born neurons and in the percentage of adult-born neurons responding to the learned odorants.

**Time between learning sessions affects stored memory.** In total 4, 14, 24 or 34 days after a first enrichment with +lim/−lim, mice were enriched with a new odorant pair, dec/dodec, tested for discrimination of the two pairs of odorants and sacrificed at the same times as in the previous experiment (groups T2' to T5') (Fig. 2a). All groups exhibited habituation behavior (Supplementary Table 2, Supplementary Fig. 4). Regarding

discrimination, for +lim/−lim, using two-way ANOVA, we observed a group effect (T1,T2',T3',T4',T5',  $F_{(4,94)} = 4.63$ ;  $p = 0.002$ ) and a trial effect ( $F_{(1,94)} = 19.99$ ;  $p < 0.0001$ ). For dec/dodec, there was no group effect ( $F_{(4,108)} = 2.19$ ;  $p = 0.08$ ) but a trial effect ( $F_{(1,108)} = 45.08$ ;  $p < 0.0001$ ) and interaction ( $F_{(4,108)} = 5.49$ ;  $p = 0.0005$ ). When the second enrichment was performed 4 days after the first one (T2'), animals were only able to discriminate the second odorant pair (Supplementary Table 2, Fig. 2b, c) suggesting that the acquisition of the second task erased the memory of the first one. This result was confirmed using another strain of mice (129 mice, Supplementary Fig. 5). As an additional control, we switched the training order, the animals were enriched first with dec/dodec and then with +lim/−lim. Result was similar to that previously obtained: animals were only able to discriminate the second learned odorant pair (+lim/−lim



in this case) (Supplementary Fig. 6). When the time between the two enrichments increased (14 days, T3'), the animals were able to discriminate the two odorants of each pair (Supplementary Table 2, Fig. 2b, c) suggesting that in this experimental configuration the formation of a new memory did not impact the memory already stored. At longer times between enrichments (24 and 34 days, respectively T4' and T5'), +lim and -lim were no longer discriminated while dec and dodec were, which is in accordance with the first experiment reporting the duration of memory retention (Supplementary Table 2, Fig. 2b, c).

BrdU-positive cell density was then assessed as in the first experiment. BrdU-positive cell density differed between groups (ANOVA, group effect,  $F_{(4,26)} = 16.1$ ;  $p < 0.0001$ ) (Fig. 2d). Post hoc Tukey corrected *t*-tests were then performed and showed that when the second enrichment was performed 4 days after the first one (T2'), the density of labeled adult-born cells was significantly decreased compared to T1 (Tukey corrected *t*-test  $p = 0.025$ ). This was accompanied by impaired +lim/-lim discrimination suggesting that, at 4 days between two learning, new memory formation altered adult-born neuron survival and already stored information. However, when the second enrichment was performed 14 days after the first (T3'), both pairs of odorants were discriminated and the density of adult-born cells remained high (Tukey corrected *t*-test, T1 versus T3':  $p = 0.2$ ). Finally, when the second enrichment occurred even later (T4' or T5'), BrdU density decreased significantly (Tukey corrected *t*-test, T3' versus T4':  $p < 0.001$ ; T5' versus T3':  $p < 0.001$ ) to a level comparable to T2' (Tukey corrected *t*-test, T4' versus T2':  $p = 0.59$ ; T5' versus T2':  $p = 0.99$ ) which is in accordance with the first experiment (Fig. 2d). To study the involvement of adult-born neurons in processing the learned information, we assessed the density of adult-born neurons responding to +lim or to dec (group effect) at the different delays between enrichments (Fig. 2e). Using two-way ANOVA, we found a group effect ( $F_{(1,42)} = 8.61$ ,  $p = 0.0054$ ), a time effect ( $F_{(1,42)} = 42.53$ ,  $p < 0.0001$ ) and an interaction ( $F_{(1,42)} = 13.08$ ,  $p < 0.0001$ ). In enriched animals in response to +lim, the density of BrdU/Zif268 cells is higher at T1 and T3' compared to T2', T4', and T5' (Time effect  $F_{(4,21)} = 73.09$ ,  $p < 0.0001$ ; Tukey corrected *t*-test  $p < 0.05$ ) which is in accordance with the performance of +lim versus -lim discrimination. In enriched animals in response to dec, the density of BrdU/Zif268 cells is higher at T3' compared to the other groups (Time effect  $F_{(4,21)} = 12.08$ ,  $p < 0.0001$ ; Tukey corrected *t*-test  $p < 0.05$ ). These changes in density relied at least partly on changes in percentage of BrdU/Zif268 double-labeled cells (Supplementary Fig. 7). For the T2', the decrease in the BrdU-Zif268 density is due more to a decrease in BrdU-positive cell density than to a decrease in the percentage of BrdU/Zif268 double-labeled cells. However, overall, the percentage of BrdU/Zif268-positive cells was higher in groups that discriminate +lim from -lim (T1 and T3') than in groups that do not discriminate (T2', T4', and T5') ( $p = 0.007$ ).

In summary, the time between the two enrichments is critical to the upkeep of the memory already stored and associated adult-born neurons survival and functional involvement. These data further suggest that the survival of at least some of these adult-born neurons is not assured as, for one week after learning, they go through a fragile state, which is vulnerable to a new learning challenge.

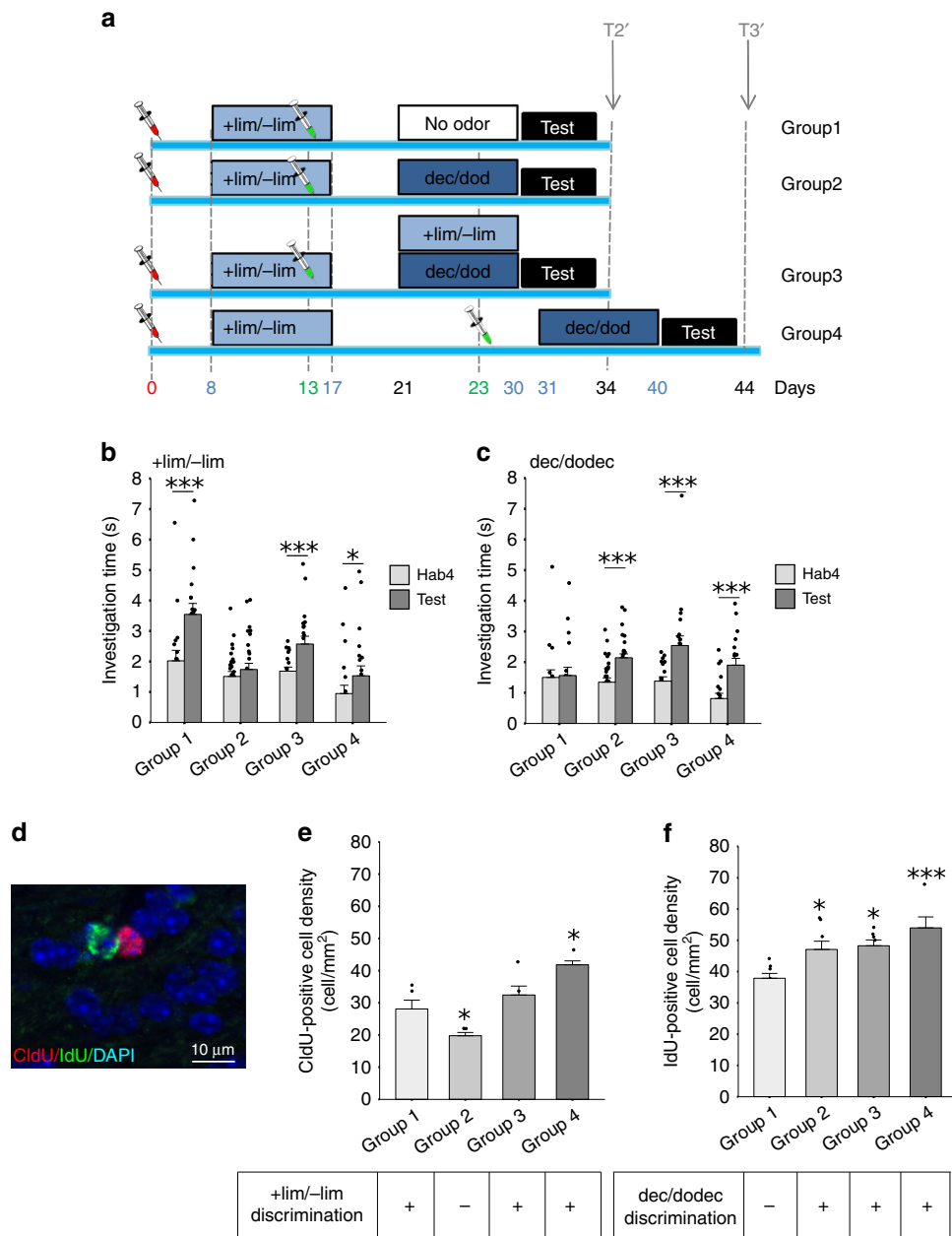
**Sensory reactivation prevents cell and memory loss.** With a short period between the two learning sessions (T2'), we found that the new memory altered previously stored information and induced apoptosis of adult-born neurons. Since cell survival is known to be input-dependent, we tested whether reactivating the network that responded to the odorants used for the enrichment

would avoid this overwriting of the memory. To do this we maintained exposure to the first pair of odorants during enrichment with the second pair (Fig. 3a).

In all groups, mice exhibited habituation behavior (Supplementary Table 3, Supplementary Fig. 8). Using two-way ANOVA, we observed significant effects of trial and group (trial:  $F_{(1,164)} = 16.88$ ;  $p < 0.0001$ , group:  $F_{(3,164)} = 12.55$ ;  $p < 0.0001$ ) (Fig. 3b) for +lim/-lim discrimination and a trial effect and a group effect (trial:  $F_{(1,164)} = 31.33$ ;  $p < 0.0001$ , group:  $F_{(3,164)} = 3.14$ ;  $p = 0.026$ ) (Fig. 3c) for dec/dodec discrimination. As in the first experiment (Fig. 1a), we observed that +lim/-lim enrichment improved discrimination between these two odorants when tested at T2' (group 1, see Supplementary Table 3, Fig. 3b, c) and that the second enrichment with dec/dodec when performed 4 days after the first one erased the ability to discriminate +lim/-lim (group 2, Fig. 3b, c, Supplementary Table 3). Interestingly, if we maintained the enrichment with +lim/-lim during the second enrichment period (group 3), the animals were able to discriminate both odorant pairs (Fig. 3b, c, Supplementary Table 3). This prevention of forgetting can be due to network reactivation or relearning. Finally, when the retention of the task was tested at T3', mice were, as previously, able to discriminate both odorant pairs (group 4, Fig. 3b, c, Supplementary Table 3).

To understand the dynamics of learning-dependent survival of adult-born neurons underlying these effects, we tagged two adult-born neuron populations differing in age using two analogues of thymidine, ChlorodeoxyUridine (CldU) and IododeoxyUridine (IdU). More precisely, we labeled a first pool of 8-day-old adult-born cells in the OB at the beginning of the first enrichment (with +lim/-lim) with CldU and a second pool of 8-day-old adult-born cells in the OB at the beginning of the second enrichment (with dec/dodec) but not present in the OB at the beginning of the first enrichment (Fig. 3a, d) with IdU. The densities of CldU- and IdU-positive cells were assessed in the OB at T2. Overlapping between CldU and IdU was never observed. Results showed differences between experimental groups for both markers (ANOVA, CldU:  $F_{(3,16)} = 19.06$ ,  $p < 0.0001$ ; IdU:  $F_{(3,24)} = 7.8$ ,  $p = 0.0008$ ). Post hoc Tukey corrected *t*-tests revealed that the level of CldU-positive cells was lower in group 2 compared to the other groups, in accordance with +lim/-lim discrimination performance (group 2 versus group 1:  $p = 0.056$ , group 2 versus group 3:  $p = 0.0029$ , group 2 versus group 4:  $p < 0.001$ ; Fig. 3e). Interestingly, the density of CldU-positive cells was not significantly higher in group 3 compared to group 1 ( $p = 0.48$ ) suggesting that the second enrichment recruited a new pool of adult-born neurons. Supporting this hypothesis, all groups that were submitted to dec/dodec enrichment after the +lim/-lim enrichment, including group 2, showed a higher level of IdU-positive cells than group 1 (respectively  $p = 0.03$ ,  $p = 0.017$  and  $p < 0.001$ ) (Fig. 3f). This strongly suggests that the second learning recruited a new population of adult-born neurons younger than that recruited during the first learning.

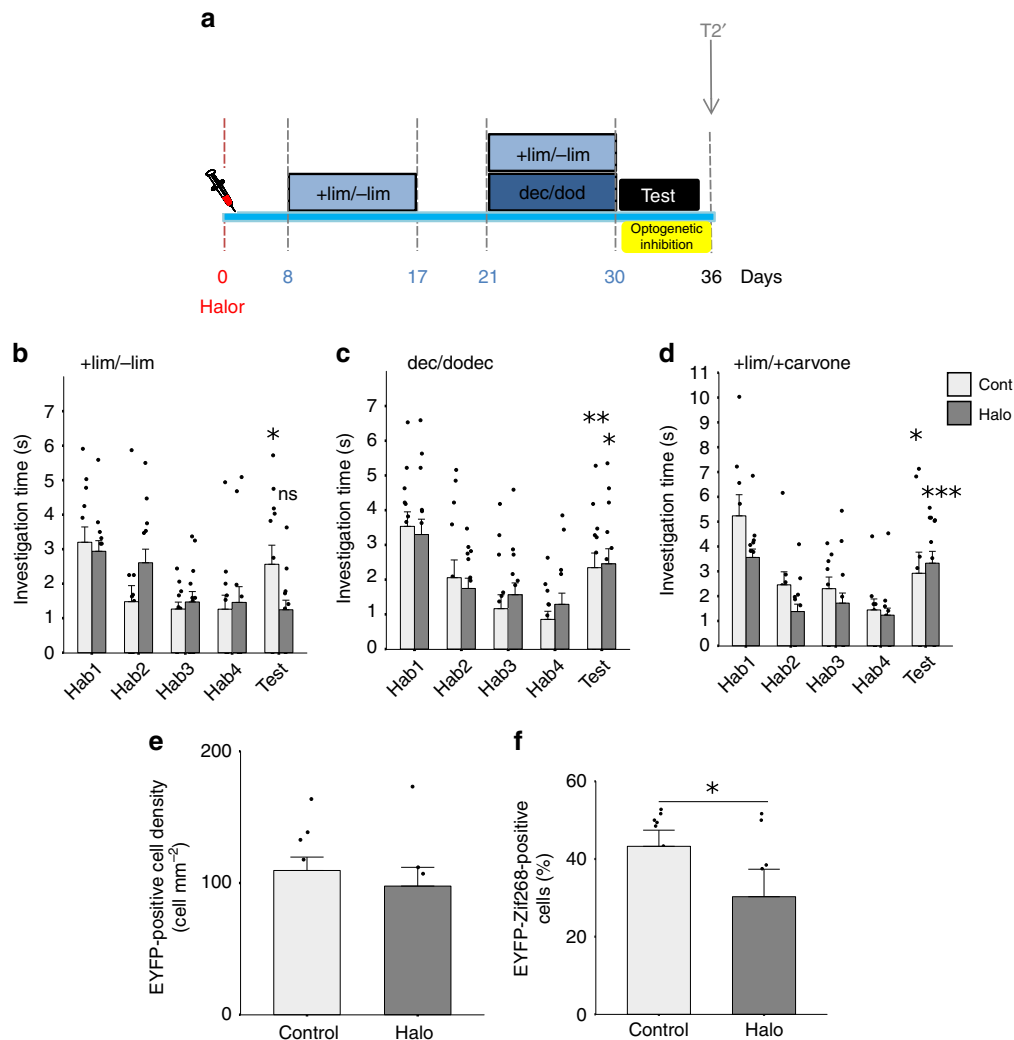
We learned from this experiment that reactivating the network of the first learned odorant pair during the second task prevented memory erasure and the disappearance of adult-born cells. Thus, for a short time between two successive learning tasks, adult-born neurons can be maintained in the network providing that the relevant sensory inputs remain in the environment. However, despite the presence of these adult-born neurons still in their critical period of integration, a distinct pool of younger adult-born neurons was recruited by the second task. It remains the question of whether the second learning relies on the last recruited adult-born neuron population (IdU-positive cells) or on both adult-born neuron populations (IdU- and CldU-positive cells). In other words, are adult-born neurons allocated to a memory trace able to underlie or contribute to subsequent learning?



