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Plasticité cérébrale dans le système olfactif :

étude du modèle des sommeliers

par Daphnée Poupon-Pourchot

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Cette thèse intitulée

Plasticité cérébrale dans le système olfactif : étude du modèle des sommeliers

Présentée par

Daphnée Poupon-Pourchot

A été évalué(e) par un jury composé des personnes suivantes

Mathieu Piché
Président-rapporteur

Johannes Frasnelli
Directeur de recherche

Hugo Théoret
Membre du jury

Géraldine Coppin
Examinatrice externe

Résumé

Cette thèse s'intéresse à la capacité du cerveau à s'adapter à un environnement changeant. Plus spécifiquement, elle s'intéresse à la plasticité cérébrale dans le système olfactif. Les sommeliers, experts dans le domaine de l'olfaction, ont constitué notre modèle.

Une première étude nous a permis d'établir un protocole afin de tester la performance olfactive des sommeliers.

Dans une deuxième étude, nous avons testé des étudiants en sommellerie au début de leur formation d'un an et demi qui mène à la profession de sommelier. Nous avons observé que ces futurs experts de l'olfaction présentaient déjà, au cours des deux premiers mois, des capacités olfactives supérieures.

Dans une troisième étude, nous avons de nouveau testé les étudiants à la fin de leur formation, afin d'examiner les effets d'un entraînement olfactif à long terme sur la performance olfactive et sur le cerveau : en plus de mesurer les capacités olfactives avec le test des Sniffin' Sticks, nous avons utilisé l'imagerie par résonance magnétique (IRM) pour évaluer l'évolution du cerveau au cours de la formation en sommellerie. Nos principales observations concernent des changements au niveau de la structure cérébrale. Premièrement, le volume du bulbe olfactif a augmenté au cours de la formation, ce qui est en accord avec la littérature disponible à propos de cette structure. Deuxièmement, nous avons observé un épaississement au niveau du cortex entorhinal mais aussi un amincissement au niveau d'autres régions du cortex. Mises en relation avec les résultats d'études antérieures, ces observations soutiennent le récent modèle de surproduction-élagage selon lequel les changements dus à la plasticité liée à l'entraînement ne sont pas linéaires mais font intervenir différents processus en plusieurs phases. Ce modèle constitue une avancée importante dans la compréhension des mécanismes impliqués dans la plasticité cérébrale et devrait être pris en compte dans les futures études sur la plasticité.

Bien que les résultats sur le plan neuroimagerie soient intéressants, les résultats de l'étude longitudinale relatifs à la performance olfactive n'étaient pas concluants sur le plan comportemental. Nous avons donc mis en place dans une quatrième étude une tâche d'identification d'odorants au sein de mélanges plus complexe et plus adaptée aux sommeliers qui

a confirmé la supériorité de leurs capacités olfactives. Nous avons aussi entraîné des novices sur cette tâche pendant cinq jours pour tester les effets d'un court entraînement olfactif.

Cette thèse est organisée sous forme de thèse par articles. Le premier chapitre correspond à l'introduction générale, qui est elle-même organisée en plusieurs grandes parties. Ces différentes parties définissent les concepts-clés de cette thèse : l'olfaction, les corrélations neuroanatomiques dans le système olfactif, la plasticité cérébrale, la plasticité liée à l'entraînement dans le système olfactif, la neuroimagerie. La dernière partie conclut l'introduction en présentant les objectifs et hypothèses de recherche. Les chapitres suivants correspondent aux articles rédigés au cours du doctorat et présentant les résultats des recherches. Le dernier chapitre constitue une discussion générale. Enfin, en annexes se trouvent deux articles publiés lors du doctorat, un chapitre à paraître dans un livre ainsi que des résultats non publiés.

Mots-clés : plasticité, olfaction, cerveau, entraînement, sommelier, bulbe olfactif, épaisseur corticale, IRM, neuroimagerie.

Abstract

This thesis is about the brain's ability to adapt to an ever-changing environment. More specifically, it is about brain plasticity in the olfactory system. We used sommeliers, who are experts in olfaction, as our model.

A first study allowed us to instate a protocol to assess sommeliers' olfactory function.

In a second study, we tested sommelier students at the start of their year-and-a-half-long training which is the prerequisite to become a professional sommelier. We observed that these future experts in olfaction already had, during the first two months of training, superior olfactory abilities.

In a third study, we tested sommelier students again at the end of their training to examine the effects of a long-term olfactory training on olfactory performance and on the brain: beside assessing olfactory performance with the Sniffin' Sticks test, we used magnetic resonance imaging (MRI) to examine the evolution of brain structure and function during sommelier training. Changes in brain structure constituted our main results. Firstly, olfactory bulb volume increased during sommelier training, which is in line with previous reports about this structure. Secondly, we observed a cortical thickness increase in the entorhinal cortex but also cortical thinning in other brain areas. Put together with findings from previous studies, these results support the recent overproduction-pruning model of plasticity according to which changes due to training-related brain plasticity are nonlinear but involve different processes and different phases. This model constitutes a great advance in the understanding of brain plasticity and its underlying mechanisms and should be considered in future studies about plasticity.

Though neuroimaging results were interesting, results from olfactory tests in our longitudinal study were not conclusive so we conducted a fourth study to test the ability to identify odorants within mixtures, a task which is more complex and suitable for sommeliers than the Sniffin' Sticks test. Sommeliers performed better. We also tested novices that we had trained on this task for five days to evaluate the effects of a short-term olfactory training.

This thesis is organized by articles. The first chapter is a general introduction, itself organized in several parts. These different parts define the major concepts of this thesis: olfaction, neuroanatomical correlations in the olfactory system, brain plasticity, plasticity in the olfactory

system, neuroimaging. The last part concludes the introduction with aims and hypotheses. The following chapters are articles written during PhD that present the results of our research. The last chapter is a general discussion of all the results. Finally, two articles published during PhD, a chapter that is to be published in a book and unpublished results are presented as appendices.

Keywords: plasticity, olfaction, brain, training, sommelier, olfactory bulb, cortical thickness, MRI, neuroimaging.

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Liste des sigles et abréviations

AC : anosmie congénitale

ANOVA : analysis of variance

AVC : accident vasculaire cérébrale

BO : bulbe olfactif (anglais : OB)

BOLD : blood oxygen level dependent

DWI : diffusion-weighted imaging

CE : cortex entorhinal

EPI : echo planar imaging

FA : fractional anisotropy

GFIt : portion triangulaire du gyrus frontal inférieur (anglais : tIFG)

GFS : gyrus frontal supérieur (anglais : SFG)

GPS : gyrus pariétal supérieur (anglais : SPG)

GTI : gyrus temporal inférieur (anglais : ITG)

IRM : imagerie par resonance magnétique (anglais : MRI)

IRMf : imagerie par resonance magnétique fonctionnelle

tIFG : triangular portion of the inferior frontal gyrus (français : GFIt)

ITG : inferior temporal gyrus (français : GTI)

ITHQ : institute de tourisme et d'hôtellerie du Québec

LCR : liquide céphalo-rachidien

M ± SD : mean ± standard deviation

MB : matière blanche

MG : matière grise

MRI : magnetic resonance imaging (français : IRM)

OB : olfactory bulb (français : BO)

PCA : principal component analysis

PEA : phenylethyl alcohol

RAVLT : Rey auditory verbal learning test

SB : surface de matière blanche

SDI : score seuil – discrimination – identification (anglais : TDI)

SFG : superior frontal gyrus (français : GFS)

SG : surface de matière grise

SPG : superior parietal gyrus (français : GPS)

T1 : first time point, at the start of training

T2 : second time point, at the end of training

TCC : trouble cranio-cérébral

TDI : score threshold – discrimination – identification (français : SDI)

TSE : turbo spin echo

UNF : unité de neuroimagerie fonctionnelle

UPSIT : University of Pennsylvania smell identification test

Dédicace à mon papy qui imagine sa petite-fille en route vers le prix Nobel

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Chapitre 1 – Introduction

L'olfaction

Odorat et traitement de l'information olfactive

Le rôle de l'odorat dans notre vie de tous les jours est largement sous-estimé. En plus d'enrichir notre perception de l'environnement en y apportant une composante supplémentaire, les odeurs jouent des rôles multiples et plus divers que ce que nous pensons généralement : en plus de nous permettre d'apprécier les fragrances du monde qui nous entoure et la flaveur des aliments qui se trouvent dans notre assiette, elles peuvent aussi servir de signaux de danger, et jouent également un rôle dans les relations, que ce soit par exemple au sein d'un couple ou entre une mère et son enfant.

L'odorat est considéré comme un sens chimique, tout comme le goût. En effet, odorat et goût reposent sur la perception de stimuli chimiques, par opposition à la vue, l'audition et le toucher qui reposent sur des stimuli physiques (la lumière, les sons et la pression, respectivement). Les odorants sont des molécules volatiles qui, en interagissant avec les neurones olfactifs de l'épithélium olfactif, permettent leur perception. Le traitement de l'information olfactive implique tout un système. En se fixant sur un récepteur d'un des six millions de neurones olfactifs (Doty *et al.*, 2006) présents dans l'épithélium olfactif dans la cavité nasale, une odeur induit un signal transmis au bulbe olfactif, où des synapses établissent le lien entre neurones olfactifs et cellules mitrales dans des structures qui s'appellent les glomérules. Le bulbe olfactif constitue ainsi le premier relais, où l'information olfactive commence à être traitée : les axones des neurones olfactifs portant un même récepteur olfactif convergent vers un même glomérule ; différents odorants activent différents glomérules au sein du bulbe olfactif, ce qui permet une activation spatiale différente pour chaque odorant (Coelho *et al.*, 2016; Gottfried, 2010; Leboucq *et al.*, 2013; Mori *et al.*, 1999). Depuis les glomérules, les axones des cellules mitrales parcourent le tractus olfactif et transmettent l'information au cortex olfactif primaire, qui se constitue du tubercule olfactif, du cortex piriforme, de l'amygdale et du cortex entorhinal, au niveau de la jonction entre lobes frontal et temporal. De nombreuses connexions lient le cortex primaire à des structures telles que le cortex orbitofrontal, l'hippocampe, l'hypothalamus, le thalamus, le cortex périrhinal,

l'insula, qui composent le cortex olfactif secondaire (Coelho *et al.*, 2016; Gottfried, 2010; Leboucq *et al.*, 2013; Patel *et al.*, 2014; Tham *et al.*, 2009). Toutes ces structures permettent de traiter les différents aspects de l'information olfactive, et de relier l'olfaction à d'autres fonctions. L'amygdale joue par exemple un rôle dans le traitement des émotions telles que la peur (Rogan *et al.*, 1997; Stein *et al.*, 2007). Hippocampe, cortex entorhinal et périrhinal sont impliqués dans le système de la mémoire épisodique (Corkin *et al.*, 1997). Le cortex orbitofrontal est impliqué dans l'intégration multisensorielle (Gottfried, 2010). Une autre structure partagée avec les autres systèmes sensoriels est le thalamus, qui sert de relais obligatoire pour moduler l'information avant qu'elle n'atteigne le cortex dans les systèmes visuel, auditif et tactile, mais pas dans le système olfactif : bien que des voies olfactives passent par le thalamus pour atteindre le cortex orbitofrontal, d'autres voies sont directes depuis le cortex olfactif primaire (Hummel *et al.*, 2006; Tham *et al.*, 2009; Zatorre *et al.*, 1992).

Variations interindividuelles : des troubles olfactifs à l'expertise

L'odorat, tout comme les autres sens, présente une variabilité interindividuelle. De nombreux facteurs génétiques et environnementaux peuvent influencer l'acuité de chacun des sens, ce qui rend la perception variable d'une personne à l'autre. En plus de varier d'un individu à l'autre, l'odorat évolue au cours du temps. Avec l'âge avancé, par exemple, les capacités olfactives diminuent (Hummel *et al.*, 2007). Il a aussi été montré que, chez les femmes, la sensibilité olfactive fluctue au cours du cycle menstruel (Doty *et al.*, 1981).

La perception olfactive peut varier tout en restant dans ce qui est défini comme étant des normes physiologiques. Cependant, environ 20% de la population se trouvent en-dessous de ces normes et souffrent de troubles olfactifs (Landis *et al.*, 2004). De multiples causes sont possibles. Les troubles les plus fréquents sont les troubles de causes sinonasale, post-virale, posttraumatique ou idiopathique. Les troubles olfactifs peuvent aussi constituer un symptôme précoce d'une maladie neurodégénérative telles que les maladies de Parkinson ou d'Alzheimer. Des troubles olfactifs peuvent également être présents dès la naissance, par exemple dans le cas d'une malformation congénitale. Il suffit qu'une structure ou un mécanisme impliqué dans le traitement de l'information olfactive soit impacté pour que l'odorat soit altéré. Les troubles peuvent être quantitatifs ou qualitatifs. Les troubles quantitatifs correspondent à une perte de sensibilité qui peut être partielle ou totale, connus sous le nom d'hyposmie ou d'anosmie, respectivement : les odeurs

ne sont que peu ou pas détectées. Les troubles qualitatifs tels que la phantosmie et la parosmie sont des altérations liées à l'identification d'odeurs. La phantosmie correspond à l'illusion de la présence d'odeurs en l'absence de sources olfactives. La parosmie se caractérise par une identification biaisée des odeurs qui sont alors généralement perçues comme plus désagréables (Coelho *et al.*, 2016). Phantosmie et parosmie touchent respectivement 1% et 2% de la population (Landis *et al.*, 2004).

Tandis que certains ont une perception altérée des odeurs, d'autres sont considérés comme des experts de l'odorat. Ce sont des professionnels tels que des parfumeurs ou des sommeliers qui ont développé leur expertise au cours de leur formation et au long de leurs années d'expérience. Leur cas sera développé dans un prochain paragraphe.

Évaluer les capacités olfactives

Différents outils permettent de mesurer les capacités olfactives et ainsi de rendre compte des variations qui existent d'une personne à l'autre ou au cours du temps. Ces outils sont des tests standardisés utilisés dans divers pays. Les plus importants exemples de tests commercialisés sont les Sniffin' Sticks et le UPSIT (University of Pennsylvania Smell Identification Test). Le Sniffin' Sticks consiste en des feutres remplis d'odeurs avec lesquels plusieurs tests peuvent être réalisés, ce qui permet d'évaluer plusieurs aspects de la performance olfactive (Hummel *et al.*, 1997). Le premier test permet d'évaluer la sensibilité à une odeur en mesurant le seuil de détection, c'est-à-dire en évaluant la concentration à partir de laquelle le participant est capable de détecter cette odeur. L'odeur utilisée peut être l'alcool phénéthylique (PEA) ou le n-butanol. Dans le deuxième test, appelé test de discrimination, trois feutres sont présentés à chaque fois au participant. Parmi ces trois feutres, deux contiennent la même odeur, par exemple l'odeur d'orange, et le troisième contient une odeur différente, par exemple l'odeur de citron. Le participant doit déterminer quel feutre contient une odeur différente. Le troisième test est un test d'identification : seize odeurs sont présentées au participant qui doit les nommer en choisissant, pour chacune, une réponse parmi une liste de quatre réponses proposées. Le participant obtient un score pour chacun de ces trois tests, et les trois scores peuvent être additionnés pour obtenir un score global appelé score SDI (Seuil – Discrimination – Identification).

Le UPSIT est un autre test communément utilisé qui permet d'évaluer la capacité du participant à identifier des odeurs. Celui-ci se présente sous la forme de quarante bandelettes que le participant

peut gratter pour ainsi libérer les odorants contenus dans des microcapsules. De même que dans le test d'identification du Sniffin' Sticks, le test se présente sous forme de questionnaire à choix multiples et le participant identifie l'odeur en choisissant sa réponse parmi un choix de quatre réponses (Doty *et al.*, 1984).

Corrélations neuroanatomiques dans le système olfactif

Corrélations neuroanatomiques chez les normosmiques

La performance olfactive varie d'un individu à l'autre et chacun a un cerveau unique, avec différentes régions cérébrales plus ou moins développées. Il se trouve que ces variations sont corrélées : chez les normosmiques, c'est-à-dire chez des personnes n'ayant pas de troubles olfactifs et donc un odorat « normal », de meilleures capacités olfactives sont associées à des structures cérébrales impliquées dans l'olfaction plus volumineuses.

La première structure concernée est le bulbe olfactif : deux équipes de chercheurs ont observé que son volume était positivement corrélé au score SDI ; plus le bulbe olfactif est volumineux, plus le score SDI est élevé, et donc meilleure est la performance olfactive. Une des deux équipes a rapporté que le volume du bulbe olfactif était plus précisément corrélé aux scores obtenus aux tests d'identification et de seuil de détection (Buschhuter *et al.*, 2008). L'autre équipe a également observé une corrélation avec le test d'identification, mais pas avec le test de seuil de détection (Seubert *et al.*, 2013).