**Fig. 3 Reactivation of learned information prevented cell death and maintained memory.** **a** Experimental design. **b, c** Behavioral results. **b** +lim and -lim are discriminated in groups 1 ( $n = 18$ ), 3 ( $n = 20$ ) and 4 ( $n = 10$ ) but not in group 2 ( $n = 18$ ). **c** Dec/dodec are discriminated in Group 2 ( $n = 18$ ), 3 ( $n = 20$ ) and 4 ( $n = 10$ ) but not in group 1 ( $n = 18$ ). **d** Example of CldU/DAPI and IdU/DAPI double labeling. **e**. Density of CldU-positive cells. A lower density is observed in group 2 compared to groups 1, 3, and 4 and a higher density in group 4 compared to groups 1, 2, and 3 ( $n = 5$ /group). **f** Density of IdU-positive cells is higher in groups 2 ( $n = 8$ ), 3 ( $n = 7$ ) and 4 ( $n = 5$ ) compared to group 1 ( $n = 8$ ). Tukey corrected  $t$ -tests  $*p \leq 0.05$ ,  $***p < 0.001$ . Data are represented as data points and mean  $\pm$  sem. Source data are provided as a Source Data file.

**Distinct adult-born neuron cohorts for distinct memories.** We used the same learning paradigm as previously (group 3; Figs. 3a and 4a) in which mice underwent a first enrichment period with +lim/-lim and 4 days later a second one with dec/dodec in the presence of +lim/-lim to avoid memory loss. Eight days before the first enrichment period, adult-born neuron progenitors were transduced with halorhodopsin-expressing or control lentiviruses coupled with EYFP expression and animals were implanted with optical fibers in the OB. Light stimulation was used to block adult-born cell activity during the test trial of the habituation/dishabituation task. Results showed that light-triggered inhibition of neurons born 8 days before the first learning task altered the discrimination of +lim/-lim (Fig. 4b, Supplementary Table 4)

but not of dec/dodec (Fig. 4c, Supplementary Table 4). Animals infected with a control virus and submitted to light stimulation behaved as group 3 of the previous experiment, suggesting no direct effect of the light. To test that light-triggered inhibition of adult-born neurons in halorhodopsin animals had no deleterious effect on spontaneous odor discrimination, we assessed the discrimination of a pair of dissimilar odorants (+lim/+carvone) and found that it was not impaired by light (Fig. 4d, Supplementary Table 4). We then analyzed the level of viral transfection in control and halorhodopsin groups and found no difference of EYFP-positive cell density ( $t$ -test,  $p = 0.5$ , Fig. 4e). We also verified the effectiveness of light-mediated inhibition of adult-born granule cells by assessing the expression of Zif268-positive cells in



**Fig. 4 Optogenetic inhibition revealed that distinct adult-born neuron cohorts underlined distinct memories.** **a** Experimental design. Adult-born neurons aged 8 days at the beginning of the first enrichment were transduced with a lentivirus expressing halorhodopsin (Halo) or with an empty virus (Cont). Light stimulation was performed during the test trial of the habituation/dishabituation task. **b-d** Behavioral results. **b** The learned discrimination of +lim/-lim is abolished by light stimulation in the Halo group ( $n = 13$ ), but not in the Control group ( $n = 12$ ). **c** Discrimination between dec/dodec is not altered by light stimulation (Cont  $n = 13$ ; Halo  $n = 14$ ). **d** Discrimination between a dissimilar odorant pair +lim/+carvone is not altered by light-triggered inhibition of adult-born neurons (Cont  $n = 9$ ; Halo  $n = 14$ ). *t*-tests \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (for difference between Hab4 and Test in **b-d**). **e** The density of EYFP-positive transduced cells in the OB is similar between Control ( $n = 9$ ) and Halo ( $n = 7$ ) animals. **f** Percentage of EYFP-Zif268-positive cells after light-triggered inhibition is decreased in Halo ( $n = 7$ ) compared to Control ( $n = 8$ ). *t*-test \* $p < 0.05$  (for difference between Halo and Control groups in **f**). Data are represented as data points and mean  $\pm$  sem. Source data are provided as a Source Data file.

EYFP-positive neurons after stimulation with light. The expression of Zif268 was significantly lower in the halorhodopsin compared to the control group (*t*-test,  $p = 0.03$ , Fig. 4f).

Hence, silencing a cohort of adult-born neurons aged between 8 and 18 days at the time of learning leads to memory erasure demonstrating the crucial role of adult-born neurons in perceptual memory. However, this manipulation did not affect the memory of a new discrimination task learned shortly afterwards, suggesting that these adult-born neurons are allocated to only one memory trace.

## Discussion

The OB is continuously receiving waves of adult-born neurons and so contains adult-born neurons of different ages and levels of maturation<sup>18</sup>. When learning happens successively, which neurons take part in underlying behavioral changes? When the time between the two learning tasks is short (4 days), part of the adult-

born neurons present in the OB after the first task are prematurely killed and memory is lost. This conclusion is based on the loss of BrdU-positive neurons and is consistent with previous reports showing that blocking adult-born cell death prevented memory erasure<sup>19</sup>. In addition to training-dependent changes in BrdU-positive cell density, the functional involvement of these cells in response to the learned odorant increased with performance for neurons aged 8 days at the beginning of learning. We further show that these two learning tasks, despite they are spaced by a short 4-day time period, induced two distinct waves of adult-born neuron survival and that optogenetically inactivating the first cohort altered the first memory, without affecting the second learning. This further reinforces the idea that the 20-day old BrdU-tagged neurons exited the time window during which they are most crucial to enrichment-induced improvement in discrimination. It is worth noting that in the short time condition, the number of adult-born cells is reduced but a substantial fraction of adult-born neurons (>20%) still respond to the learned odorant even though

discrimination is lost. This state differs from that observed for longer between-task times where both density of adult-born neurons and their responsiveness to the learned odorants are strongly reduced. This suggests only a partial withdrawal of the remaining adult-born neurons from processing the first learned odorants in the short time condition, sufficient however to prevent discrimination. Nevertheless, we conclude that cohorts of neurons of different ages, but still within their critical period, are required to allow the encoding of successive learning experiences. Importantly, when the time between tasks is longer (14 days), no cell loss occurred and performances were maintained. Altogether, these data provide evidence for an unexpectedly early and sharp functional transition within the critical period. The critical period of adult-born neurons which, based on their vulnerability to cell death, morphological development and synaptic current development and plasticity, has been reported to last from 30 days to 60 days post neuronal birth<sup>6,10,20–23</sup>. Within this critical period, our data suggest that availability of adult-born neurons for encoding new experience lasts no longer than 20 days.

When previously learned information is reintroduced into the environment, we observed no premature cell death and the second learning did no longer erase the first one. To maintain the first learned discrimination, the network could either use adult-born neurons already integrated into this network or recruit a new cohort of adult-born neurons. Optogenetic inactivation of the 8-day old cohort of adult-born neurons present in the OB at the beginning of the first training impaired memory of the first task. This strongly suggests that performance relies on adult-born neurons recruited in the initial task suggesting that the memory is recalled rather than re-encoded. Hence, reactivated memories are not over-written and forgotten. On the contrary, neurons encoding obsolete information (i.e. not present in the environment) could be sentenced to death after about 45 days unless another learning task occurs soon after, causing interference and leading to earlier neuronal death. Our data thus suggest that adult-born neurons are sensitive to interference only during their first 3 weeks and that if further learning occurs when adult-born neurons present in the OB are more than 21 days old, they are resistant to death and memory persists. On the other hand, a second learning task causes neurons aged less than 21 days old to die and erases the encoded memory. This difference could be due to the state of synaptic integration of the adult-born neurons<sup>20</sup> which are more or less sensitive to competition from later waves of adult-born neurons.

Much of what we experience is ultimately forgotten, but memories for some events persist. Here, we report that modulation of the OB circuitry, as a function of the environment and dependent on the fate of maturing adult-born neurons, is responsible for the balance between the transience or persistence of memory. This is made possible thanks to distinct neuronal populations encoding temporally distinct experiences.

## Methods

**Mice.** Adult C57BL/6J mice (8 weeks old, male, Charles River, L'arbresles, France) and 129 mice (8 weeks old, male, Charles River, L'arbresles, France) were used in this study. They were housed in standard laboratory cages with water and food ad libitum and were kept on a 12-hr light/dark cycle at a constant temperature of 22 °C. All behavioral training was conducted in the afternoon (12:00–18:00). Experiments were done following procedures in accordance with the European Community Council Directive of 22 September 2010 (2010/63/UE) and approved by the National Ethics Committee (Agreement DR2013-48(vM)). Every effort was made to minimize animal suffering.

Different animals were used in different experimental groups. Histological data were obtained from a sample of animals used for behavioral tasks.

**Experimental designs.** Regarding the first experiment, mice were injected with a DNA marker, BrdU, in order to label a cohort of adult-born neurons. Eight days later, they began the enrichment procedure with (+)limonene (+lim) and (–)limonene (–lim) for one hour daily over 10 days. At the end of the enrichment, mice were

tested on their spontaneous discrimination between +lim and –lim and also between another pair of perceptually similar odorants (decanal (dec) and dodecane (dodec)). Discrimination was assessed using an olfactory habituation/dishabituation task. Animals were sacrificed 24, 34, 44, 54 or 64 days after BrdU injections (Fig. 1a).

Regarding the second experiment, eight days after BrdU injection, a different set of mice was similarly enriched with +lim and –lim but this was followed by a second 10-day enrichment period with dec and dodec either 4, 14, 24, 34 or 44 days after the first. At the end of both enrichments, mice were tested on their spontaneous discrimination between the two odorants of each of the pairs using an olfactory habituation/dishabituation task and animals were sacrificed 24, 34, 44, 54 and 64 days post BrdU injections (Fig. 2a).

In the third experiment, we used two other DNA markers (analogues of BrdU), 5'-chloro-2'-deoxyuridine (CldU) and 5'-iodo-2'-deoxyuridine (IdU), to label two different populations of adult-born cells. CldU was injected 8 days before the first enrichment (again with +lim and –lim) while IdU was injected 8 days before the second enrichment period. The second enrichment period varied among groups: no enrichment (group 1); dec and dodec (group 2 and group 4), +lim and –lim plus dec and dodec (group 3). The time between the two enrichments was either 4 or 14 days. As previously, discrimination was assessed using a habituation/dishabituation task and animals were sacrificed 34 or 44 days post-CldU injection (Fig. 3a).

In the fourth experiment using group 3 experimental configuration from the previous experiment, we performed targeted lentiviral-induced halorhodospin channel expression (NpHR3.0) in the subventricular zone, and used optogenetics to specifically inhibit the population of adult-born neurons arriving in the OB at the beginning of the +lim/–lim enrichment (Fig. 4a).

**Perceptual learning.** This implicit olfactory learning consisted in passive exposure to odorants (enrichment). For the olfactory enrichment, 100 µL of pure odorant were placed in two separate tea balls of the home cages for one hour daily over 10 days. For the multiple enrichment (experiment 3, group 3), the two pairs of odorants were presented with an interval of 1 h (SI Methods).

We assessed the spontaneous discrimination between two pairs of similar odorants: +lim/–lim and dec/dodec using olfactory habituation/dishabituation. A test session consisted of four 50-s odor presentations of a first odorant (Hab) at 5 min intervals, followed by one 50-s presentation of the second odorant of the pair (Test). Investigation was defined as active sniffing within 1 cm of the tea ball (SI Methods)<sup>8,24,25</sup>. Exploration assessments were done blind with regard to the experimental group.

**Data analysis.** Data analysis was performed using R software (CRAN). Normality was assessed using the Kolmogorov-Smirnov test. Global two-way ANOVAs were performed to evaluate changes in discrimination abilities between groups. Then intra-group one-way RM-ANOVAs and unilateral paired *t*-tests were performed to determine whether the mice exhibited habituation (trial effect) as well as discrimination (by comparing Hab4 and Test). Discrimination was indicated by a significant increase in investigation time during the test trial. Discrimination index was calculated as  $[1 - (\text{Hab4}/\text{Test})]^{26}$  (SI Methods). Sample sizes were determined based on previous reports<sup>8,14,27</sup>. Animal assignment to the various experimental groups was randomized.

**Adult-born cell density assessment.** Bromodeoxyuridine (BrdU) (Sigma-Aldrich) was injected 8 days before the enrichment period (50 mg kg<sup>-1</sup> in saline, 3x at 2-h intervals; i.p.)<sup>14,22</sup>.

24, 34, 44, 54 and 64 days post BrdU injection, five mice were taken randomly from each experimental group for sacrifice and BrdU, IdU and CldU immunohistochemistries were carried on as described in SI Methods<sup>14,22</sup>.

The method used for BrdU-, IdU- and CldU-positive cell counting is described in SI Methods<sup>14,28</sup>. The mean positive cell density of each array was calculated and averaged within each experimental group. Between-groups comparisons of the mean cell density were performed by ANOVA followed by post hoc *t*-tests with Tukey corrections. Unilateral *t*-tests were performed for comparisons between two groups. The level of significance was set to 0.05.

**BrdU/Zif268 experiment.** To investigate immediate early gene expression in response to odorant exposure, mice were presented with a tea ball containing 100 µl of pure +lim or dec for 1 h, 1 h before sacrifice. A rabbit anti-Zif268 (1:1000, Santa Cruz Biotechnology) and a rat anti-BrdU (1:100, Harlan Sera-Lab) were used. The appropriate secondary antibodies, coupled to Alexa Fluor 633 and 488 (Invitrogen) were used for revelation of the different markers. BrdU/Zif268 density was calculated by combining the mean percentage of BrdU/Zif268 positive cells per group to individual density of BrdU positive cells. All cell counts were conducted blind with regard to the experimental group.

**Optogenetics in freely behaving mice.** Hundred and fifty nanoliters of pLenti-hSyn-eNpHR3.0-EYFP lentivirus ( $9.22 \times 10^6$  IU ml<sup>-1</sup>) or 300 nL of control pLenti-hSyn-EYFP ( $1.1 \times 10^6$  IU ml<sup>-1</sup>, expressing only the reporter gene EYFP<sup>29</sup>) injections were done bilaterally in the subventricular zone, with the following coordinates relative to bregma: antero-posterior + 1 mm, medio-lateral ± 1 mm, dorso-ventral –2.3 mm and at a rate of 150 nL min<sup>-1</sup>. Just after virus infusions, mice were



implanted with bilateral optic fibers (200 nm core diameter, 0.22 N.A., Doric Lenses) in the OB, with the following coordinates respective to bregma: antero-posterior +4.6 mm, medio-lateral  $\pm 0.75$  mm, dorso-ventral -2 mm. Mice were injected with a ketoprofen solution (2 mg kg<sup>-1</sup>) after the surgery as well as during the following days and allowed to recover with food and water ad libitum.

During the habituation/dishabituation mice were automatically stimulated (crystal laser, 561 nm, 10–15 mW, continuous stimulation) only during the test trial (Test) when they entered a 2.5 cm diameter zone around the tea ball.

In order to control of light-triggered inhibition, 36 days post-surgery and lentiviral infusion, mice were stimulated during the hour before sacrifice with light patterns mimicking the average light stimulating pattern during the test trial (0.75 s light ON, 5 s light OFF for 1 h). After brain sectioning (see above), EYFP and Zif268 double immunohistochemistry was performed: incubation with rabbit Zif268 antibody (1:1,000, Santa Cruz, ref. Sc-189), chicken GFP antibody (1:1,000, Anaspec TEBU, ref. 29, 55423). The density of EYFP, Zif268<sup>+</sup> and double-stained cells were counted on 1–2 slices under the fiber implantation to allow assessment of the inhibition. Statistical significance was assessed using a unilateral t-test.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

### Data availability

The data associated to all main figures are provided as a Source Data file.

### Code availability

The codes are provided in supplementary file entitled Supplementary Software 1.