Les corrélations entre performance olfactive et cerveau ne se limitent pas au bulbe olfactif : des corrélations ont été trouvées au niveau de régions cérébrales olfactives telles que le cortex piriforme, le cortex entorhinal, le cortex orbitofrontal et l'insula ; plus le cortex de ces régions est épais, meilleures sont les capacités olfactives (Frasnelli *et al.*, 2010; Seubert *et al.*, 2013). Puisque le cortex est plus épais, les sillons sont plus profonds ; la profondeur du sillon olfactif, qui se situe entre le gyrus rectus et le gyrus orbitofrontal médian sur la face inférieure du lobe frontal, est ainsi positivement corrélée à la performance olfactive (Hummel *et al.*, 2003). Des corrélations ont aussi été observées au niveau de régions qui ne sont généralement pas associées à l'olfaction. C'est le cas du cortex occipital, principalement impliqué dans la vision, et du cortex somato-moteur, responsable de la motricité et des mouvements (Frasnelli *et al.*, 2010). Les corrélations sont spécifiques à différents tests olfactifs : la performance au test de seuil de détection ainsi que le

score SDI sont prédits par le volume du cortex orbitofrontal ; les scores obtenus au test d'identification sont liés au cortex piriforme, au cortex entorhinal et au cortex occipital ; le résultat du test de discrimination dépend de la taille du cortex orbitofrontal, de l'insula et du cortex somato-moteur (Frasnelli *et al.*, 2010; Seubert *et al.*, 2013).

Des corrélations entre performance olfactive et neuroanatomie existent donc, mais ni l'un ni l'autre ne sont fixes. Capacités olfactives et cerveau évoluent au cours du temps et les corrélations persistent : des variations de la performance olfactive sont associés à des variations de la structure et de la fonction du cerveau. Par exemple, tandis que les capacités olfactives diminuent avec l'âge, les structures cérébrales ont aussi tendance à rétrécir avec les années (Buschhuter *et al.*, 2008). Divers autres facteurs peuvent moduler performance olfactive et structure du cerveau et mettre en jeu diverses formes de plasticité. L'interaction entre olfaction et cerveau se fait dans les deux sens : des modulations au niveau de la fonction olfactive peuvent modeler le cerveau, c'est le cas par exemple pour l'entraînement olfactif, ou pour une obstruction unilatérale d'une narine qui mène à une diminution du volume du bulbe olfactif (Askar *et al.*, 2015). Au contraire, des changements dans le cerveau peuvent impacter l'olfaction, comme par exemple dans le cas d'un trouble cranio-cérébral, d'une maladie neurodégénérative ou d'une anosmie congénitale.

Olfaction et cerveau en conditions pathologiques

L'étude des troubles de l'odorat a permis un grand apport de connaissances sur la relation entre olfaction et structure cérébrale. En effet, diverses causes peuvent affecter l'odorat et avoir un impact sur le cerveau, ou au contraire affecter le cerveau et se répercuter sur l'odorat, et cela permet d'examiner le système olfactif de manière à extraire des informations qui ne pourraient être obtenues en étudiant le système olfactif en conditions physiologiques. En effet, il est par exemple plus simple de comprendre la fonction d'une région cérébrale lorsque cette région a subi une lésion et que la conséquence de cette lésion sur l'odorat peut être directement observé. Les troubles olfactifs constituent ainsi des modèles pour comprendre le lien entre olfaction et structure cérébrale.

Le cas du traumatisme cranio-cérébral

Un accident, une chute ou un coup porté à la tête peuvent provoquer un traumatisme cranio-cérébral (TCC). Ce TTC peut engendrer différentes conséquences en fonction de la force du choc et des zones du cerveau affectées. Des troubles olfactifs peuvent résulter d'un TCC. En effet, les TCC constituent une des causes les plus fréquentes de troubles olfactifs, et la prévalence de ces troubles

est assez importante puisque jusqu'à deux tiers des patients souffrant d'un TCC de gravité modérée à élevée voient leur odorat altéré (Bakker *et al.*, 2016; Costanzo *et al.*, 1986; Costanzo *et al.*, 1991; Drummond *et al.*, 2017; Frasnelli *et al.*, 2016; Kim *et al.*, 2017).

Ces chocs peuvent avoir différents effets et toucher le système olfactif à différents niveaux : l'épithélium olfactif peut être endommagé ; les nerfs olfactifs qui rejoignent le bulbe olfactif en traversant la lame criblée sont fixés tandis que le cerveau n'est pas complètement immobilisé au sein de la boîte crânienne, ce qui a pour conséquence la possibilité que les nerfs olfactifs soient rompus lorsqu'un choc secoue le cerveau ; des contusions ou hémorragies peuvent endommager les différentes structures qui constituent cortex olfactifs primaire et secondaire, ainsi que les connexions qui existent entre elles (Coelho *et al.*, 2016; Costanzo *et al.*, 2006).

L'évolution des troubles olfactifs au cours du temps est variable. En fonction de la gravité du TCC et du type de blessures occasionnées, un rétablissement de l'odorat est possible grâce à l'importante capacité de régénération du système olfactif (Costanzo *et al.*, 2006; Frasnelli *et al.*, 2016; Jimenez *et al.*, 1997). L'épithélium olfactif peut par exemple être régénéré grâce à la maturation des cellules basales en nouveaux neurones qui peuvent se développer de manière à ce que leur axone parvienne au bulbe olfactif, et ainsi établir de nouvelles connexions fonctionnelles (Yee *et al.*, 1995). Cette régénération n'est possible que dans certains cas : si le TCC est d'une sévérité trop importante ou les blessures trop graves, la régénération des nerfs olfactifs peut par exemple être bloquée par la formation de tissus cicatriciels ou de la gliose (Costanzo *et al.*, 1992). Le rétablissement des capacités olfactives n'est alors pas possible.

Le bulbe olfactif semble particulièrement impliqué : quasiment 90% des patients atteints de troubles olfactifs posttraumatiques présentent des altérations du bulbe olfactif (Yousem *et al.*, 1996b; Yousem *et al.*, 1999). Dans le cas d'anosmies posttraumatiques, ces altérations du bulbe olfactif concernent l'ensemble des patients (Liu *et al.*, 2008). Le volume de cette structure est corrélé à la performance olfactive (Liu *et al.*, 2017; Rombaux *et al.*, 2006b; Rombaux *et al.*, 2012; Yousem *et al.*, 1996a; Yousem *et al.*, 1999). De plus, il semble constituer un prédicteur de la probabilité de récupération. En effet, en plus d'être corrélé à la performance olfactive, un volume plus important au moment du diagnostic est associé à une amélioration plus importante de la fonction olfactive (Rombaux *et al.*, 2012).

Le bulbe olfactif n'est pas la seule structure impactée : le lobe sous-frontal et le lobe temporal sont également altérés, dans environ 60% et 30% des cas, respectivement (Yousem *et al.*, 1996a; Yousem *et al.*, 1999). Des contusions dans les lobes frontaux et temporaux sont associées à une altération des capacités à discriminer et identifier des odeurs, tandis que la capacité à les détecter reste intacte (Costanzo *et al.*, 1991; Levin *et al.*, 1985). Des lésions situées plus précisément dans le cortex orbitofrontal sont associées à des troubles de discrimination, d'identification, ainsi que de mémoire olfactive (Gottfried, 2010). Une lésion au niveau du cortex olfactif secondaire tandis que les aires primaires sont intactes peut causer une parosmie : le patient détecte l'odeur mais, à un plus haut niveau de traitement, l'information olfactive est déformée (Lotsch *et al.*, 2016).

Le cas de la maladie de Parkinson

La maladie de Parkinson est une maladie neurodégénérative qui se caractérise principalement par des symptômes moteurs. La maladie est déclarée lors de l'apparition de ces symptômes, mais d'autres symptômes non-moteurs apparaissent plusieurs années plus tôt, lors de la phase dite prémotrice. Parmi ces symptômes figurent des troubles du sommeil, la dépression, ainsi que des troubles olfactifs (Tremblay *et al.*, 2017).