Received: 8 January 2019; Accepted: 12 November 2019;

Published online: 06 December 2019

## References

- Altman, J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J. Comp. Neurol.* **137**, 433–457 (1969).
- Lois, C. & Alvarez-Buylla, A. Long-distance neuronal migration in the adult mammalian brain. *Science* **264**, 1145–1148 (1994).
- Ming, G. & Song, H. Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223–250 (2005).
- Ming, G. & Song, H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron* **70**, 687–702 (2011).
- Alonso, M. et al. Olfactory discrimination learning increases the survival of adult-born neurons in the olfactory bulb. *J. Neurosci.* **26**, 10508–10513 (2006).
- Mouret, A. et al. Learning and survival of newly generated neurons: when time matters. *J. Neurosci.* **28**, 11511–11516 (2008).
- Lazarini, F. et al. Cellular and behavioral effects of cranial irradiation of the subventricular zone in adult mice. *PLoS ONE* **4**, e7017 (2009).
- Moreno, M. M. et al. Olfactory perceptual learning requires adult neurogenesis. *Proc. Natl Acad. Sci. USA* **106**, 17980–17985 (2009).
- Moreno, M. M. et al. Action of the noradrenergic system on adult-born cells is required for olfactory learning in mice. *J. Neurosci.* **32**, 3748–3758 (2012).
- Nissant, A., Bardy, C., Katagiri, H., Murray, K. & Lledo, P.-M. Adult neurogenesis promotes synaptic plasticity in the olfactory bulb. *Nat. Neurosci.* **12**, 728–730 (2009).
- Sultan, S. et al. Learning-dependent neurogenesis in the olfactory bulb determines long-term olfactory memory. *FASEB J.* **24**, 2355–2363 (2010).
- Sultan, S., Lefort, J. M., Sacquet, J., Mandaïron, N. & Didier, A. Acquisition of an olfactory associative task triggers a regionalized down-regulation of adult born neuron cell death. *Front. Neurosci.* **5**, 52 (2011).
- Grelat, A. et al. Adult-born neurons boost odor-reward association. *Proc. Natl Acad. Sci. USA* **115**, 2514–2519 (2018).
- Mandaïron, N. et al. Opposite regulation of inhibition by adult-born granule cells during implicit versus explicit olfactory learning. *eLife* **7**, e34976 (2018).
- Akers, K. G. et al. Hippocampal neurogenesis regulates forgetting during adulthood and infancy. *Science* **344**, 598–602 (2014).
- Frankland, P. W. & Josselyn, S. A. Hippocampal neurogenesis and memory clearance. *Neuropsychopharmacology* **41**, 382–383 (2016).
- Richards, B. A. & Frankland, P. W. The persistence and transience of memory. *Neuron* **94**, 1071–1084 (2017).
- Carleton, A. et al. Making scents of olfactory neurogenesis. *J. Physiol.-Paris* **96**, 115–122 (2002).
- Sultan, S., Rey, N., Sacquet, J., Mandaïron, N. & Didier, A. Newborn neurons in the olfactory bulb selected for long-term survival through olfactory learning are prematurely suppressed when the olfactory memory is erased. *J. Neurosci.* **31**, 14893–14898 (2011).
- Kelsch, W., Lin, C.-W., Mosley, C. P. & Lois, C. A critical period for activity-dependent synaptic development during olfactory bulb adult neurogenesis. *J. Neurosci.* **29**, 11852–11858 (2009).
- Yamaguchi, M. & Mori, K. Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb. *PNAS* **102**, 9697–9702 (2005).
- Mandaïron, N. et al. Neurogenic correlates of an olfactory discrimination task in the adult olfactory bulb. *Eur. J. Neurosci.* **24**, 3578–3588 (2006).
- Ge, S., Yang, C.-H., Hsu, K.-S., Ming, G.-L. & Song, H. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron* **54**, 559–566 (2007).
- Mandaïron, N., Stack, C., Kiselycznyk, C. & Linster, C. Broad activation of the olfactory bulb produces long-lasting changes in odor perception. *Proc. Natl Acad. Sci. USA* **103**, 13543–13548 (2006).
- Cleland, T. A., Morse, A., Yue, E. L. & Linster, C. Behavioral models of odor similarity. *Behav. Neurosci.* **116**, 222–231 (2002).
- Rey, N. L., Sacquet, J., Veyrac, A., Jourdan, F. & Didier, A. Behavioral and cellular markers of olfactory aging and their response to enrichment. *Neurobiol. Aging* **33**, 626.e9–626.e23 (2012).
- Forest, J. et al. Role of adult-born versus preexisting neurons born at p0 in olfactory perception in a complex olfactory environment in mice. *Cereb. Cortex* <https://doi.org/10.1093/cercor/bhz105> (2019).
- Mandaïron, N., Sacquet, J., Jourdan, F. & Didier, A. Long-term fate and distribution of newborn cells in the adult mouse olfactory bulb: influences of olfactory deprivation. *Neuroscience* **141**, 443–451 (2006).
- Kermen, F. et al. Topographical representation of odor hedonics in the olfactory bulb. *Nat. Neurosci.* <https://doi.org/10.1038/nn.4317> (2016).

## Acknowledgements

This work was supported by CNRS, Inserm, Lyon 1 University and Ecole Normale Supérieure de Lyon. We would like to thank C. Benetollo from the Neurogenetic and Optogenetic Platform of the CRNL for lentiviral production and G. Froment, D. Nègre and C. Costa from the lentivector production facility /SFR BioSciences de Lyon (UMS3444/US8).

## Author contributions

J.F., M.M., A.D., and N.M. designed the research. J.F., M.M., A.Z., M.C., J.S., and L.C. performed the research. J.F., M.M., M.R., A.D., and N.M. wrote the paper.

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary information** is available for this paper at <https://doi.org/10.1038/s41467-019-13521-7>.

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**ANNEXE 3** : "Rôle des neurones nés à l'âge adulte par rapport aux neurones préexistants nés à P0 dans la perception olfactive dans un environnement olfactif complexe chez la souris." (publiée)

## ORIGINAL ARTICLE

# Role of Adult-Born Versus Preexisting Neurons Born at P0 in Olfactory Perception in a Complex Olfactory Environment in Mice

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## Abstract

Olfactory perceptual learning is defined as an improvement in the discrimination of perceptually close odorants after passive exposure to these odorants. In mice, simple olfactory perceptual learning involving the discrimination of two odorants depends on an increased number of adult-born neurons in the olfactory bulb, which refines the bulbar output. However, the olfactory environment is complex, raising the question of the adjustment of the bulbar network to multiple discrimination challenges. Perceptual learning of 1 to 6 pairs of similar odorants led to discrimination of all learned odor pairs. Increasing complexity did not increase adult-born neuron survival but enhanced the number of adult-born neurons responding to learned odorants and their spine density. Moreover, only complex learning induced morphological changes in neurons of the granule cell layer born during the first day of life (P0). Selective optogenetic inactivation of either population confirmed functional involvement of adult-born neurons regardless of the enrichment complexity, while preexisting neurons were required for complex discrimination only.

**Key words:** adult neurogenesis, olfaction, optogenetic, perceptual learning, structural plasticity



## Introduction

The rodent olfactory bulb (OB) is supplied during adulthood with new inhibitory interneurons (Altman 1969; Lois and Alvarez-Buylla 1994; Ming and Song 2005) that shape the message carried outside the OB by the mitral cells (Winner et al. 2002; Malvaut and Saghatelian 2016). These adult-born neurons play a central role in tuning the animal's discrimination abilities through perceptual learning (Mandairon et al. 2006b; Moreno et al. 2009). Perceptual learning is defined as a significant improvement in discrimination abilities induced by previous non-reinforced experiences (Gilbert et al. 2001), reflecting a dynamic adjustment by which animals discriminate relevant stimuli within their immediate environment. In the context of olfaction, mice can improve their performances of discrimination between perceptually similar odorants in a relative odor-specific manner thanks to passive enrichment with these odorants (Mandairon et al. 2006b; Moreno et al. 2009). This perceptual learning not only increases adult-born neuron survival and their spine density (Daroles et al. 2015; Mandairon et al. 2018) but requires their presence in the OB (Moreno et al. 2009). Importantly, in order to correctly guide the animal's behavior within a natural environment, the olfactory system needs to cope with its complexity, that is an environment in which multiple odorants are present within the same time window. What part do adult-born neurons play in perceptual learning when a complex olfactory environment challenges the network? In other words, does complex learning require more adult-born neurons and/or an enhanced structural plasticity of these neurons? Moreover, in order to understand the specificity of the role of adult-born neurons, it is crucial to compare their functional properties and structural plasticity with those of neurons born during the ontogenesis of the OB [labeled at P0 and so-called hereafter preexisting neurons (Bayer 1983)].

Newly integrated adult-born cells show specific electrophysiological properties compared with resident neurons (formed by a mix of older adult-born neurons and preexisting neurons) such as increased excitability, increased long-term potentiation (Carleton et al. 2003; Nissant et al. 2009), as well as a higher responsiveness to odorant stimulation (Livneh et al. 2014). Moreover, following olfactory enrichment in adulthood, adult-born neurons become more selective to the enriched odorants and thus acquire functional features distinct from those of older resident neurons (Magavi et al. 2005; Livneh et al. 2014).

The newly integrated adult-born neurons also show specific response properties compared specifically with preexisting neurons (Lemasson et al. 2005; Valley et al. 2013; Breton-Provencher et al. 2016). Recent studies using chemo- or optogenetic manipulations on adult-born versus preexisting neurons showed the differential involvement of preexisting versus adult-born neurons in reward-associated olfactory learning (Muthusamy et al. 2017; Grelat et al. 2018). However, the respective roles of adult-born compared with preexisting neurons in other learning contexts still remain unclear.

To answer these questions, we used a perceptual learning paradigm in different configurations in terms of complexity. More precisely, young adult mice were exposed to 1, 2, 3, or 6 pairs of odorants during the enrichment period. We assessed the effect of these different enrichment protocols on mice ability to discriminate. We also assessed adult-born cell survival, responsiveness to the learned odorants, and

structural plasticity. In addition, we analyzed the uniqueness of adult-born neuron plasticity by comparing it with that present in preexisting neurons. Finally, to determine the functional role of each population on discrimination performances after perceptual learning, we measured the effect of selective optogenetic inactivation of the populations of preexisting or adult-born neurons on learned discrimination.

## Materials and Methods

### Animals

A total of 103 inbred male C57BL/6J young adult mice born and raised in our laboratory were used in this study. Mice were housed in a controlled environment under a 12-h light/dark cycle with food and water *ad libitum*. All behavioral training (enrichment) was conducted between 8 AM and 8 PM, while behavioral testing was conducted in the afternoon (12 AM–7 PM) on young adult mice aged 2–3 months. All efforts were made to minimize the number of animals used and their suffering during the experimental procedure in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC). Protocols were approved by the French Ethical Committee [DR2013–48 (vM)]. Mice were obtained from multiple independent litters over several months, and mice from the same litter were assigned as much as possible to different experimental groups.

### Overview of the Experiment

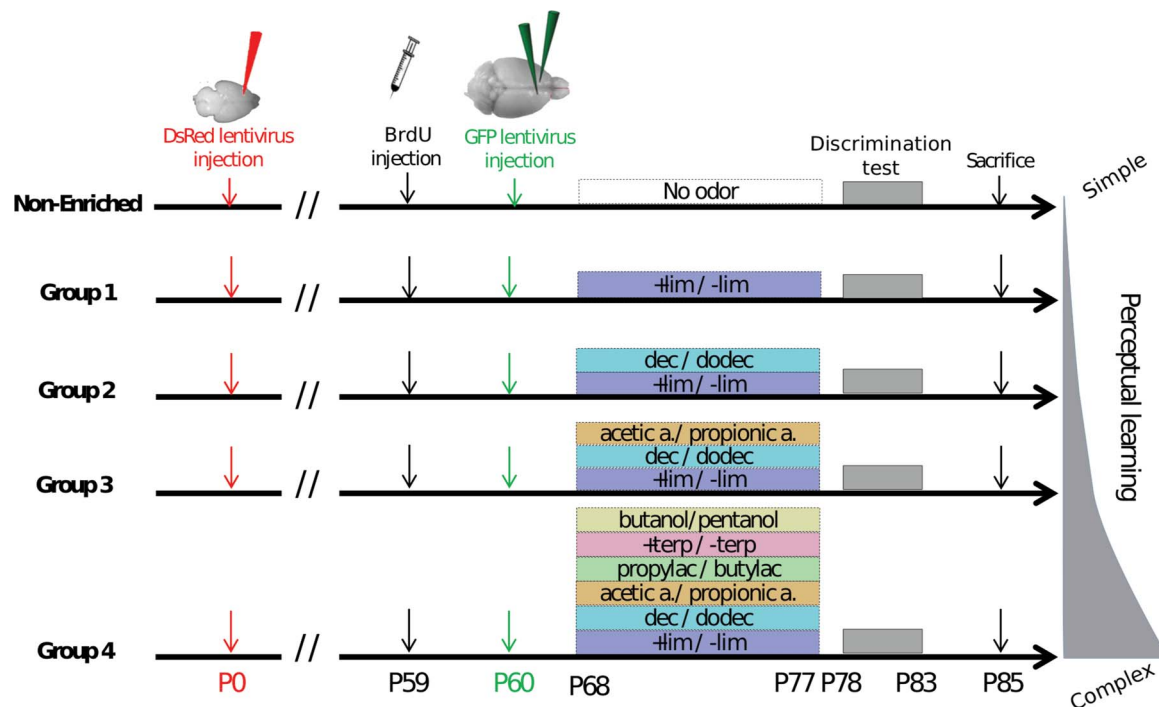
A total of 5 experimental groups of mice were formed based on the number of odorant pairs used for the enrichment (from 0 to 6 odorant pairs; Fig. 1). Using a habituation/cross-habituation test, we assessed each group for their discrimination performances, adult-born cell survival using Bromodeoxyuridine (BrdU) injections, and structural plasticity using a DsRed-expressing lentivirus injection at P0 for preexisting neuron labeling and a green fluorescent protein (GFP)-expressing lentivirus injection at P60 for adult-born neurons labeling followed by *ad hoc post mortem* fine neuronal morphology analysis. Mice were sacrificed 85 days after birth (Fig. 1).

### Detailed Procedures

#### Behavioral Experiments

**Enrichment.** Odor enrichment consisted of exposure to odorant pairs (from 1 to 6, depending on the experimental group; Fig. 1) for 1 h per day over 10 consecutive days. For each odorant pair, the 2 odorants were presented simultaneously on 2 swabs containing 100  $\mu$ L of pure odorant placed in 2 tea balls hanging from the cover of the cage. For multiple enrichments, odorant pairs were presented with an interval of at least 1 hour. Odorant pairs were presented in the same order during the 10-day enrichment for a given cage, but this order was randomized between cages. The different experimental groups were as follows: Group 1 enriched with +lim/–lim; Group 2 with +lim/–lim and dec/dodec; Group 3 with +lim/–lim, dec/dodec, and acetic a./propionic a.; Group 4 with +lim/–lim, dec/dodec, acetic a./propionic a., buta/penta, propyl/butyl, and +terp/–terp (Fig. 1 and Supplementary Table 1). Control non-enriched (NE) mice were subjected to the same protocol and under the same conditions except that the tea balls contained only mineral oil.

**Olfactory habituation/cross-habituation test.** We used a habituation/cross-habituation test to assess olfactory discrimination



**Figure 1.** Experimental design. In mice, preexisting neurons (born during ontogenesis) were labeled by injecting DsRed-expressing lentivirus in the lateral ventricle at post-natal day 0 (P0). Adult-born neurons were labeled by injecting BrdU (i.p.) at P59 and GFP-expressing lentivirus in the subventricular zone at P60 (2 months old). Then, 5 groups of mice were formed depending on the number of odorant pairs used for the 10-day enrichment period (control NE: Group NE; enriched with 1 odor pair: Group 1; enriched with 2 odorant pairs: Group 2; enriched with 3 odorant pairs: Group 3; enriched with 6 odorant pairs: Group 4). Animals were enriched for 1 h per day with each odorant pair. After the enrichment period, mice were tested on their ability to discriminate the odorants of each odorant pair using an olfactory habituation/cross-habituation test. Mice were sacrificed 85 days after their birth.

(Moreno et al. 2009). Briefly, this task assessed whether mice were able to spontaneously discriminate between 2 odorants by habituating them to 1 odorant and measuring their cross-habituation to the second odorant. We tested the discrimination between the 2 odorants of each pair used for the enrichment (Group 1 was tested for discrimination between +lim/–lim; Group 2 +lim/–lim and dec/dodec; Group 3 +lim/–lim, dec/dodec, and acetic a./propionic a.; Group 4 +lim/–lim, dec/dodec, acetic a./propionic a., buta/penta, propyl/butyl, and +terp/–terp). All experiments took place in a clean cage similar to the home cage. Odorants were presented by placing 60  $\mu$ L of the diluted odorant (1 Pa) (see Supplementary Table 1) onto a filter paper (Whatman #1) that was put inside a tea ball hanging from the side of the cage. A behavioral session consisted of 1 presentation of mineral oil then 4 odorant presentations of the habituation odorant (Ohab1–4) followed by 1 presentation of the test odorant (Otest). Each odorant presentation lasted 50 s and was separated by 5 min. Each odorant of each pair was used alternatively as the habituation or test odorant. Investigation time (defined as the mouse being maximally 1 cm away from the tea ball and actively sniffing in its direction) of each odorant was manually recorded, and a difference between Ohab4 and Otest indicated discrimination.

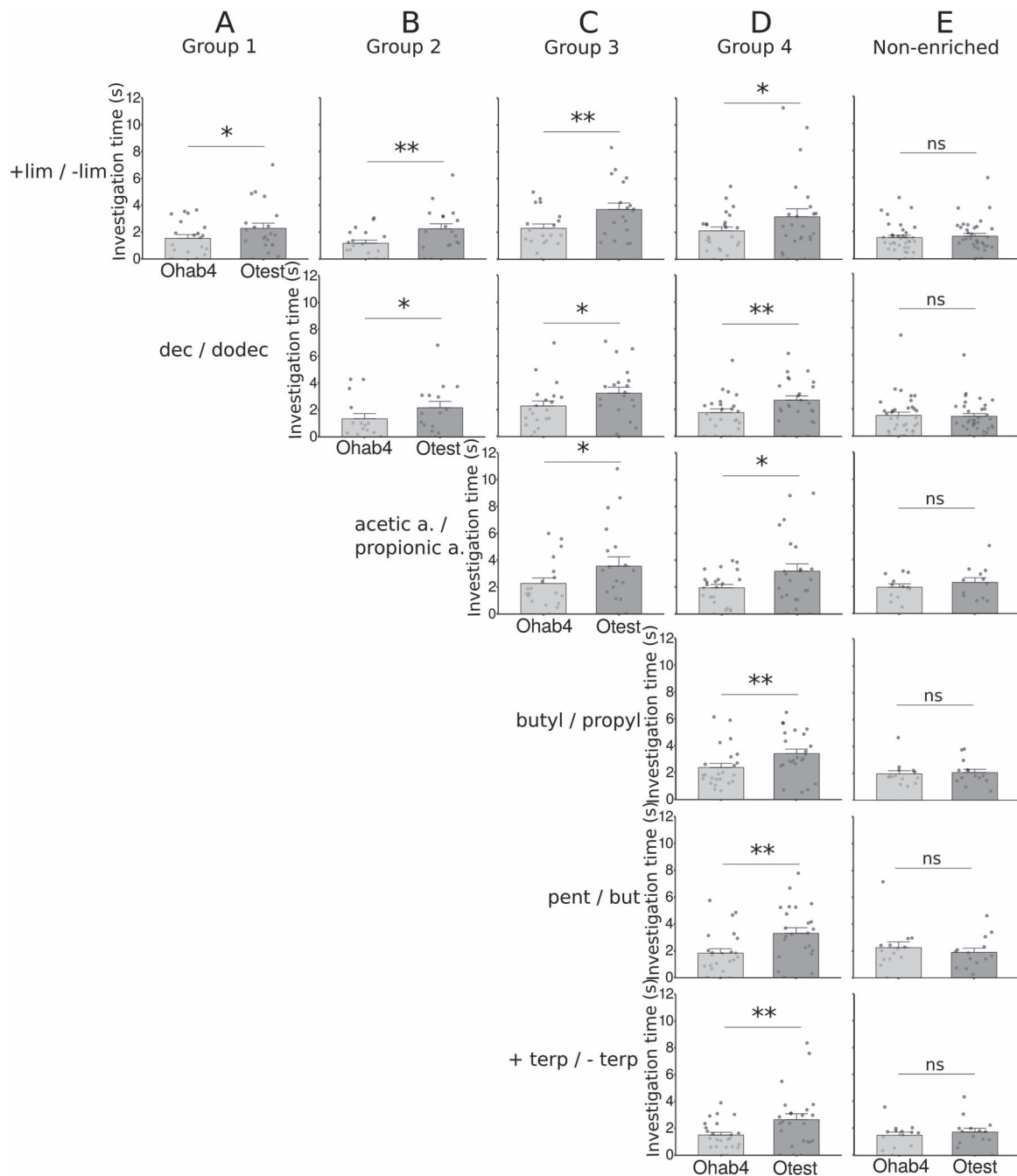
#### Adult-born Neuron Quantification in the OB

**BrdU injection.** Mice were injected with BrdU (Sigma) (50 mg/kg in saline, 3 $\times$  at 2-h intervals, i.p.) 9 days before the beginning of the enrichment period (27 days before sacrifice) in order to have a cohort of labeled adult-born cells in the OB at the beginning of

the enrichment period (Fig. 1) as previously described (Moreno et al. 2009).

**Sacrifice.** To investigate the expression of the immediate-early gene *Zif268* in response to the learned odorants, enriched mice were exposed to 100  $\mu$ L of pure learned odorants (odorants used for the enrichment presented simultaneously) for 1 h using tea balls. A control NE group (NE<sub>1</sub>–NE<sub>4</sub>) corresponding to each experimental group (group 1–4) was exposed to the same odorants on the day of sacrifice. These NE groups were composed of mice randomly selected from the NE group presented in Figure 1. Then, 1 h after the end of odorant stimulation, the mice were deeply anesthetized (pentobarbital, 0.25 mL/30 g) and killed by intracardiac perfusion of 40–50 mL of fixative (4% paraformaldehyde in phosphate buffer, pH 7.4). Their brains were removed, postfixed, cryoprotected in sucrose (20%), frozen rapidly, and then stored at –20 °C. Brains were sectioned with a cryostat (Reichert-Jung, NuBlock) at 14  $\mu$ m and 40  $\mu$ m (3 $\times$ 14  $\mu$ m followed by 2 $\times$ 40  $\mu$ m sectioning) for cell counts and morphological analysis, respectively.

**BrdU immunohistochemistry.** 14  $\mu$ m thick sections from mice randomly selected in each group were incubated in Target Retrieval Solution (Dako) for 20 min at 98 °C. After cooling, they were treated with pepsin (0.43 U/mL in 0.1 N HCl, Sigma) for 3 min. Sections were transferred to a blocking solution [5% normal horse serum (Sigma) with 5% Bovine Serum Albumine (BSA) and 0,125% Triton X-100] and were then incubated overnight at 4 °C in a mouse anti-BrdU antibody (1/100, Chemicon), followed by a biotylated anti-mouse secondary antibody (1/200, Vector Laboratories) for 2 h. The sections were then processed



**Figure 2.** Increasing the complexity of perceptual learning leads to the discrimination of more odorant pairs. Only the last habituation trial (Ohab4) and the test trial (Otest) are represented to illustrate discrimination. (A) After enrichment with +lim and -lim (Group 1), the investigation time during the test trial is superior to that of the Ohab4 trial, indicating discrimination between these 2 odorants (see [Supplementary Table 2](#) for detailed statistics). (B) A similar result is observed after enrichment with +lim/-lim and dec/dodec (Group 2) indicating discrimination between the 2 odorants of the 2 pairs. (C) Enrichment with +lim/-lim, dec/dodec, and acetic a./propionic a. (Group 3) allows discrimination between the 2 odorants of the 3 pairs. (D) Enrichment with 6 pairs of odorants (Group 4), that is +lim/-lim, dec/dodec, acetic a./propionic a., propyl/butyl, pent/but, and +terp/-terp, allows discrimination between the 2 odorants in all 6 pairs. (E) In NE control animals (NE Group), no difference is observed between Ohab4 and Otest, for any of the 6 odorant pairs (+lim/-lim, dec/dodec, acetic a./propionic a., propyl/butyl, pent/but, and +terp/-terp) indicating no discrimination. \* $P < 0.05$ . \*\* $P < 0.01$ . Data are expressed as mean  $\pm$  SEM, and all data points are represented. Each dot represents a trial.

through an avidin-biotin-peroxidase complex (ABC Elite Kit, Vector Laboratories). After dehydration in graded ethanols, the sections were defatted in xylene and cover-slipped in DEPEX (Sigma).

**Triple-labeling immunohistochemistry.** To determine the phenotype of BrdU-positive cells in the OB and their functional involvement, triple labeling was performed using rat anti-BrdU (1:100, Harlan Sera Laboratory), mouse anti-NeuN (1:500, Chemicon),

and rabbit anti-Zif268 (1:1000, Santa Cruz Biotechnology) antibodies. Appropriate secondary antibodies coupled with Alexa 546 for revelation of BrdU, Alexa 633 for NeuN, and Alexa 488 for Zif268 were used (Molecular Probes).

**Cell counting.** All cell counts were conducted blind with regard to the experimental mouse group. BrdU data were collected with the help of a mapping software (Mercator Pro, Explora Nova), coupled with a Zeiss microscope. The BrdU-positive cells were counted in the entire granule cell layer of the OB on about 9 sections (14  $\mu\text{m}$  thick, 216  $\mu\text{m}$  intervals) of 5–8 mice/group. The number of positive cells was divided by the surface of the granule cell layer to yield the total density of labeled cells (labeled profiles/ $\mu\text{m}^2$ ).

Triple-labeled sections were examined using pseudo-confocal scanning microscopy equipped with an Apotome (Zeiss). BrdU-positive cells were examined for co-labeling with NeuN and Zif268 (30 cells/animal,  $n = 3\text{--}9$  animals/group), yielding a percentage of activated neurons (Zif268<sup>+</sup>) over the whole adult-born neuron population (BrdU<sup>+</sup> and NeuN<sup>+</sup>).

#### Neuronal Morphology Analysis

**Preexisting neuron labeling.** At P0, mice were anesthetized on ice and injected in the lateral ventricle with 1  $\mu\text{L}$  of Lenti-PGK-DsRed lentivirus ( $6.11 \times 10^8$  UI/ml, Lenti-PGK-DsRed was generated by the Functional Neurogenetic and Optogenetic platform of the Lyon Neuroscience Research Center by excision of the GFP sequence from the Lenti-PGK-GFP and its replacement by a DsRed sequence). The injection was done using a glass micropipette linked to a Hamilton syringe connected to a programmable syringe controller (infusion rate: 0.2  $\mu\text{L}/\text{s}$ ).

**Adult-born neuron labeling.** Prior to surgery, the same mice previously injected at P0 were anesthetized at the age of 2 months 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). 100 nL of Lenti-PGK-GFP lentivirus was injected bilaterally ( $2 \times 10^9$  UI/ml, provided through Addgene #12252 by the Trono Lab) into the subventricular zone (with respect to the bregma: AP, +1 mm; ML,  $\pm 1$ ; DV,  $-2.3$  mm) at a rate of 150 nL/min. Mice received antalgic ketoprofen (2 mg/kg) at the end of surgery and once every day until they regained the lost weight. Mice were allowed 8 days to recover from the surgery before behavioral testing.

**Immunohistochemistry.** Preexisting (DsRed-positive) and adult-born (GFP-positive) neurons activated by the learned odorants were identified by Zif268 expression. Triple labeling was performed on 40  $\mu\text{m}$ -thick sections by using chicken anti-GFP (1:1000, Anaspec), mouse anti-DsRed (1:200, Santa Cruz Biotechnology), and rabbit anti-Zif268 antibodies (1:1000, Santa Cruz Biotechnology). Appropriate secondary antibodies coupled with Alexa 546 for DsRed revelation, Alexa 488 for GFP, and Alexa 633 or Alexa 546 for Zif268 were used.

**Morphological data analysis.** For each neuron, we first determined whether it expressed Zif268 using images taken at both 40 $\times$  and 63 $\times$  magnification (lateral and z-axis resolutions were, respectively, 160 nm and 280 nm at 40 $\times$  and 100 nm and 200 nm at 63 $\times$ ). Only Zif268-positive neurons were kept for further analysis. For the analysis of dendritic arborizations, images were taken with 20 $\times$  (lateral and z-axis resolutions were 320 nm and 400 nm) and 40 $\times$  objectives. The length of all the distal dendritic segments of the apical distal domain, of the proximal dendritic segment of the apical domain, or of all the dendritic segments of the basal domain was measured

based on previous reports (Kelsch et al. 2008). Then, spine densities on apical and basal dendrites were assessed on images taken with a 100 $\times$  objective (lateral and z-axis resolutions were 60 nm and 200 nm, respectively). To calculate spine densities, apical distal and basal dendritic segments were randomly chosen from the apical distal and the basal dendritic arbor, respectively. The average length of dendrite used for the calculation was for the apical dendrites,  $85.55 \pm 1.98$   $\mu\text{m}$  [mean  $\pm$  standard error of the mean (SEM)] per adult-born neuron,  $80.11 \pm 1.97$  (mean  $\pm$  SEM) per preexisting neuron and for the basal dendrites,  $70.14 \pm 2.62$   $\mu\text{m}$  (mean  $\pm$  SEM) per adult-born neuron and  $43.59 \pm 2.29$  (mean  $\pm$  SEM) per preexisting neuron. The spine density of the proximal apical dendrite was calculated within the 100  $\mu\text{m}$  from the soma. The average length of dendrite used for the calculation of the proximal spine density was  $64.69 \pm 0.93$   $\mu\text{m}$  (mean  $\pm$  SEM) in adult-born neurons and  $53.98 \pm 1.51$   $\mu\text{m}$  (mean  $\pm$  SEM) in preexisting neurons. All images were acquired using a Zeiss pseudo-confocal system (Mandairon et al. 2018).

Neuron stitching was performed using Vias (<http://research.mssm.edu/cnic/tools-vias.html>) and neuron reconstruction using NeuronStudio software (Wearne et al. 2005; Rodriguez et al. 2008) (<http://research.mssm.edu/cnic/tools-ns.html>). Neuronstudio allows 3D reconstructions of dendrites and spines from confocal z-series stacks on a spatial scale. Dendritic length was measured semi-automatically, and spines were manually identified with the help of the 3D reconstruction. All morphological analyses were conducted blind with regard to the mouse group. Number of animals, neurons per animals, total neurons analyzed, and number of dendrites per neurons for adult-born and preexisting neurons are reported in Supplementary Tables 3 and 4, respectively.

#### Optogenetics in Freely Behaving Mice

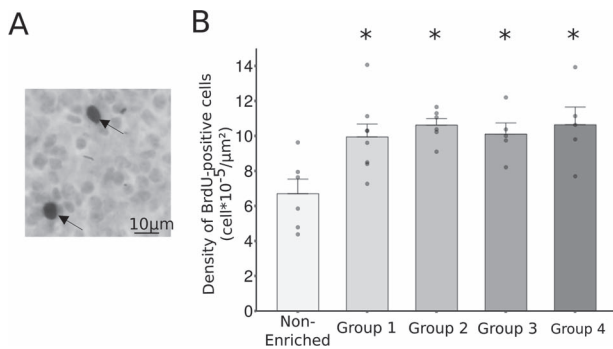
**Experimental design.** We used perceptual learning in its simplest (one pair of odorant) and most complex (6 pairs of odorants) configurations in order to unravel the specific contribution of preexisting versus adult-born neurons in the expression of the learned discrimination ( $n = 39$ ; Fig. 3). Of the 39 mice, 14 were removed from the analysis either from optical fiber technical problems ( $n = 3$ ), absence of halorhodopsin-expressing neurons in the OB ( $n = 7$ ), or inappropriate optical fiber implantation site ( $n = 4$ ).