La maladie de Parkinson est entre autres causée par la formation de corps de Lewy à l'intérieur des neurones : l'alpha-synucléine est une protéine qui, dans la maladie, est mal repliée lors de sa synthèse et devient insoluble. Cela mène à une accumulation qui constitue les corps de Lewy, des dépôts intracellulaires qui peuvent mener à la dégénérescence des neurones (Kalia *et al.*, 2015). Ce sont ces corps de Lewy qui seraient à l'origine des troubles olfactifs observés dans la maladie de Parkinson. En effet, selon le modèle de Braak, ce phénomène progresse de manière temporelle et spatiale en plusieurs étapes, et débute dans le système olfactif, plus précisément dans le noyau olfactif antérieur, une structure qui démarre dans le bulbe olfactif et s'étend le long du tractus olfactif jusqu'au cortex orbital (Braak, Del Tredici, *et al.*, 2003; Braak, Rub, *et al.*, 2003). Cela mène à une très forte prévalence des troubles olfactifs chez les patients : plus de 90% sont touchés, ce qui fait des troubles olfactifs un des symptômes non-moteurs les plus fréquents dans la maladie de Parkinson (Doty, 2012; Haehner, Boesveldt, *et al.*, 2009; Takeda, 2013). L'altération de la fonction olfactive précède l'apparition des symptômes moteurs, et donc le diagnostic, d'au moins quatre ans (Ross *et al.*, 2008), ce qui a mené les troubles olfactifs a être plus amplement étudiés dans l'espoir de les utiliser comme outil diagnostique précoce de la maladie.

Que ce soit dans les tâches d'identification, de discrimination, et de seuil de détection, la performance des patients est significativement moins bonne que celle des participants contrôles du même âge (Barz *et al.*, 1997; Boesveldt *et al.*, 2008; Tissingh *et al.*, 2001; Wang *et al.*, 2011). La performance olfactive dans la tâche de discrimination est corrélée à l'avancée de la maladie, ce qui n'est pas le cas de la tâche d'identification (Boesveldt *et al.*, 2008; Doty *et al.*, 1988; Tissingh *et al.*, 2001).

La performance olfactive peut généralement être améliorée grâce à un entraînement olfactif, qui consiste à sentir des odeurs chaque jour. Tandis que, pour des troubles olfactifs dont la cause est autre que la maladie de Parkinson, les capacités dans les tâches d'identification et de discrimination sont nettement améliorées et un effet faible à modéré est observé pour le seuil de détection, l'entraînement olfactif a un effet seulement sur la tâche de discrimination dans le cas des troubles olfactifs causés par la maladie de Parkinson (Haehner *et al.*, 2013; Sorokowska, Drechsler, *et al.*, 2017).

Le cas de la maladie d'Alzheimer

Une autre maladie neurodégénérative est la maladie d'Alzheimer qui se caractérise par trois principaux groupes de symptômes : des symptômes cognitifs tels que perte de mémoire, problèmes de langage et difficultés dans la prise de décision et la planification, des symptômes psychiatriques tels que dépression et hallucinations, et des symptômes liés à la difficulté d'exécuter les tâches du quotidien (Burns *et al.*, 2009). Ces symptômes sont dus à l'accumulation de protéines : les peptides β-amyloïdes s'amassent autour des neurones et forment des plaques amyloïdes tandis que la protéine tau s'accumule à l'intérieur des neurones et forme des dégénérescences neurofibrillaires. Peptides β-amyloïdes et protéine tau constituent les deux marqueurs biologiques de la maladie. L'accumulation de la protéine tau en présence de peptides β-amyloïdes présage le développement de symptômes cognitifs caractéristiques de la maladie d'Alzheimer (Galasko *et al.*, 2017).

Tout comme pour la maladie de Parkinson, la fonction olfactive est altérée dans la maladie d'Alzheimer et les troubles olfactifs constituent un symptôme précoce (Devanand *et al.*, 2008; Djordjevic *et al.*, 2008; Mesholam *et al.*, 1998). Une méta-analyse des diverses études de la fonction olfactive réalisées chez des patients a montré que la maladie d'Alzheimer était principalement associée à des troubles de l'identification et de la discrimination d'odeurs (Silva *et al.*, 2018). Bien que l'identification soit l'aspect de la fonction olfactive le plus altéré dans la

maladie d'Alzheimer, d'autres troubles olfactifs sont observés, comme par exemple une mémoire olfactive réduite (Gilbert *et al.*, 2004). Quant à la sensibilité olfactive, bien que peu de résultats soient disponibles par rapport au seuil olfactif, les quelques études montrent que la sensibilité olfactive est également réduite chez les patients, et que le degré de déficience est associé au degré de démence (Djordjevic *et al.*, 2008; Doty *et al.*, 1987; Murphy *et al.*, 1990).

L'altération de la performance olfactive est causée par les plaques amyloïdes et les dégénérescences neurofibrillaires qui se déposent à divers niveaux du système olfactif. En périphérie, des peptides β-amyloïdes se déposent dans l'épithélium olfactif des patients (Wilson *et al.*, 2007). La protéine tau forme des dégénérescences neurofibrillaires dans le bulbe olfactif (Attems *et al.*, 2005). Fréquence et densité de ces dégénérescences fibrillaires dans le bulbe olfactif sont grandement corrélées à celles qui se trouvent dans le cortex entorhinal (Christen-Zaech *et al.*, 2003; Price *et al.*, 1991). Ces dégénérescences neurofibrillaires se forment très précocement, avant l'apparition de symptômes cliniques, et seraient la cause des troubles olfactifs observés (Devanand *et al.*, 2008; Djordjevic *et al.*, 2008; Wilson *et al.*, 2007). Lorsque la maladie évolue, les dégénérescences neurofibrillaires s'étendent dans le cortex entorhinal, le cortex périrhinal, l'hippocampe et l'amygdale. Les aires olfactives sont donc très touchées par la pathologie (Attems *et al.*, 2014; Braak *et al.*, 1992; Hyman *et al.*, 1991; Murphy, 2019; Ohm *et al.*, 1987; Struble *et al.*, 1992). Un faible volume de l'hippocampe et une faible épaisseur du cortex entorhinal sont associés à un déficit dans l'identification d'odeurs (Growdon *et al.*, 2015).

Les lésions ont aussi un impact sur l'activité cérébrale. Chez les patients, l'activation du cortex piriforme, du cortex entorhinal, de l'amygdale et de l'insula est réduite lors de la réalisation d'une tâche olfactive passive (Kareken *et al.*, 2004; Wang *et al.*, 2010), lors d'une tâche de détection (Vasavada *et al.*, 2017), ou encore lors de l'évaluation de la qualité d'un stimulus olfactif (Li *et al.*, 2010). L'activation cérébrale étant lente et réduite, le traitement de l'information olfactive est inefficace et des mécanismes de compensation sont mis en place, ce qui mène à des différences visibles au niveau de la connectivité fonctionnelle lors de la tâche de mémoire olfactive : d'autres réseaux neuronaux sont recrutés (Haase *et al.*, 2013). Les mécanismes de compensation peuvent cependant avoir un effet délétère : pour contrer les conséquences de la présence des plaques amyloïdes, des tâches cognitives complexes qui requièrent des efforts peuvent mener à une hyperactivation de certaines aires cérébrales (Mormino *et al.*, 2012). Or, cela peut avoir un effet

néfaste. En effet, les individus à risque pour la maladie d'Alzheimer qui ont des troubles olfactifs sont susceptibles de faire plus d'efforts lors de tâches olfactives complexes, ce qui résulte en une hyperactivation des aires olfactives et cognitives. L'hyperactivation favoriserait la dégénérescence qui, au fil du temps, mène à une diminution de l'épaisseur du cortex entorhinal et du volume de l'hippocampe, ce qui accentue le déclin de la performance olfactive dans les tâches d'identification et de mémoire olfactive et amplifie donc le processus autodestructeur (Murphy, 2019).

Le cas de l'anosmie congénitale

Tandis que diverses conditions peuvent affecter l'odorat au cours de la vie, certains sont directement nés sans odorat. C'est ce qui est appelé l'anosmie congénitale (AC).

Le bulbe olfactif étant peu ou pas développé est la cause de l'anosmie. Les recherches en neuroanatomie dans le cadre de l'AC se basent sur l'hypothèse que l'absence de stimuli olfactifs a des effets dans d'autres régions cérébrales et, en effet, il a été observé que le sillon olfactif était moins profond et le cortex dans cette région était plus épais (Manara *et al.*, 2014). L'augmentation de l'épaisseur corticale autour du sillon olfactif a été rapportée par deux autres études (Frasnelli *et al.*, 2013; Ottaviano *et al.*, 2015). L'épaisseur corticale est négativement corrélée au score obtenu dans la tâche olfactive. Plus le bulbe olfactif est petit, plus l'augmentation de l'épaisseur corticale dans cette région est importante.