**Surgery.** To be able to inhibit adult-born neurons or preexisting neurons in adult mice, we performed surgery either at P0 or at P60 in the same way as explained above for DsRed and GFP lentivirus injections except the injected virus was a Lenti-hSyn-eNpHR3.0-EYFP lentivirus ( $9.22 \times 10^6$  IU/ml, provided through Addgene #26775 by the Diesseroth Lab) (Kermen et al. 2016). Adult mice were then implanted with bilateral optical fibers (200 nm core diameter, 0.22 N.A., Doric Lenses) in the OB (with respect to the bregma: AP, +4.6 mm; ML,  $\pm 0.75$  mm; DV,  $-2$  mm).

**Behavior.** Mice were light stimulated (crystal laser, 561 nm, 10–15 mW, continuous stimulation) during the test trial (Otest) of the habituation/cross-habituation task. More specifically, light stimulation was automatically triggered when the mouse's nose approached within 2.5 cm of the tea ball (VideoTrack, Viewpoint) and stopped automatically when the nose exited the zone.

**Control of light-triggered inhibition.** For the optogenetic experiment, mice were sacrificed as previously described with the exception that just before sacrifice they were randomly affected





**Figure 3.** Perceptual learning increased adult-born granule cell density independently of the number of learned odorant pairs. (A) Example of BrdU-positive immunolabeled cells (arrows). Scale bar = 10  $\mu\text{m}$ . (B) Groups enriched with 1 odorant pair (Group 1,  $n = 8$ ), 2 odorant pairs (Group 2,  $n = 6$ ), 3 pairs (Group 3,  $n = 5$ ), or 6 pairs (Group 4,  $n = 5$ ) have a significantly higher BrdU-positive cell density compared with the NE group (NE,  $n = 6$ ). No difference in BrdU-positive cell density was observed between the 4 enriched groups. \* $P < 0.05$  in comparison with the NE group. Data are expressed as mean  $\pm$  SEM, and all data points are represented. Each dot represents an animal.

to one of the following conditions: light stimulation associated with odorant stimulation or odorant stimulation alone. For the light stimulation group, this followed a pattern mimicking the average light stimulation pattern during the Otest session (0.5 s light ON, 2 s light OFF, pattern repeated over 1 h). After brain sectioning (see above), EYFP and Zif268 double immunohistochemistry was performed as described previously (Kermen et al. 2016). EYFP-positive and Zif268-positive cells were counted in the OB sampled on 1 to 3 sections (40  $\mu\text{m}$  thick, interval 80  $\mu\text{m}$ ) located under the optic fiber implantation site to allow assessment of the inhibition efficiency.

In addition, we measured in the same area the percentage of transduced cells relative to the total granule cell layer population.

### Statistical Analyses

All data were analyzed using in-house made scripts written in R and/or Python. The level of significance was set to  $\alpha = 0.05$ .

**Behavioral data.** Preliminary tests including Shapiro–Wilk test for normality checking (performed for each group, for each odorant, on each ensemble of habituation or test trial) and Mauchly’s test for sphericity were performed on the data. This resulted in the use of nonparametric tests as deviation from normality, and/or deviation from sphericity was observed. For each group, mice investigation time during habituation (Ohab1–4) of each odorant pair was analyzed using Friedman tests. Investigation time between Ohab4 and Otest for each group and each pair was compared using unilateral Wilcoxon tests. Only mice that investigated the odorant for at least 1 s at Ohab1 were included in the analysis (9 trials were excluded among 388 total trials).

**Adult-born neurons quantification.** BrdU-positive cell densities were compared between all experimental groups using a one-way analysis of variance (ANOVA) (group as factor) followed by Holm-corrected post hoc t-tests (normality of residuals and homoscedasticity were validated using Shapiro–Wilk and Levene tests, respectively). Triple-labeled sections were analyzed by two-way ANOVA (group and odorant stimulation

before sacrifice as factors, normality, and homoscedasticity were checked as above) followed by regression analysis and post hoc t-tests to compare the enriched groups with their respective controls.

**Morphological data analysis.** Morphological data (spine density and dendritic length) were compared using one-way ANOVA (with groups as factor) followed by post hoc t-tests using Holm–Bonferroni correction (Aickin and Gensler 1996) and using contrasts comparing enriched groups with the control group.

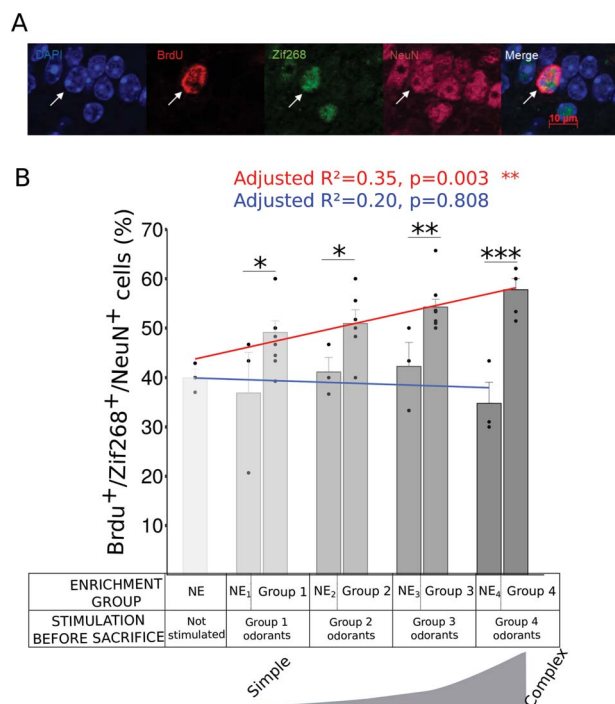
## Results

### Increasing the Complexity of Perceptual Learning Did Not Proportionally Increase Adult-born Cell Survival but Enhanced the Functional Recruitment of Adult-born Neurons

Groups of mice were submitted to different perceptual learning paradigms varying in the number of odorant pairs used during the olfactory enrichment period (from 1 to 6 pairs of odorants, Fig. 1). Enrichment consisted of successive presentations of odorant pairs during a day and the repetition of this process for over 10 days. After this enrichment period, we assessed whether mice were able to discriminate the odorants from each odorant pairs using a habituation/cross-habituation test. In this test, mice were submitted to 4 habituation trials (Ohab trials) with 1 odorant of the pair and a test trial (Otest) with the other odorant of the pair. Decreased investigation time of the odorant across Ohab trials reflected habituation to the odorant. Increased investigation time between Ohab4 and Otest reflected discrimination between the 2 odorants of the pair. A total of 5 experimental groups were constituted: control NE, enriched with 1 odorant pair (Group 1), enriched with 2 odorant pairs (Group 2), enriched with 3 odorant pairs (Group 3), and enriched with 6 odorant pairs (Group 4) (Fig. 1 and Supplementary Table 1).

Significant habituation was observed for all groups as evidenced by the reduction in investigation time across trials (Friedman tests, see Supplementary Table 2 and Supplementary Figure 1). Regarding discrimination, all enriched groups showed discrimination of the odorant pair(s) used for enrichment (see Supplementary Table 1 for abbreviations): +lim/–lim (Group 1) (Fig. 2A); +lim/–lim and dec/dodec (Group 2) (Fig. 2B). +lim/–lim, dec/dodec, and acetic a./propionic a. (Group 3) (Fig. 2C). It is worth noting that enrichment with +lim/–lim and dec/dodec did not improve discrimination of acetic a./propionic a. (see Supplementary Figure 2). Finally, the most complex enrichment (6 pairs: +lim/–lim, dec/dodec, acetic a./propionic a., propyl/butyl, penta/buta, and +terp/–terp) (Group 4) allowed mice to discriminate between the odorants of all 6 pairs (Fig. 2D). Importantly, the control NE group showed no improvement in discrimination abilities between any of the tested odorant pairs (Fig. 2E). These results showed that mice exposed to complex olfactory environment improved their performances of discrimination for at least 6 odorant pairs at a time.

We then asked whether increasing the number of odorant pairs mice passively learned to discriminate will increase the number of surviving adult-born neurons. Therefore, we labeled a cohort of adult-born cells integrating in the OB at the beginning of the enrichment, by injecting BrdU 9 days before the enrichment period. This delay allows neuroblasts to migrate from the subventricular zone to the OB. We assessed adult-born cells density in the granule cell layer since perceptual



**Figure 4.** Increasing the complexity of perceptual learning increased the functional recruitment of adult-born neurons. (A) Example of a BrdU/Zif268/NeuN-positive neuron (arrows). (B) Enrichment with 1 (Group 1,  $n=9$ ), 2 (Group 2,  $n=6$ ), 3 (Group 3,  $n=9$ ), or 6 (Group 4,  $n=5$ ) pairs of odorants increased the percentage of adult-born neurons (BrdU/NeuN), responding to the learned odorants (Zif268) compared with their respective NE group. NE groups were stimulated on the day of sacrifice with 1 (NE<sub>1</sub>), 2 (NE<sub>2</sub>), 3 (NE<sub>3</sub>), or 6 (NE<sub>4</sub>) odorant pairs;  $n=3$  for each NE group. Linear regression analysis indicated that the percentage of BrdU/NeuN/Zif268-positive cells is increasing with enrichment complexity (red line), while this is not the case for control groups (blue line). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . See Figure 1 for details on the odorants used. Data are expressed as mean  $\pm$  SEM, and all data points are represented. Each dot represents an animal.

learning had previously been shown to modulate the number of adult-born granule cells, whereas it was not the case for the number of adult-born glomerular interneurons (Moreno et al. 2009). We found a significant difference between experimental groups (one-way ANOVA,  $F_{(4,25)} = 4.77$ ;  $P = 0.005$ ; Fig. 3). More precisely, BrdU-positive cell density increased in all enriched groups compared with the control NE group (Holm-corrected post hoc t-tests—Group 1 vs. NE:  $P = 0.025$ ; Group 2 vs. NE:  $P = 0.011$ ; Group 3 vs. NE:  $P = 0.036$ ; Group 4 vs. NE:  $P = 0.014$ ). However, no significant difference between enriched groups was observed ( $P > 0.9$  for all comparisons), indicating that the density of surviving adult-born cells is not dependent on the number of learned odorant pairs.

Since we found no increase in the density of surviving adult-born cells even when the enrichment became more complex, we hypothesized that a higher proportion of the surviving adult-born neurons could be recruited to process the learned odorants as the number of pairs mice were exposed to increased. To test this hypothesis, we assessed the percentage of adult-born granule cells expressing Zif268 in response to the learned odorants as an index of cellular activation (Moreno et al. 2012). Before sacrificing the animals, we exposed them to their respective learned odorants +lim/–lim for Group 1; +lim/–lim and dec/dodec for Group 2; +lim/–lim, dec/dodec, and acetic a./propionic a.

for Group 3; +lim/–lim, dec/dodec, acetic a./propionic a., propyl/butyl, buta/penta, and +terp/–terp for Group 6. We also tested an NE control for each experimental group that was stimulated with the same odorants on the day of sacrifice but that had not been previously enriched with the odorants (NE<sub>1</sub>–NE<sub>4</sub>).

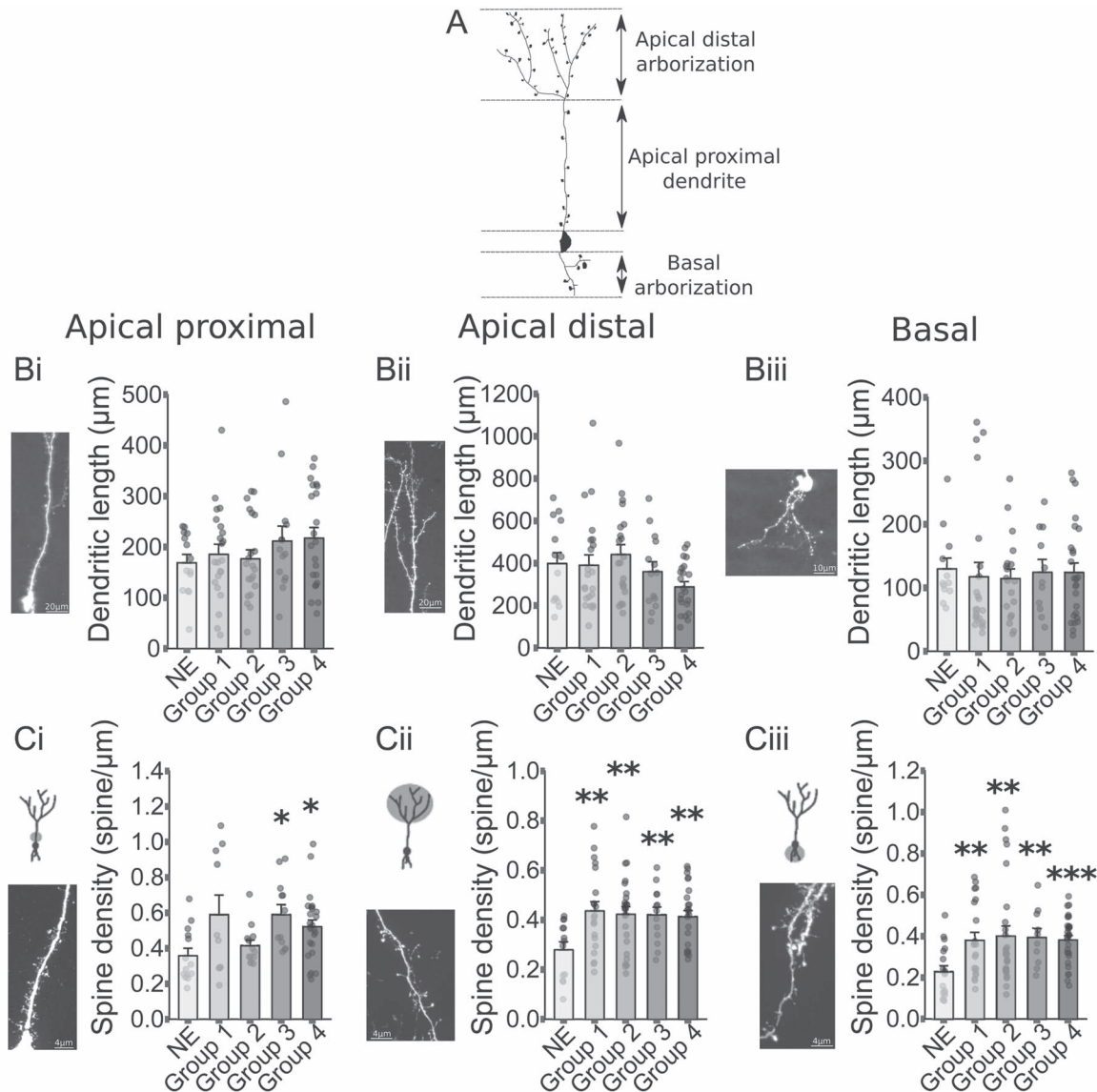
First, we observed the effect of enrichment on the responsiveness of adult-born neurons to olfactory stimulation measured as the percentage of BrdU-positive cells co-expressing Zif268 and NeuN (two-way ANOVA, enrichment effect  $F_{(4,35)} = 10.70$ ,  $P = 0.00009$ ) (Fig. 4). Each group was different from its respective control (unilateral t-tests; Group 1 vs. its control:  $t = -2.05$ ,  $P = 0.034$ ; Group 2 vs. its control:  $t = -2.18$ ,  $P = 0.033$ ; Group 3 vs. its control:  $t = -3.20$ ,  $P = 0.005$ ; Group 4 vs. its control:  $t = -5.30$ ,  $P = 0.0009$ ). In addition, the more complex the enrichment, the more BrdU/Zif268/NeuN-positive cells were retrieved (linear regression: adjusted- $R^2 = 0.35$ ,  $P = 0.003$ ). This was not seen in the control NE groups (linear regression: adjusted- $R^2 = -0.20$ ,  $P = 0.81$ ). Thus, the correlation suggests that enrichment complexity and adult-born neuron responsiveness are linked, even though other factors may interact as well.