L'AC est ainsi associée à des augmentations de l'épaisseur corticale dans les aires olfactives. Ce résultat semble en désaccord avec le fait qu'épaisseur corticale et volume de matière grise sont généralement corrélés positivement à la performance olfactive (Frasnelli *et al.*, 2010; Royet *et al.*, 2013; Seubert *et al.*, 2013). Cependant, des résultats similaires sont obtenus par exemple dans le système visuel : les aveugles congénitaux présentent un cortex visuel plus épais (Kupers *et al.*, 2014). L'explication proposée concerne l'élagage synaptique : ce processus consiste en une réduction du nombre de connexions synaptiques pour favoriser les connexions les plus utiles, et est induit par l'information reçue. En absence de stimuli olfactifs, il n'y a pas d'élagage synaptique dans les cortex olfactifs dont l'épaisseur et la densité augmentent donc.

La plasticité cérébrale

Se modifier pour s'adapter

Le monde autour de nous change sans cesse. Pour nous permettre de nous adapter à ces changements, le cerveau se modifie. La plasticité cérébrale représente cette capacité du système nerveux à moduler sa structure et son activité en réponse à divers stimuli intrinsèques et extrinsèques. Le cerveau n'est donc pas figé et immuable, mais se modifie constamment et évolue de manière à répondre au mieux à nos besoins. La plasticité cérébrale a divers rôles : essentielle à l'établissement et au maintien de circuits neuronaux, elle contribue au développement du cerveau, elle est impliquée dans l'apprentissage et la mémoire, elle permet l'acquisition de nouvelles compétences ainsi que l'adaptation suite à une lésion cérébrale ou à la perte d'un membre ou d'un sens (Hubener *et al.*, 2010; Mateos-Aparicio *et al.*, 2019; Pascual-Leone *et al.*, 2011; Squire *et al.*, 2004).

Des modifications structurales et fonctionnelles à différents niveaux

De nombreux facteurs tels que des molécules endogènes (par exemple les hormones, les neurotransmetteurs, les facteurs de croissance), le stress, l'apprentissage, le vieillissement, nos mouvements, nos perceptions sensorielles, ou encore nos décisions et nos pensées, peuvent amener le cerveau à se modifier (Fuchs *et al.*, 2014; Pascual-Leone *et al.*, 2005). Les modifications peuvent s'effectuer au niveau moléculaire, au niveau cellulaire, au niveau d'un circuit ou même du réseau. Neurones et synapses se modifient de manière structurale et fonctionnelle. La plasticité synaptique structurale implique des modifications morphologiques de l'axone, des dendrites et des épines dendritiques, et mène à la formation de nouvelles synapses ou à l'élagage synaptique. Les synapses peuvent être fonctionnellement renforcées ou affaiblies, ce qui permet de moduler l'efficacité des connexions entre les neurones (Mateos-Aparicio *et al.*, 2019). Un autre mécanisme, la neurogenèse, consiste en la formation de nouveaux neurones (Pascual-Leone *et al.*, 2011). La plasticité n'implique pas seulement les neurones mais également de leur environnement : la gliogenèse régule les cellules gliales qui assurent le soutien, la nutrition et la protection des neurones, la myélinisation d'axones non myélinisés ou des modifications de la gaine de myéline déjà existante module l'efficacité de la transmission du signal nerveux, et des changements vasculaires permettent d'améliorer localement l'oxygénation du cerveau (Zatorre *et al.*, 2012). À l'échelle macroscopique,

les réseaux neuronaux peuvent être remodelés : les patterns spatiotemporels d'activation peuvent changer si, par exemple, des régions qui ne sont pas fonctionnellement connectées sont incorporées à un réseau (Ganguly *et al.*, 2013; Pascual-Leone *et al.*, 2011).

La plasticité développementale

La plasticité cérébrale joue un rôle primordial dès le tout début de notre vie et permet la constitution d'un cerveau unique à chacun. La plupart des neurones sont formés durant la vie prénatale et au cours des huit premiers mois après la naissance. Dès la naissance et pendant l'enfance, de très nombreuses connexions sont établies entre les neurones (Kolb *et al.*, 2014). Cette synaptogenèse est orchestrée par un ensemble de facteurs génétiques et environnementaux : durant l'enfance, chaque expérience crée de nouvelles connexions. Des facteurs aussi divers que les perceptions sensorielles, le stress, le régime alimentaire ou encore la relation avec les parents, influencent le câblage du cerveau (Kolb *et al.*, 2011).

Neurones et synapses sont produits en excès. La moitié des neurones générés sont éliminés par apoptose, ce qui permet d'ajuster la population de neurones à la taille nécessaire ou aux besoins fonctionnels, et permet également d'éliminer de nombreux neurones dont les axones se sont dirigés vers la mauvaise cible (Burek *et al.*, 1996; Cowan *et al.*, 1984; Oppenheim, 1991). En effet, le grand nombre de neurones entraîne une compétition pour les facteurs trophiques essentiels à leur survie et, en n'atteignant pas la bonne cible, les neurones sont privés de facteurs de croissance normalement produits par le tissu-cible ; en l'absence de ces signaux de survie, l'apoptose est déclenchée (Mazarakis *et al.*, 1997). L'élagage synaptique est un autre processus qui permet de réguler les connexions entre les neurones sans pour autant causer la mort cellulaire : ce sont les synapses qui sont éliminées (Cowan *et al.*, 1984; Huttenlocher, 1979; Huttenlocher *et al.*, 1987). Jusqu'à deux tiers des synapses seraient élaguées (Chechik *et al.*, 1999), ce qui permet d'affiner les réseaux neuronaux et d'optimiser leur efficacité (Kolb *et al.*, 2011; Low *et al.*, 2006). L'élagage synaptique se fait majoritairement durant l'adolescence et jusqu'à environ 30 ans, âge auquel le nombre de synapses semble se stabiliser (Kolb *et al.*, 2014). Ce sont les neurones qui assurent la régulation de leurs propres synapses en les renforçant ou en les éliminant (Turrigiano *et al.*, 1998). Les synapses ne sont pas éliminées au hasard. Chaque expérience pertinente active certains circuits neuronaux. Les neurones renforcent les synapses qui sont le plus souvent activées, et éliminent les synapses les plus faibles (Chechik *et al.*, 1998). L'ensemble de ces mécanismes permet chez chaque

individu le développement d'un cerveau unique, modelé à partir du patrimoine génétique, du mode de vie et des expériences, avec des réseaux optimisés pour une plus grande efficacité.

La plasticité développementale est un des plus importants types de plasticité, raison pour laquelle elle est mentionnée dans cette introduction. Elle n'est cependant pas l'objet de cette thèse.

La plasticité cérébrale chez l'adulte

Pendant longtemps, la plasticité a été considérée comme un processus qui n'a lieu que chez l'enfant mais au cours des dernières décennies, de nombreuses études ont montré que, même si le cerveau est plus malléable chez l'enfant, les réseaux neuronaux évoluent toujours à l'âge adulte. La plasticité cérébrale est essentielle pour l'adaptation de l'adulte dans diverses situations.

Adaptation du cerveau à la suite d'une amputation ou d'une lésion cérébrale

Un exemple majeur de plasticité cérébrale concerne les patients qui ont subi une amputation d'un membre. En temps normal, le cerveau reçoit des informations sensorielles provenant de l'ensemble du corps. Le cortex sensorimoteur primaire est organisé de telle manière que chaque partie du corps est représentée : différentes régions corticales reçoivent les informations sensorielles provenant de différentes parties du corps. Lors de l'amputation d'une main, par exemple, la zone du cortex sensorimoteur qui répond normalement aux informations sensorielles provenant de la main ne reçoit plus aucune information. L'absence d'afférences mène à une réorganisation corticale structurale et fonctionnelle : la zone du cortex sensorimoteur qui est privée d'afférences est recrutée par les régions corticales voisines. Des études réalisées chez le singe ont ainsi montré que l'aire cérébrale initialement associée à la main amputée évolue et répond aux afférences provenant du bas du visage (Florence *et al.*, 1998; Pons *et al.*, 1991). Chez l'humain, des réorganisations semblables du cortex sensorimoteur primaire ont été mises en évidence à la suite d'amputations (Cohen *et al.*, 1991; Flor *et al.*, 1995; Grusser *et al.*, 2001; Karl *et al.*, 2001). Puisque les aires sensorimotrices primaires sont interconnectées à d'autres régions cérébrales, les réorganisations locales du cortex sensorimoteur primaire sont à l'origine d'une cascade de réorganisations corticales qui ont lieu à plus grande échelle et qui peuvent impacter d'autres fonctions telles que la perception visuospatiale dans le cas d'une amputation de la main (Bramati *et al.*, 2019; Makin *et al.*, 2015). Ces réorganisations corticales sont réversibles : les modifications cérébrales dues à une amputation sont inversées après une greffe (Giraux *et al.*, 2001).

À la suite d'une amputation, une aire cérébrale privée d'afférences peut donc être réorganisée de manière à assurer une fonction différente. Dans le cas d'une lésion cérébrale, c'est l'inverse : un tissu cérébral est endommagé et incapable de remplir sa fonction. C'est ainsi qu'un traumatisme cranio-cérébral (TCC) ou un accident vasculaire cérébral (AVC) peut mener par exemple à la perte de l'usage d'un membre ou la perte de la parole. La récupération peut alors se faire par restauration ou par compensation. Une restauration est possible si le tissu neuronal perturbé récupère ses fonctions, ce qui permet de restaurer le comportement associé tel qu'avant la lésion. La compensation implique le recrutement de nouveaux circuits neuronaux et l'acquisition de nouvelles fonctions ou comportements pour remplacer ceux perdus suite à la lésion (Kleim, 2011). La récupération peut être spontanée, ou peut nécessiter une réadaptation qui permet par l'entraînement de réapprendre ce qui a été perdu (Chen *et al.*, 2010).

La récupération spontanée se fait dans les trois à six mois suivant la lésion et implique soit une restauration du tissu endommagé, soit une réorganisation corticale. La restauration du tissu endommagé est possible lorsque les changements du métabolisme et de la circulation sanguine, l'inflammation et l'œdème dus à la lésion se résorbent, ce qui permet au tissu cérébral de retrouver un état fonctionnel (Warraich *et al.*, 2010). La restauration a été mise en évidence dans des cas où des fonctions motrices ou cognitives telles que le langage ou l'attention étaient atteintes (Kleim, 2011). Lorsqu'elle entraîne une réorganisation corticale, la récupération spontanée implique des mécanismes de compensation. Neurogenèse, pousse axonale, plasticité synaptique, changements de l'excitabilité des neurones et formation de nouveaux vaisseaux sanguins peuvent être observés autour de la lésion (Kerr *et al.*, 2011; Nudo, 2011). La réorganisation corticale peut mener au recrutement d'aires homologues dans l'autre hémisphère, par exemple dans le cas d'une lésion de l'aire de Broca dédiée au langage de l'hémisphère gauche, l'homologue de l'aire de Broca dans l'hémisphère droit peut être temporairement recruté jusqu'à ce que l'aire de l'hémisphère gauche puisse récupérer ses fonctions (Chen *et al.*, 2010).

Lorsque la récupération spontanée est impossible ou insuffisante, un entraînement peut déclencher des processus de plasticité cérébrale et permettre une réadaptation. Des mécanismes de compensation permettent alors le recrutement de nouvelles aires cérébrales ou la mise en place de nouveaux réseaux neuronaux pour assurer les fonctions perdues suite à la lésion, notamment grâce à la synaptogenèse et la plasticité synaptique (Chen *et al.*, 2010). Cette réorganisation corticale est

facilitée par la redondance qui existe dans le cerveau et entre autres dans les aires corticales primaires : dans le cortex moteur primaire, les aires somatosensorielles, le cortex visuel primaire et le cortex auditif primaire, différentes aires répondraient à des stimuli similaires (Warraich *et al.*, 2010). Cette redondance s'observe également à plus grande échelle, avec différentes régions cérébrales qui peuvent être dédiées à une fonction similaire, ce qui facilite l'intégration d'une information mais offre également une possibilité de récupération après une lésion (Warraich *et al.*, 2010).

Apprentissage et expertise

La plasticité cérébrale chez l'adulte n'est pas impliquée seulement en cas de changement brutal tel qu'une lésion cérébrale ou une amputation. Des changements de comportement ou l'expérience peuvent également mener à des modifications au niveau neurobiologique. L'entraînement peut permettre le réapprentissage de fonctions perdues à la suite d'une lésion, mais il peut aussi tout simplement permettre l'apprentissage. En effet, même chez l'adulte, l'entraînement peut mener à l'acquisition de nouvelles capacités et à leur amélioration, ce qui s'accompagne de modifications au niveau du cerveau.

Puisque le cerveau est subdivisé en régions spécialisées dans diverses fonctions, les modifications se produisent dans des régions cérébrales qui sont spécifiques à la nature de l'entraînement. En s'entraînant à effectuer une tâche encore et encore, des régions spécifiques sont mobilisées de manière répétée. Cela mène principalement au renforcement de synapses existantes ou à la création de nouvelles synapses, ce qui se caractérise généralement par des changements de taille ou de densité de ces régions cérébrales (Fu *et al.*, 2011; May, 2011; Zatorre *et al.*, 2012). La mobilisation des circuits neuronaux concernés devient alors plus efficace, ce qui facilite l'exécution de la tâche en question.

Diverses études ont montré des liens entre entraînement et modifications cérébrales. Ces études comparent généralement un groupe expérimental suivant un entraînement d'une certaine durée à un groupe contrôle, tous deux étant testés au début et à la fin de l'entraînement pour voir l'évolution du cerveau. C'est ainsi que des groupes ont été entraînés à diverses tâches sur des périodes de temps plus ou moins longues. Une étude dans laquelle un groupe suivait un entraînement de jonglage d'une durée de trois mois a ainsi montré qu'à la fin de l'entraînement, une augmentation du volume de matière grise était visible dans des régions cérébrales impliquées dans le traitement et le

stockage de l'information visuelle relative au mouvement (Draganski *et al.*, 2004). Dans une autre étude impliquant le jonglage, un entraînement de seulement sept jours était suffisant pour que des effets sur le cerveau soient visibles, ce qui a conduit les auteurs à suggérer que l'apprentissage d'une tâche nouvelle avait plus d'impact sur le cerveau que l'entraînement continu d'une tâche déjà apprise (Driemeyer *et al.*, 2008). Des résultats similaires ont été obtenus avec un entraînement impliquant un exercice de piano ; après seulement cinq jours, des changements fonctionnels étaient visibles dans le cortex moteur (Pascual-Leone *et al.*, 1995). Les entraînements peuvent également être cognitifs : un entraînement de la mémoire de travail sur une durée de deux mois a par exemple impacté la connectivité structurale dans des régions adjacentes au sillon intrapariétal et dans la partie adjacente du corps calleux, des régions connues comme étant impliquées dans la mémoire de travail (Takeuchi *et al.*, 2010). Même les capacités sociales telles que l'attention, l'empathie, la compassion, la compréhension des croyances et des intentions d'autrui, peuvent être entraînées et, en moins de trois mois, des épaississements du cortex sont visibles dans les régions spécifiques à ces fonctions (Valk *et al.*, 2017). L'entraînement permet donc l'acquisition de nouvelles compétences dans divers domaines et mène à des modifications cérébrales structurales et fonctionnelles en seulement quelques semaines voire quelques jours.

L'entraînement mène éventuellement à l'expertise. La plasticité peut par exemple être observée chez des musiciens ou sportifs de haut niveau, ou encore chez des personnes exerçant une certaine profession si cette profession requiert des aptitudes particulières. Une étude pionnière a été réalisée chez des chauffeurs de taxis londoniens et a révélé qu'ils présentaient des spécificités au niveau de leur cerveau, et plus précisément au niveau de l'hippocampe. L'hippocampe est une structure cérébrale dont un des rôles est de faciliter la mémoire spatiale lors de déplacements. Cette structure est subdivisée en deux régions : l'hippocampe antérieur, responsable de mémoriser les informations spatiales de nouveaux environnements, et l'hippocampe postérieur, mobilisé lorsque des informations spatiales déjà mémorisées sont utilisées. Chez les chauffeurs de taxis, l'hippocampe postérieur est plus volumineux tandis que l'hippocampe antérieur est plus petit, et ces différences s'accentuent avec le nombre d'années d'expérience : plus un chauffeur de taxi exerce sa profession longtemps, plus son hippocampe postérieur est volumineux et son hippocampe antérieur est petit. En effet, avec les années d'expérience, le chauffeur connaît de mieux en mieux Londres : les nouvelles informations spatiales sont donc de moins en moins nombreuses et l'hippocampe antérieur est de moins en moins mobilisé, tandis que la carte de la ville qu'il a

enregistrée dans sa mémoire est de plus en plus détaillée et requiert donc de plus en plus de place, d'où un hippocampe postérieur de plus en plus volumineux (Maguire *et al.*, 2000). Similairement, chez les joueurs de badminton professionnels, l'adaptation du cerveau favorise un mouvement plus coordonné, tandis que chez les musiciens, elle facilite la synchronisation des deux mains (Amunts *et al.*, 1997; Di *et al.*, 2012). Le cerveau des radiologistes présente également des spécificités ; ces différences sont liées à un sens de l'observation plus aiguisé qui leur est nécessaire pour remarquer tout détail important sur une radiographie (Harley *et al.*, 2009).