We further tested whether this result could be due to the different durations of odor enrichment between groups (1 h per day for Group 1 vs. 6 h per day for Group 4). To test this, we enriched a new group of mice with +lim/–lim 6 h per day for 10 days (similar duration of enrichment compared with Group 4). The percentage of BrdU/Zif268/NeuN-positive cells did not differ from that in Group 1 (see Supplementary Figure 3; unilateral t-test:  $t = -1.00$ ,  $P = 0.82$ ), suggesting that it was the diversity of the odorants presented during the enrichment and not the duration of the exposure that affected adult-born neuron recruitment.

Finally, to ensure that the increased BrdU/Zif268/NeuN co-labeling resulted from increased odor responsiveness and not from a change in the basal level of BrdU/Zif268/NeuN co-expression in the enriched groups, we assessed BrdU/Zif268/NeuN co-labeling in the basal condition (without odor stimulation) in animals enriched with 6 odorant pairs and compared it with that in NE animals. We found no difference between these 2 experimental groups (unilateral t-test:  $t = 0.32$ ,  $P = 0.38$ ; see Supplementary Figure 4), suggesting that the observed change in BrdU/Zif268/NeuN-positive levels was not due to an overall increase in adult-born granule cell activity in the enriched OB.

We observed no effect of enrichment or olfactory stimulation on the day of sacrifice on the rate of neuronal differentiation of adult-born cells (two-way ANOVA: enrichment effect  $F_{(4,35)} = 1.32$ ;  $P = 0.48$ ; stimulation effect:  $F_{(4,35)} = 0.66$ ,  $P = 0.62$ ; see Supplementary Figure 5A). Quantification of Zif268-positive cell density showed an effect of enrichment but no effect of odor stimulation before sacrifice (see Supplementary Figure 5B) (two-way ANOVA, enrichment effect:  $F_{(4,35)} = 6.83$ ,  $P = 0.00036$ ; stimulation effect:  $F_{(4,35)} = 1.27$ ,  $P = 0.3$ ; followed by unilateral t-tests: Group 1 vs. its control:  $t = -2.46$ ,  $P = 0.016$ ; Group 2 vs. its control:  $t = -2.02$ ,  $P = 0.041$ ; Group 3 vs. its control:  $t = -1.90$ ,  $P = 0.043$ ; Group 4 vs. its control:  $t = -2.27$ ,  $P = 0.032$ ), thus showing that enrichment increased overall granule cell activity independently of the number of learned odorants.

Together, these results indicate that even if the number of adult-born granule cells saved by learning reached a plateau, the pool of surviving granule cells are increasingly called upon to process the learned odorants as perceptual learning complexity increased in young adult mice.



**Figure 5.** Increasing the complexity of perceptual learning increased structural plasticity of adult-born neurons. (A) Schematic representation of a granule cell and its dendritic domains. (B) No group differences for the length of apical proximal (Bi) ( $n_{\text{neuron}}$  from NE to Group 4 = 14, 24, 21, 14, 22), apical distal (Bii) ( $n_{\text{neuron}}$  from NE to Group 4 = 14, 23, 21, 14, 22), and basal dendritic arborization (Biii) ( $n_{\text{neuron}}$  from NE to Group 4 = 12, 23, 19, 11, 26). (C) Spine densities were increased after perceptual learning at the apical proximal (Ci) ( $n_{\text{neuron}}$  from NE to Group 4 = 14, 10, 13, 12, 25), apical distal (Cii) ( $n_{\text{neuron}}$  from NE to Group 4 = 13, 20, 25, 13, 24), and basal domains (Ciii) ( $n_{\text{neuron}}$  from NE to Group 4 = 18, 22, 27, 10, 29). Regarding the apical proximal domain, increased spine density was observed only in Groups 3 and 4, meaning after complex perceptual learning. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  for comparison with the NE group, Holm-corrected t-tests. Data are expressed as mean  $\pm$  SEM, and all data points are represented. Each dot represents a neuron.

### Increasing the Complexity of Perceptual Learning Increased Structural Plasticity of Adult-born Neurons and Induces Limited Morphological Changes in Preexisting Neurons

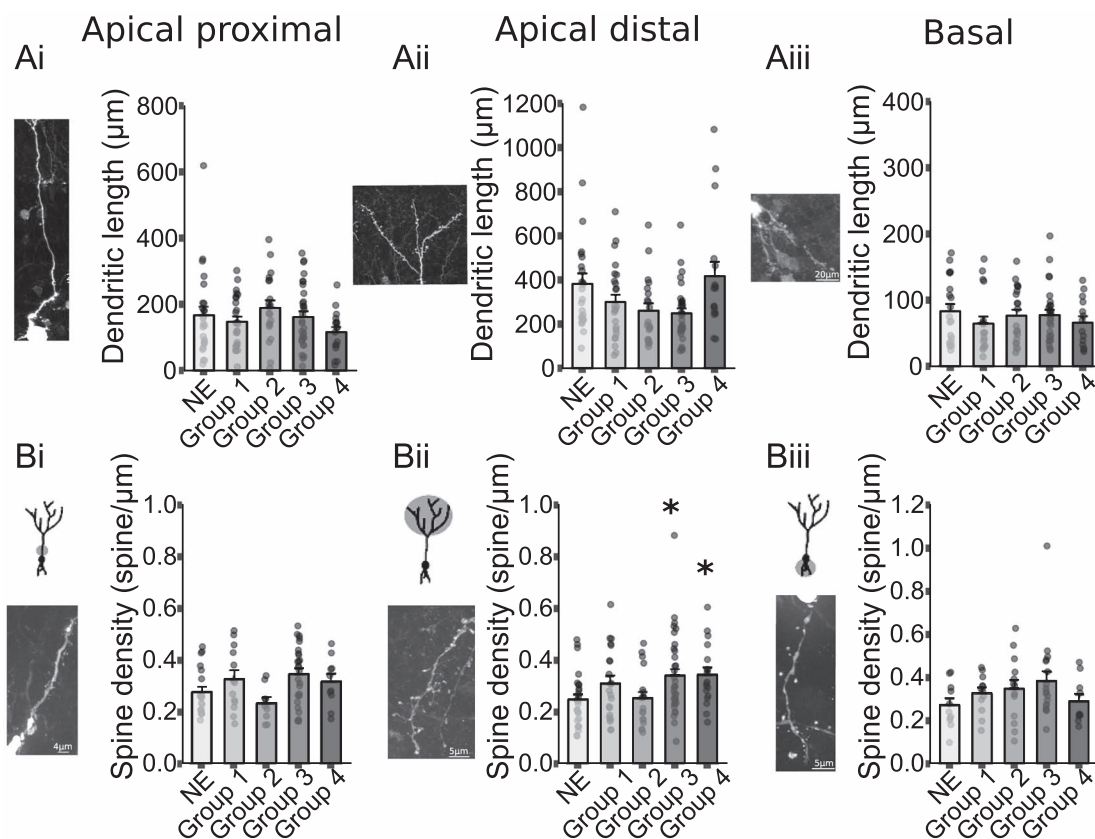
To better understand the increased functional recruitment of adult-born neurons with perceptual learning complexity, we looked for associated structural modifications in these neurons. To determine whether perceptual learning had an effect on the morphology of odor-responding adult-born neurons (Zif268-positive), we injected a lentivirus expressing GFP at postnatal day 60 (P60) in the subventricular zone 8 days before learning (Fig. 1). Animals were sacrificed 25 days post-injection,

and we analyzed dendritic arborization and spine density of the apical distal domain [the site of interactions with mitral/tufted (M/T) cells] as well as the apical proximal and basal domains (sites of centrifugal inputs) (Fig. 5A) (Mandairon et al. 2018) of Zif268-positive neurons.

Enrichment did not affect dendritic length of any domain (one-way ANOVAs, apical proximal,  $F_{(4,89)} = 0.95$ ,  $P = 0.44$ ; Fig. 5Bi; apical distal,  $F_{(4,88)} = 1.92$ ,  $P = 0.11$ ; Fig. 5Bii; basal,  $F_{(4,86)} = 0.09$ ,  $P = 0.98$ ; Fig. 5Biii).

However, we found that perceptual learning increased the spine density of all dendritic domains (one-way ANOVAs, apical proximal,  $F_{(4,69)} = 3.48$ ,  $P = 0.01$ ; Fig. 5Ci; apical distal,  $F_{(4,90)} = 3.03$ ,  $P = 0.022$ ; Fig. 5Cii; apical proximal basal,  $F_{(4,101)} = 3.24$ ,  $P = 0.015$ ;





**Figure 6.** Perceptual learning induced limited morphological changes in preexisting neurons. (A) No effect of perceptual learning was observed on the length of the apical proximal dendrites (Ai) ( $n_{\text{neuron}}$  from NE to Group 4 = 25, 25, 20, 31, 17), the apical distal dendritic arborization (Aii) ( $n_{\text{neuron}}$  from NE to Group 4 = 25, 25, 20, 31, 17), or the basal dendritic arborization (Aiii) ( $n_{\text{neuron}}$  from NE to Group 4 = 20, 18, 21, 27, 14). (B) Spine densities after perceptual learning at the apical proximal (Bi) ( $n_{\text{neuron}}$  from NE to Group 4 = 21, 13, 9, 26, 10), apical distal (Bii) ( $n_{\text{neuron}}$  from NE to Group 4 = 27, 21, 18, 34, 18), and basal domains (Biii) ( $n_{\text{neuron}}$  from NE to Group 4 = 11, 12, 14, 18, 10). Increased spine density in the apical distal domain was seen after complex perceptual learning only (Groups 3 and 4). Regarding the apical proximal domain and basal domains, no increased spine densities were observed after learning. \* $P < 0.05$  for comparison with the NE group. Data are expressed as mean  $\pm$  SEM, and all data points are represented. Each dot represents a neuron.

Fig. 5Ciii). A detailed analysis of the data revealed that, at the apical proximal domain, we observed an increased spine density on adult-born Zif268-positive neurons only in Groups 3 and 4 compared with NE (Holm-corrected post hoc t-tests; Group 3 vs. NE  $P = 0.01$ , Group 4 vs. NE  $P = 0.02$ ; Fig. 5Ci). In the apical distal domain, the increased spine density is observed in all groups (Holm-corrected post hoc t-tests; Group 1 vs. NE  $P = 0.011$ , Group 2 vs. NE  $P = 0.011$ , Group 3 vs. NE  $P = 0.011$ , Group 4 vs. NE  $P = 0.011$ ; Fig. 6Cii). The same global increase is observed for the basal domain (Holm-corrected post hoc t-tests; Group 1 vs. NE  $P = 0.008$ , Group 2 vs. NE  $P = 0.008$ , Group 3 vs. NE  $P = 0.008$ , Group 4 vs. NE  $P = 0.0005$ ; Fig. 5Ciii).

These results suggested that when the environmental complexity was low (enrichment with 1 and 2 odorant pairs), only apical distal and basal domains showed morphological modifications (i.e. increased spine density), whereas when the environmental complexity increased (enrichment with 3 and 6 odorant pairs), morphological changes affected the apical proximal domain in addition to apical distal and basal domains. Thus as the environmental complexity increased, morphology changed in a non-linear manner and not uniformly across the different dendritic domains.

These same mice were beforehand injected with a lentivirus expressing DsRed at postnatal day 0 (P0) (Fig. 1). Because of that,

it was possible to investigate the structural plasticity induced in odor-responding preexisting neurons of the OB and evaluate the specificity of learning-induced morphological changes in adult-born neurons. We found no morphological difference between experimental groups (NE and Group 1–4) regarding the length of the apical proximal and basal dendritic domains (one-way ANOVAs;  $F_{(4,113)} = 1.46$ ,  $P = 0.22$ ; Fig. 6Ai;  $F_{(4,95)} = 0.6$ ,  $P = 0.66$ ; Fig. 6Aiii). Regarding the apical distal domain, the analysis revealed a group effect (one-way ANOVA,  $F_{(4,113)} = 3.39$ ,  $P = 0.012$ ), but there was no significant difference for comparisons between NE and enriched groups (Holm-corrected post hoc t-tests,  $P > 0.06$ ; Fig. 6Aii).

An increase of spine density in the apical distal domain was observed in Groups 3 and 4 compared with the NE group (one-way ANOVA;  $F_{(4,113)} = 3.12$ ,  $P = 0.02$ ; Holm-corrected post hoc t-tests: Group 3 vs. NE  $P = 0.027$ , Group 4 vs. NE  $P = 0.027$ ; Fig. 6Bii). For the apical proximal spine density, a group effect was found (one-way ANOVA,  $F_{(4,74)} = 2.77$ ,  $P = 0.03$ ), but there was no significant difference for comparisons between NE and enriched groups (Holm-corrected post hoc t-tests,  $P > 0.08$ ; Fig. 6Bi). Spine density in the basal domain was not affected by enrichment (one-way ANOVA,  $F_{(4,60)} = 1.39$ ,  $P = 0.25$ ; Fig. 6Biii).

Taken together, these results suggest that the morphology of adult-born neurons is modified as soon as they are engaged in a

simple (single odorant pair) perceptual learning task, while the morphology of preexisting neurons is modified only when the perceptual learning becomes complex (3 or 6 odorant pairs).

### Optogenetically Inhibiting Preexisting or Adult-born Neurons in Freely Moving Mice Reveals Their Functionally Distinct Involvement in Simple and Complex Perceptual Learning

Based on the previous morphological results, we hypothesized that inhibiting adult-born neurons after simple and complex perceptual learning would alter learned discrimination, while inhibiting preexisting neurons would only affect the expression of learned discrimination following the complex learning. To test this hypothesis, we used 4 new groups of mice (Fig. 7). The first group was infused with a halorhodopsin- and EYFP-expressing lentivirus in the subventricular zone at P60 to transduce and, later on, silence adult-born neurons. Half of the animals of this group were submitted to simple environmental enrichment (1 odorant pair; similar to Group 1) and the other half to complex environmental enrichment (6 odorant pairs; similar to Group 4). A second group of mice was infused with the same lentivirus in the lateral ventricle at P0 to transduce preexisting neurons and silence them later on in adulthood. This group was also divided in 2: 1 enriched with 1 odorant pair and 1 enriched with 6 odorant pairs (Fig. 7). As previously, mice discrimination was tested after enrichment, using habituation/cross-habituation tests assessing perceptual learning. Optogenetic inactivation of transduced neurons was achieved in freely behaving mice only during the test trial of the habituation/cross-habituation paradigm by automatically triggering the light stimulation (see methods) each time the animal approached the odor source. All mice were also tested in a light OFF condition.

As expected, since light was never on during this phase of the test, habituation proceeded normally (see Supplementary Figure 6 and Supplementary Table 5). Regarding discrimination, we found that inhibiting adult-born neurons during the test trials abolished the improvement of discrimination induced by simple and complex enrichment (Fig. 8; Supplementary Table 5). More specifically, animals enriched with +lim/–lim were able to discriminate these 2 odorants in the light OFF condition but not under light triggered inactivation of adult-born neurons (Fig. 8A and C; Supplementary Table 5). The same phenomenon was observed for complex enrichment; animals were able to discriminate the 6 learned odorant pairs in the light OFF condition, but this was no longer observed in the light ON condition (Fig. 8B and D; Supplementary Table 5).

We assessed the level of viral transduction by first analyzing the density of EYFP-positive cells in the OB under the optical fibers and found the same density of transduced neurons in the groups submitted to simple and complex enrichments (bilateral t-test:  $P = 0.098$ ; see Supplementary Figure 7Ai). We also measured the proportion of transduced neurons (EYFP positive) relative to the whole cell population (DAPI positive). Groups injected at P0 showed higher percentages (simple  $3.8\% \pm 0.21$ , complex  $2.8\% \pm 0.29$ ) than groups injected at P60 (simple  $2.14\% \pm 0.23$ , complex  $1.59\% \pm 0.23$ ). Statistical analysis indicated that percentage of transduced neurons was superior in groups injected at P0 compared with groups injected at P60 (ANOVA,  $F_{(1,21)} = 30.35$ ,  $P < 0.0001$ ). Within the group injected at P0, the group tested in the simple paradigm exhibited a percentage of transduced cells higher than that of the complex group ( $P = 0.029$ , Holm-corrected t-test). It is worth noting that the group showing

the highest density of transduced cells is the group in which light has no behavioral effect. Thus, these differences can probably not account for the reported effects of light. Second, the effectiveness of light-mediated adult-born granule cell inhibition was assessed by counting Zif268-positive cells among EYFP-positive cells in the same region. A decrease in the percentage of EYFP/Zif268-positive cells was observed in the light ON condition compared with the light OFF confirming that light actually inhibited transduced adult-born neurons (unilateral t-test: simple learning,  $P = 0.028$ , complex learning,  $P = 0.043$ ; see Supplementary Figure 7Aii).