La plasticité intermodale : le modèle de la cécité

Un sens qui manque

La perception de notre environnement résulte de l'interaction de nos différents sens, qui nous permettent de percevoir ses différents aspects et d'y répondre de manière appropriée : différents systèmes nous permettent de capter des stimuli de différentes natures qui sont ensuite traités en suivant des voies spécifiques dans le cerveau, avant d'être intégrés pour nous donner une vue d'ensemble. Cependant, dans certains cas, un sens est dysfonctionnel, et les individus concernés se retrouvent donc privés de certaines informations. C'est le cas chez les aveugles, qui sont privés d'un sens qui est majeur chez l'humain : la vue. Diverses causes peuvent entraîner une cécité, qui peut être précoce ou tardive. De nombreuses études se sont intéressées aux aveugles et à comment ils palliaient ce déficit. Des mécanismes d'adaptation leur permettant d'obtenir l'information dont ils ont besoin pour interagir avec leur environnement ont été mis en évidence. Ces études concernaient majoritairement leurs capacités auditives et tactiles avant que les chercheurs s'intéressent également à leurs capacités olfactives.

Le système visuel

Dans le système visuel, les photons atteignent la rétine et induisent un signal transmis via les nerfs optiques. Tandis que l'olfaction est ipsilatérale, signifiant que les odeurs perçues par la narine gauche seront traitées dans l'hémisphère gauche, la vue est controlatérale : le champ visuel est divisé en deux hémichamps et, au sein du chiasma optique, les fibres se croisent de manière à ce que les informations de l'hémichamp visuel droit soient traitées dans l'hémisphère gauche et inversement. À la sortie du chiasma optique, l'information visuelle passe par le corps géniculé latéral du thalamus avant d'être projetée sur le cortex strié, qui constitue l'aire visuelle primaire (Bishop, 2011). À partir du cortex strié, deux voies transmettent l'information visuelle : la voie

ventrale mène au cortex temporal inférieur, tandis que la voie dorsale mène au cortex pariétal inférieur. Chacune de ces voies est spécialisée. La fonction primaire de la voie ventrale est d'analyser les propriétés de l'objet telles que sa forme et sa texture. L'interaction du cortex temporal inférieur avec les systèmes mnésiques permet ainsi l'identification de l'objet perçu. La voie dorsale traite principalement l'information relative à sa localisation et à sa position dans l'espace (Mishkin *et al.*, 1983; Ungerleider *et al.*, 1994; Zachariou *et al.*, 2014; Zafar *et al.*, 2015).

Audition et toucher chez l'aveugle

Les fonctions auditives et tactiles chez l'aveugle ont amplement été étudiées. Au niveau comportemental, il est ressorti que les aveugles avaient une perception auditive et tactile plus fine que les voyants (Goldreich *et al.*, 2003, 2006; Gougoux *et al.*, 2004; Van Boven *et al.*, 2000; Voss *et al.*, 2004; Wan *et al.*, 2010a, 2010b).

La neuroimagerie a permis d'observer que le cortex occipital, siège du traitement de l'information visuelle, était activé chez l'aveugle lors de tâches auditives et tactiles (Buchel *et al.*, 1998; Burton *et al.*, 2002; Sadato *et al.*, 1996; Theoret *et al.*, 2004; Weeks *et al.*, 2000).

Ces résultats semblent confirmer l'existence d'une compensation intermodale chez les individus privés de la vue. Ces observations ont mené les chercheurs à s'interroger sur le système olfactif chez l'aveugle.

L'olfaction chez l'aveugle

Sniffin' Sticks et UPSIT ont été utilisés pour évaluer plusieurs aspects de la performance olfactive des aveugles.

Dans la tâche de seuil de détection, qui est principalement perceptuelle, aucune différence significative entre les deux groupes n'a été observée dans la plupart des études (Cornell Karnekull *et al.*, 2016; Luers *et al.*, 2014; Rosenbluth *et al.*, 2000; Schwenn *et al.*, 2002; Sorokowska, 2016; Wakefield *et al.*, 2004), bien que certaines aient relevé un seuil de détection plus bas chez les aveugles (Beaulieu-Lefebvre *et al.*, 2011; Comoglu *et al.*, 2015; Cuevas *et al.*, 2010).

Dans la tâche de discrimination, les résultats sont partagés : tandis que les résultats suggèrent que les aveugles surpassent les voyants dans certaines études (Comoglu *et al.*, 2015; Cuevas *et al.*, 2010; Renier *et al.*, 2013; Rombaux *et al.*, 2010), d'autres n'observent aucune différence

significative entre les deux groupes (Beaulieu-Lefebvre *et al.*, 2011; Luers *et al.*, 2014; Schwenn *et al.*, 2002; Sorokowska, 2016).

Les résultats des deux études dans lesquelles la mémoire olfactive a été testée concordent : les aveugles ne sont pas meilleurs que les voyants dans cette tâche (Cornell Karnekull *et al.*, 2016; Sorokowska & Karwowski, 2017).

Différents résultats ont été obtenus dans les deux formes de la tâche d'identification. En effet, dans le cas de l'identification à choix multiples, l'ensemble des études s'accordent à dire que la cécité n'améliore pas la performance (Beaulieu-Lefebvre *et al.*, 2011; Comoglu *et al.*, 2015; Cuevas *et al.*, 2010; Gagnon *et al.*, 2015; Luers *et al.*, 2014; Rosenbluth *et al.*, 2000; Schwenn *et al.*, 2002; Smith *et al.*, 1993; Sorokowska, 2016). Il a même été rapporté dans une étude que les voyants étaient meilleurs que les aveugles (Sorokowska & Karwowski, 2017). Cependant, dans le cas de l'identification libre, une grande majorité des études a montré que les aveugles avaient plus de facilités que les voyants (Cuevas *et al.*, 2009; Gagnon *et al.*, 2015; Murphy *et al.*, 1986; Renier *et al.*, 2013; Rombaux *et al.*, 2010; Rosenbluth *et al.*, 2000; Wakefield *et al.*, 2004). Seule une équipe n'a pas obtenu de résultats significatifs (Sorokowska, 2016; Sorokowska & Karwowski, 2017).

Bien que tous les résultats ne concordent pas, la fonction olfactive semble plus performante chez les aveugles dans le cadre de certaines tâches : les aveugles ne semblent pas être meilleurs que les voyants dans les tâches d'identification à choix multiples ou de mémoire olfactive, et les résultats sont très partagés quant à leurs capacités de détection et de discrimination, mais leur capacité à identifier une odeur en ne disposant d'aucun indice sémantique est supérieure. Il a également été observé que leur temps de réponse était plus court, suggérant qu'ils nécessitent moins de temps pour analyser l'information olfactive (Cuevas *et al.*, 2009; Gagnon *et al.*, 2015).

Dans d'autres études, des tests autres que les Sniffin' Sticks ou l'UPSIT ont été utilisés. Les aveugles ont par exemple été testés sur leur capacité à catégoriser des odeurs de vins, et les résultats ont montré qu'ils n'étaient pas meilleurs que les voyants (Manescu *et al.*, 2018). L'article relatif à cette étude est présenté en Annexe 2. Dans une autre étude, des échantillons de sueur ont été obtenus de donneurs qui regardaient des films qui provoquaient l'amusement, le dégoût, la peur, ou l'excitation sexuelle. Les participants sentaient les différents échantillons et devaient identifier les émotions qui y étaient associées. La capacité des aveugles congénitaux à identifier la peur était significativement meilleure que celle des voyants, et une tendance allant dans le même sens a été

observée pour le dégoût. Ces résultats suggèrent que les aveugles peuvent identifier, à partir d'odeurs, des émotions ayant une importante valeur écologique plus efficacement que les voyants (Iversen *et al.*, 2015).