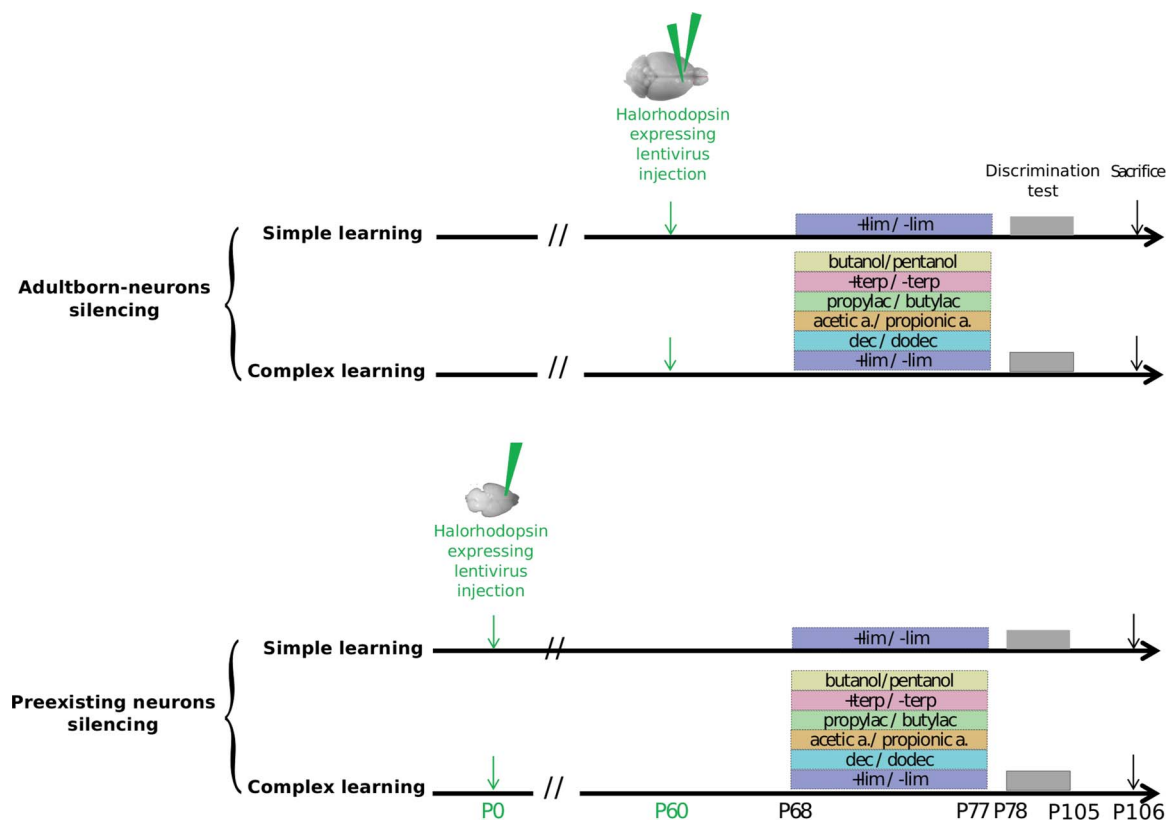
In contrast to adult-born neurons, light-triggered inactivation of preexisting neurons did not alter discrimination of the learned odorants after simple enrichment (Fig. 9A and C, Supplementary Table 5). However, inhibiting preexisting neurons after complex enrichment impaired discrimination of most odorant pairs (Fig. 9B and D, Supplementary Table 5). The level of viral transduction was also analyzed in preexisting neurons. The density of EYFP-positive cells in the OB was similar between simple and complex learning groups (bilateral t-test,  $P = 0.13$ ; see Supplementary Figure 7Bi), and a decrease in the percentage of EYFP/Zif268-positive cells was observed in the light ON compared with the light OFF condition (unilateral t-tests: simple learning,  $P = 0.048$ ; complex learning,  $P = 0.015$ ; see Supplementary Figure 7Bii).

As an additional control, the discrimination of dissimilar odorants was not altered by the inhibition of either adult-born or preexisting neurons in either the simple or complex enriched groups (see Supplementary Figure 8 and Supplementary Table 6).

These results reveal that adult-born and preexisting neurons can both undergo structural plasticity upon perceptual learning, but their involvement in the task is governed by distinct conditions. In this context, adult-born neurons are necessary for both simple and complex learned discrimination, while preexisting neurons are required only for the expression of complex learned discrimination.

## Discussion

Perceptual learning contributes to the representation of the sensory environment that guides animal's behavior. An essential component of the plasticity of this representation in the olfactory system is adult neurogenesis, that is renewal of interneurons within a preexisting neuronal network. Perceptual learning requires the presence of OB adult-born neurons and increases their survival (Moreno et al. 2009). In this study, we did not find a higher level of surviving adult-born cells with increasing environmental complexity, suggesting that a plateau of adult-born cell survival is reached after simple perceptual learning. Furthermore, among the adult-born neurons surviving after perceptual learning, only some of them respond to the learned odorants (Moreno et al. 2009; Mandairon et al. 2011; Sultan et al. 2011). Our findings show a positive linear relationship between the proportion of adult-born neurons expressing Zif268 in response to olfactory stimulation and the number of discriminated pairs of odorants. Interestingly, the learning-dependent survival increase reported here is close to the rate of adult-born neurons saved by different olfactory learning paradigms [associative and perceptual learning tasks (Alonso et al. 2006; Mouret et al. 2008; Moreno et al. 2009; Kermen et al. 2010; Sultan et al. 2010; Mandairon et al. 2011)], suggesting that this survival increase might not be specific to a task but simply a correlate of environmental modifications. Thus learning type or



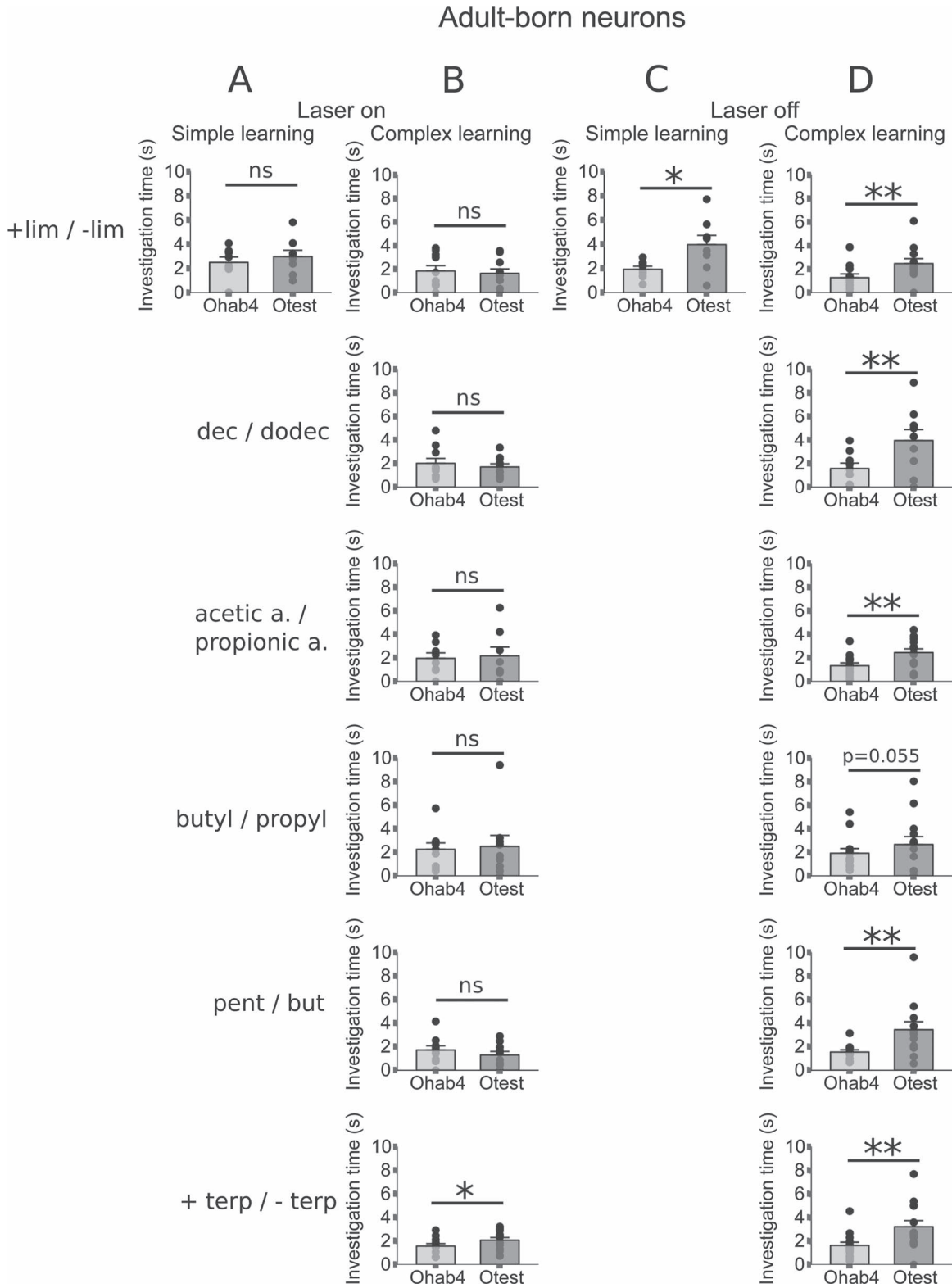
**Figure 7.** Design of the optogenetic experiment. Halorhodopsin-expressing lentivirus was injected in P0 mice to infect preexisting neurons or at P60 to infect adult-born neurons. 2 groups of mice were trained in a simple perceptual learning [1 injected at P0 ( $n=6$ ) and 1 at P60 ( $n=4$ )], and 2 groups of mice were trained in a complex perceptual learning [1 injected at P0 ( $n=8$ ) and 1 at P60 ( $n=7$ )]. After enrichment, mice were tested on their ability to discriminate the learned odorant pairs using an olfactory habituation/cross-habituation test. Light-stimulation was triggered in freely moving mice during the test trial of the habituation/cross-habituation task, when mice approached the odor source. The same groups of mice were also tested in a light OFF condition.

environmental change complexity is not coded by the survival rate of adult-born neurons. However, our results strongly suggest that the information about environmental complexity could be carried by functionally recruiting more adult-born neurons from the pool of available ones.

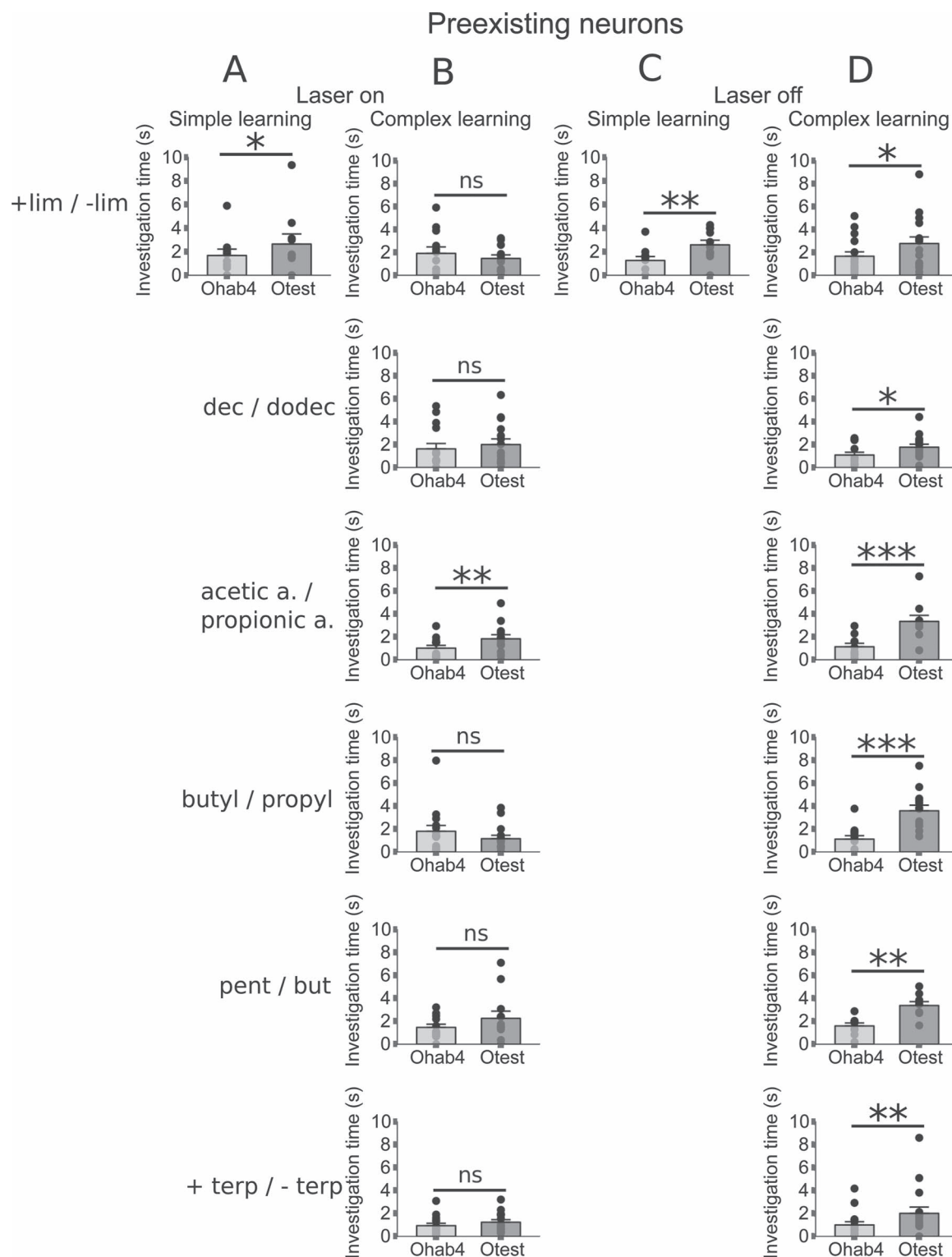
One limitation of our study comes from the fact that not all mice were tested on their ability to discriminate odorants of all 6 pairs. Previous studies showed that enrichment with +lim/-lim did not induce discrimination of dec/dodec, indicating that perceptual learning is relatively specific of the odorants used during exposure (Mandaïron et al. 2006c; Moreno et al. 2009), in line with the specific activation patterns they evoke in the OB (Linster et al. 2001). Furthermore, we showed that enrichment with +lim/-lim and dec/dodec does not induce discrimination of acetic a./propionic a., strongly supporting the hypothesis that discrimination of more odorant pairs is actually allowed by a more complex environment. However, from 3 pairs of odorants onwards, the OB stimulation is broad and activates overlapping regions of the OB leading to a less specific improvement in discrimination. This is similar to what was observed following global odor enrichment (Rey et al. 2012) or pharmacological activation of the OB (Mandaïron et al. 2006c).

Beyond the number of surviving adult-born cells or the proportion of them that is activated, another parameter of plasticity shown to be involved in improved discrimination is the structural plasticity of adult-born neurons (Daroles et al.

2015; Mandaïron et al. 2018). Cell activity is a major regulator of neuronal morphology (Lu 2003; Kelsch et al. 2010). In line with this, we first found that simple and complex perceptual learning increased the density of dendritic spines at the apical distal, apical proximal, and basal domains of adult-born granule cells. Optogenetically inhibiting these neurons prevented discrimination after simple perceptual learning (in accordance with Moreno et al. 2009) and also after complex perceptual learning. We also report that simple olfactory perceptual learning does not modify the morphology of preexisting cells although an increase of spine density can be observed at the apical domain after complex perceptual olfactory learning. Importantly, these modifications seem to correlate with our observation that inhibition of preexisting neurons prevented discrimination of the learned odorants only after complex but not simple perceptual learning. Based on these observations, we can hypothesize that morphological modifications are the cause of the discrimination changes observed at the behavioral level. There are several ways they could underlie perceptual learning. First, apical dendrites are the site of reciprocal dendrodendritic synapses between granule and mitral cells (Price and Powell 1970; Shepherd 1972), thus a higher number of spines could elevate the previously reported inhibitory drive on mitral cells (Moreno et al. 2009; Mandaïron et al. 2018) and explain why the absence or inactivity of these same cells prevents discrimination (Moreno et al. 2009). Second, the apical proximal and basal



**Figure 8.** Optogenetically inhibiting adult-born neurons prevented discrimination after both simple and complex perceptual learning. In light On condition, mice were not able to discriminate learned odorant pairs during the discrimination test after both simple (A) and complex (B) learning. In light OFF conditions, the same mice were able to discriminate learned odorant pairs after both (C) simple and (D) complex learning. Data are expressed as mean  $\pm$  SEM, and all data points are represented. \* $P < 0.05$ , \*\* $P < 0.01$ . Each dot represents a trial.



**Figure 9.** Optogenetically inhibiting preexisting neurons did not prevent discrimination after simple learning but did alter discrimination after complex learning. After simple perceptual learning (A), inhibiting preexisting neurons did not prevent discrimination, whereas (B) inhibiting them after complex perceptual learning altered the discrimination of most odorant pairs. In light OFF condition, (C) and (D), odorant pairs were discriminated after both types of learning. Data are expressed as mean  $\pm$  SEM, and all data points are represented. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Each dot represents a trial.



domains both receive cortico-bulbar projections from olfactory cortices (Nissant et al. 2009; Lepousez et al. 2014) as well as neuromodulatory inputs such as noradrenergic, cholinergic, and serotonergic (Fletcher and Chen 2010). Because the noradrenergic system acts on adult-born cells to allow olfactory perceptual learning (Moreno et al. 2012) and gates dendrodendritic inhibition onto mitral cells (Balu et al. 2007) while not being exclusive of other influences such as cholinergic (Ravel et al. 1994; Linster and Cleland 2002, 2016; Mandairon et al. 2006a; de Almeida et al. 2013), this increase in proximal and basal inputs could enhance noradrenergic influences on granule cell activity. Hence, as environmental complexity increases, spine density modifications (both apical, proximal, and basal) probably lead to a remapping of synaptic weights, thereby synergistically producing the linearly increased functional involvement of adult-born neurons as the environment gets more and more complex. Increased activation of adult-born interneurons together with increased inhibitory connectivity with mitral cells could serve as a means of decorrelation and mediate a refinement of the representations of each odorant in a pair.

The modifications of both basal and apical proximal spine densities observed here are similar to a previous study showing that associative olfactory learning can increase the excitatory drive from the olfactory cortex onto adult-born granule cells (Lepousez et al. 2014). However, we also observed changes in spine density at the apical distal domain. One possible explanation for this difference could simply be the nature of the task (reinforced vs. non-reinforced discrimination learning) as this has recently been shown to induce opposite changes in the apical connectivity of adult born cells (Mandairon et al. 2018).

It has been proposed that adult-born neurons confer two distinct but complementary forms of circuit plasticity to the OB network: an early form based on individual neuron survival and a later form based on the fine tuning of synaptic strength (Livneh et al. 2014). Our experimental data support this proposition by showing the absence of an increase in adult-born survival, regardless of task complexity, accompanied by an increase in the functional recruitment and structural plasticity of adult-born neurons. The present experiments further show that a third level of network plasticity is supported by preexisting neurons more particularly in complex learning when adjustments of the network supported by adult-born neurons may not be sufficient to adjust to the environmental context.

All the above as well as previous studies (see for review Hardy and Saghatelian 2017) indicate the existence of fundamental differences between adult-born and preexisting neurons. Preexisting neurons and their associated synapses seem more likely to be stable over time, whereas adult-born neurons constitute a more plastic and changing neuronal population that would first and foremost be the substrate of adaptive learning.

While the occurrence of adult neurogenesis in adult Human OB was subject to debate regarding its presence (Curtis et al. 2007), current consensus is that neurogenesis is absent beyond the age of 2 in the human OB (Sanai et al. 2007, 2011; Bergmann et al. 2012). This raises the interesting issue of how the human bulbar network adjusts to allow the plasticity of olfactory perception. Indeed humans improve their discrimination abilities with experience (Jehl et al. 1995). Our data indicating that neurons born during ontogenesis can also support structural plasticity and improved discrimination suggest that such plasticity could be at play in humans.

In conclusion, in this mice study, we have demonstrated both the necessity and sufficiency of adult-born neurons to simple perceptual learning as well as their necessity for complex perceptual learning. This reinforces their essential role in behavioral olfactory adaptation. For the first time, we have also shown that preexisting neurons do not participate in OB modifications associated with simple perceptual learning but can still be recruited both functionally and structurally to contribute to learning in cases of environmental complexity.

## Funding

Centre National de la Recherche Scientifique (CNRS); Institut National de la Santé et de la Recherche Médicale (Inserm); Lyon 1 University; French Ministry for Research (to J.F.).

## Notes

We would like to thank G. Froment, D. Nègre, and C. Costa from the lentivector production facility SFR BioSciences de Lyon (UMS3444/US8) and Région Auvergne-Rhône Alpes (to CT). The authors declare no conflict of interest. J.F., M.R., A.D., N.M. designed the experiments. J.F., L.C., M.M., C.T., I.C., J.S., K.M., N.M. performed the experiments. C.B., M.R. provided the viruses used. J.F., A.D., N.M. wrote the manuscript.

## References

- Aickin M, Gensler H. 1996. Adjusting for multiple testing when reporting research results: the Bonferroni vs Holm methods. *Am J Public Health*. 86:726–728.
- Alonso M, Viollet C, Gabellec M-M, Meas-Yedid V, Olivio-Marin J-C, Lledo P-M. 2006. Olfactory discrimination learning increases the survival of adult-born neurons in the olfactory bulb. *J Neurosci*. 26:10508–10513.
- Altman J. 1969. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J Comp Neurol*. 137: 433–457.
- Balu R, Pressler RT, Strowbridge BW. 2007. Multiple modes of synaptic excitation of olfactory bulb granule cells. *J Neurosci*. 27:5621–5632.
- Bayer SA. 1983. 3H-thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. *Exp Brain Res*. 50:329–340.
- Bergmann O, Liebl J, Bernard S, Alkass K, Yeung MSY, Steier P, Kutschera W, Johnson L, Landén M, Druid H et al. 2012. The age of olfactory bulb neurons in humans. *Neuron*. 74: 634–639.
- Breton-Provencher V, Bakhshetyan K, Hardy D, Bammann RR, Cavarretta F, Snapyan M, Côté D, Migliore M, Saghatelian A. 2016. Principal cell activity induces spine relocation of adult-born interneurons in the olfactory bulb. *Nat Commun*. 7:12659.
- Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo P-M. 2003. Becoming a new neuron in the adult olfactory bulb. *Nat Neurosci*. 6:507–518.
- Curtis MA, Kam M, Nannmark U, Anderson MF, Axell MZ, Wikkelso C, Holtås S, van R-MWMC, Björk-Eriksson T, Nordborg C et al. 2007. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science*. 315(5816):1243–1249

- Daroles L, Gribaudo S, Doulazmi M, Scotto-Lomassese S, Dubacq C, Madaïron N, Greer CA, Didier A, Trembleau A, Caillé I. 2015. Fragile X mental retardation protein and dendritic local translation of the alpha subunit of the calcium/calmodulin-dependent kinase II messenger RNA are required for the structural plasticity underlying olfactory learning. *Biol Psychiatry*. 80:149–159
- de Almeida L, Idiart M, Linster C. 2013. A model of cholinergic modulation in olfactory bulb and piriform cortex. *J Neurophysiol*. 109:1360–1377.
- Fletcher ML, Chen WR. 2010. Neural correlates of olfactory learning: critical role of centrifugal neuromodulation. *Learn Mem*. 17:561–570.
- Gilbert CD, Sigman M, Crist RE. 2001. The neural basis of perceptual learning. *Neuron*. 31:681–697.
- Grelat A, Benoit L, Wagner S, Moigneu C, Lledo P-M, Alonso M. 2018. Adult-born neurons boost odor–reward association. *Proc Natl Acad Sci U S A*. 115:2514–2519.
- Hardy D, Saghatelian A. 2017. Different forms of structural plasticity in the adult olfactory bulb. *Neurogenesis*. 4: e1301850.
- Jehl C, Royet JP, Holley A. 1995. Odor discrimination and recognition memory as a function of familiarization. *Percept Psychophys*. 57:1002–1011.
- Kelsch W, Lin C-W, Lois C. 2008. Sequential development of synapses in dendritic domains during adult neurogenesis. *Proc Natl Acad Sci U S A*. 105:16803–16808.
- Kelsch W, Sim S, Lois C. 2010. Watching synaptogenesis in the adult brain. *Annu Rev Neurosci*. 33:131–149.
- Kermen F, Midroit M, Kuczewski N, Forest J, Thévenet M, Sacquet J, Benetollo C, Richard M, Didier A, Madaïron N. 2016. Topographical representation of odor hedonics in the olfactory bulb. *Nat Neurosci*. 19:876–878
- Kermen F, Sultan S, Sacquet J, Madaïron N, Didier A. 2010. Consolidation of an olfactory memory trace in the olfactory bulb is required for learning-induced survival of adult-born neurons and long-term memory. *PLoS One*. 5(8): e12118.
- Lemasson M, Saghatelian A, Olivo-Marin J-C, Lledo P-M. 2005. Neonatal and adult neurogenesis provide two distinct populations of newborn neurons to the mouse olfactory bulb. *J Neurosci*. 25:6816–6825.
- Lepousez G, Nissant A, Bryant AK, Gheusi G, Greer CA, Lledo P-M. 2014. Olfactory learning promotes input-specific synaptic plasticity in adult-born neurons. *Proc Natl Acad Sci USA*. 111:13984–13989.
- Linster C, Cleland TA. 2002. Cholinergic modulation of sensory representations in the olfactory bulb. *Neural Netw*. 15:709–717.
- Linster C, Cleland TA. 2016. Neuromodulation of olfactory transformations. *Curr Opin Neurobiol*. 40:170–177.
- Linster C, Johnson BA, Yue E, Morse A, Xu Z, Hingco EE, Choi Y, Choi M, Messiha A, Leon M. 2001. Perceptual correlates of neural representations evoked by odorant enantiomers. *J Neurosci*. 21:9837–9843.
- Livneh Y, Adam Y, Mizrahi A. 2014. Odor processing by adult-born neurons. *Neuron*. 81:1097–1110.
- Lois C, Alvarez-Buylla A. 1994. Long-distance neuronal migration in the adult mammalian brain. *Science*. 264:1145–1148.
- Lu B. 2003. BDNF and activity-dependent synaptic modulation. *Learn Mem*. 10:86–98.
- Magavi SSP, Mitchell BD, Szentirmai O, Carter BS, Macklis JD. 2005. Adult-born and preexisting olfactory granule neurons undergo distinct experience-dependent modifications of their olfactory responses in vivo. *J Neurosci*. 25:10729–10739.
- Malvaut S, Saghatelian A. 2016. The role of adult-born neurons in the constantly changing olfactory bulb network. *Neural Plast*. 2016:e1614329.
- Madaïron N, Ferretti CJ, Stack CM, Rubin DB, Cleland TA, Linster C. 2006a. Cholinergic modulation in the olfactory bulb influences spontaneous olfactory discrimination in adult rats. *Eur J Neurosci*. 24:3234–3244.
- Madaïron N, Kuczewski N, Kermen F, Forest J, Midroit M, Richard M, Thevenet M, Sacquet J, Linster C, Didier A. 2018. Opposite regulation of inhibition by adult-born granule cells during implicit versus explicit olfactory learning. *Elife*. 7: e34976.
- Madaïron N, Sacquet J, Garcia S, Ravel N, Jourdan F, Didier A. 2006b. Neurogenic correlates of an olfactory discrimination task in the adult olfactory bulb. *Eur J Neurosci*. 24:3578–3588.
- Madaïron N, Stack C, Kiselycznyk C, Linster C. 2006c. Broad activation of the olfactory bulb produces long-lasting changes in odor perception. *Proc Natl Acad Sci U S A*. 103:13543–13548.
- Madaïron N, Sultan S, Nouvian M, Sacquet J, Didier A. 2011. Involvement of newborn neurons in olfactory associative learning? The operant or non-operant component of the task makes all the difference. *J Neurosci*. 31:12455–12460.
- Ming G, Song H. 2005. Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci*. 28:223–250.
- Moreno MM, Bath K, Kuczewski N, Sacquet J, Didier A, Madaïron N. 2012. Action of the noradrenergic system on adult-born cells is required for olfactory learning in mice. *J Neurosci*. 32:3748–3758.
- Moreno MM, Linster C, Escanilla O, Sacquet J, Didier A, Madaïron N. 2009. Olfactory perceptual learning requires adult neurogenesis. *Proc Natl Acad Sci U S A*. 106:17980–17985.
- Mouret A, Gheusi G, Gabellec M-M, de CF, Olivo-Marin J-C, Lledo P-M. 2008. Learning and survival of newly generated neurons: when time matters. *J Neurosci*. 28:11511–11516.
- Muthusamy N, Zhang X, Johnson CA, Yadav PN, Ghashghaei HT. 2017. Developmentally defined forebrain circuits regulate appetitive and aversive olfactory learning. *Nat Neurosci*. 20:20–23.
- Nissant A, Bardy C, Katagiri H, Murray K, Lledo P-M. 2009. Adult neurogenesis promotes synaptic plasticity in the olfactory bulb. *Nat Neurosci*. 12:728–730.
- Price JL, Powell TPS. 1970. The mitral and short axon cells of the olfactory bulb. *J Cell Sci*. 7:631–651.
- Ravel N, Elaagouby A, Gervais R. 1994. Scopolamine injection into the olfactory bulb impairs short-term olfactory memory in rats. *Behav Neurosci*. 108:317.
- Rey NL, Sacquet J, Veyrac A, Jourdan F, Didier A. 2012. Behavioral and cellular markers of olfactory aging and their response to enrichment. *Neurobiol Aging*. 33:626.e9–626.e23.
- Rodriguez A, Ehlenberger DB, Dickstein DL, Hof PR, Wearne SL. 2008. Automated three-dimensional detection and shape classification of dendritic spines from fluorescence microscopy images. *PLoS One*. 3:e1997.
- Sanai N, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. 2007. Comment on “human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension”. *Science*. 318:393.
- Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai H-H, Wong M, Gupta N, Berger MS, Huang E, Garcia-Verdugo J-M et al. 2011. Corridors of migrating neurons in the human brain and their decline during infancy. *Nature*. 478:382–386.



- Shepherd GM. 1972. Synaptic organization of the mammalian olfactory bulb. *Physiol Rev.* 52:864–917.
- Sultan S, Mandairon N, Kermen F, Garcia S, Sacquet J, Didier A. 2010. Learning-dependent neurogenesis in the olfactory bulb determines long-term olfactory memory. *FASEB J.* 24:2355–2363.
- Sultan S, Rey N, Sacquet J, Mandairon N, Didier A. 2011. New-born neurons in the olfactory bulb selected for long-term survival through olfactory learning are prematurely suppressed when the olfactory memory is erased. *J Neurosci.* 31:14893–14898.
- Valley MT, Henderson LG, Inverso SA, Lledo P-M. 2013. Adult neurogenesis produces neurons with unique GABAergic synapses in the olfactory bulb. *J Neurosci.* 33:14660–14665.
- Wearne SL, Rodriguez A, Ehlenberger DB, Rocher AB, Henderson SC, Hof PR. 2005. New techniques for imaging, digitization and analysis of three-dimensional neural morphology on multiple scales. *Neuroscience.* 136:661–680.
- Winner B, Cooper-Kuhn CM, Aigner R, Winkler J, Kuhn HG. 2002. Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb: neurogenesis in the adult rat olfactory bulb. *Eur J Neurosci.* 16:1681–1689.