Le cerveau des aveugles

L'activation cérébrale a été observée lors d'une simple tâche olfactive dans laquelle les participants avaient simplement à indiquer s'ils avaient perçu l'odeur qui leur était envoyée. Chez les aveugles, les activations du cortex orbitofrontal droit, du cortex entorhinal, de l'hippocampe, du thalamus dorsal médian, et également du cortex occipital, étaient plus fortes que chez les voyants. Aucune région n'a montré une activation plus forte chez les voyants que chez les aveugles. Les principales différences sont donc observées dans les aires olfactives de haut niveau et les aires visuelles, qui sont plus fortement activées en réponse à un stimulus olfactif chez les aveugles (Kupers *et al.*, 2011).

Autre que le cortex occipital, lors de tâches de catégorisation et de discrimination, il a été montré que, chez les aveugles, le gyrus fusiforme droit était activé. De plus, son activation était corrélée à la performance olfactive. Le gyrus fusiforme est normalement impliqué dans la voie ventrale du système visuel. Cette observation montre que la voie ventrale n'a pas besoin d'afférence visuelle pour développer son rôle dans le traitement de l'identité du stimulus, et qu'en absence de stimulus visuel, elle est recrutée pour traiter des stimuli de nature différente. Il est possible que son rôle soit conservé, et que son activation chez les aveugles soit donc à l'origine de leur meilleure capacité à identifier les odeurs (Renier *et al.*, 2013).

En plus de la neuroimagerie fonctionnelle, certaines études se sont intéressées à la neuroanatomie chez l'aveugle. La principale observation faite au sujet du système olfactif concerne le bulbe olfactif qui a, chez les aveugles, un volume plus important (Rombaux *et al.*, 2010), ce qui a été rapporté à plusieurs reprises comme étant, de manière générale, corrélé à une meilleure performance olfactive (Buschhuter *et al.*, 2008; Royet *et al.*, 2013; Seubert *et al.*, 2013). Cette augmentation du volume du bulbe olfactif serait la conséquence directe d'une utilisation plus importante de l'odorat (Araneda *et al.*, 2016).

Le processus permettant aux aires visuelles d'être activées chez les aveugles par des stimuli non-visuels est la plasticité intermodale : le cerveau s'adapte et les réseaux se réorganisent, permettant à une région cérébrale d'acquérir une nouvelle fonction à la suite de la perte d'un sens (Frasnelli *et*

al., 2011). Ainsi, chez les aveugles, le cortex visuel est recruté dans diverses fonctions non-visuelles telles que l'olfaction, l'audition et le toucher.

Plasticité liée à l'entraînement dans le système olfactif

L'entraînement olfactif chez les normosmiques

L'entraînement olfactif pour améliorer ses capacités olfactives

Bien que la majorité des facteurs influençant les capacités olfactives soient hors de notre contrôle, l'odorat peut être travaillé : l'entraînement olfactif, qui consiste à répéter un exercice olfactif de manière régulière tel que sentir un lot d'odeurs chaque jour, peut permettre d'améliorer l'odorat. Une des premières mises en évidence date d'il y a plus de 30 ans, lorsqu'une équipe a observé en testant la sensibilité au benzaldéhyde trois fois de suite que la sensibilité à l'odeur s'améliorait entre le premier et le troisième test (Rabin *et al.*, 1986). Des résultats similaires ont été observés dans une étude lors de laquelle la sensibilité au benzaldéhyde a été testée 30 fois avec un intervalle de deux jours entre chaque test : la sensibilité au benzaldéhyde s'est améliorée. Cet effet était cependant spécifique à l'odeur utilisée lors de l'entraînement car la sensibilité à d'autres odeurs n'était pas affinée (Dalton *et al.*, 2002). Une étude menée chez les enfants a montré que sentir quatre odeurs de manière quotidienne pendant trois mois permettait d'augmenter la sensibilité et d'améliorer la capacité à identifier ces quatre odeurs (Mori *et al.*, 2015). Dans une autre étude, des participants adultes ont suivi un entraînement olfactif de six semaines réalisé directement au laboratoire et constitué de différentes tâches olfactives. À la fin de cet entraînement, les participants avaient de meilleures capacités olfactives, surtout pour l'identification d'odeurs (Al Ain *et al.*, 2019). L'article rapportant les résultats de cette étude est présenté en Annexe 1.

S'entraîner à sentir une odeur en particulier peut permettre de devenir sensible à une odeur qui ne pouvait pas auparavant pas être sentie. L'anosmie spécifique est un phénomène non pathologique qui se caractérise par l'insensibilité à une certaine odeur. La plus connue est l'anosmie à l'androsténone, une phéromone chez le porc qui est également présente dans l'urine et la sueur chez l'humain (Araneda *et al.*, 2004). La perception de cette odeur est génétiquement déterminée (Wysocki *et al.*, 1984). Tandis que la perception de la majorité des odeurs dépend de divers récepteurs olfactifs, l'androsténone a une affinité spécifique pour le récepteur olfactif OR7D4 codé par un unique gène dont la variabilité définit la perception de l'odeur : en fonction du génotype,

l'odeur peut être perçue comme intense et désagréable (identifiée comme une odeur d'urine ou de sueur), peu intense et plutôt agréable (alors décrite comme florale), ou peut ne pas être perçue du tout (Araneda *et al.*, 2004; Keller *et al.*, 2007). Cependant, bien que la perception de cette odeur soit initialement génétiquement déterminée, environ la moitié de ceux qui ne perçoivent pas cette odeur peuvent y être sensibilisés à la suite d'une exposition répétée (Moller *et al.*, 1999; Wang *et al.*, 2004; Wysocki *et al.*, 1989). L'anosmie spécifique ne se limite pas à l'androsténone. Plus récemment, dans une étude où 20 odeurs étaient utilisées, il a été rapporté qu'un quart des participants étaient insensibles à au moins une des odeurs, et qu'une exposition répétée à ces odeurs sur une durée de trois mois menait à une augmentation de la sensibilité à ces odeurs qui, au début, n'étaient pas perçues (Croy *et al.*, 2015).

Effets de l'entraînement olfactif sur le cerveau chez les normosmiques

Dans la première partie de l'introduction sur l'olfaction, il a été montré que l'entraînement olfactif pouvait mener à une amélioration de la performance olfactive. Cette amélioration s'accompagne de changements dans le cerveau. En effet, l'entraînement olfactif peut mener à des modifications structurales. Sentir quotidiennement quatre odeurs pendant quatre mois mène par exemple à une augmentation du volume du bulbe olfactif (Negoias *et al.*, 2017). Dans l'étude précédemment mentionnée lors de laquelle des participants ont suivi un entraînement olfactif de six semaines réalisé directement au laboratoire, une amélioration des capacités olfactives a été notée, notamment pour l'identification d'odeurs, et il a été observé que le cortex de plusieurs régions s'était épaissi, par exemple au niveau de la partie triangulaire du gyrus frontal inférieur, généralement rapporté comme étant une zone active après une stimulation olfactive (Al Ain *et al.*, 2019).

L'entraînement olfactif dans le cas d'un trouble olfactif

L'entraînement olfactif comme piste thérapeutique

L'entraînement olfactif s'est révélé être si efficace pour améliorer les capacités olfactives qu'il est considéré comme piste thérapeutique pour les patients hyposmiques. En effet, plusieurs études ont rapporté les effets d'entraînements olfactifs réalisés chez des patients qui ont perdu leur odorat à la suite d'une maladie, une infection ou un accident. L'entraînement consistait à sentir quatre odeurs chaque jour pendant trois à huit mois. Les patients ont été testés au début et à la fin de l'entraînement, non seulement sur la sensibilité aux quatre odeurs utilisées, mais sur la performance olfactive en général grâce aux Sniffin' Sticks. Au cours de l'entraînement olfactif, la sensibilité

aux quatre odeurs utilisées s'est améliorée mais l'effet n'était pas généralisé au PEA (Haehner *et al.*, 2013; Hummel *et al.*, 2009). Cependant, une amélioration significative a été notée dans les tests de discrimination et d'identification (Altundag *et al.*, 2015; Damm *et al.*, 2014; Fleiner *et al.*, 2012; Geissler *et al.*, 2014; Haehner *et al.*, 2013; Hummel *et al.*, 2009; Konstantinidis *et al.*, 2013). Cela fait de l'entraînement olfactif une piste prometteuse pour les patients hyposmiques car sentir quelques odeurs de manière quotidienne ne permettrait pas seulement d'augmenter la sensibilité à ces odeurs-là, mais également d'améliorer l'attention et le traitement cognitif des odeurs en général (Haehner *et al.*, 2013).

Effets de l'entraînement olfactif sur le cerveau chez les patients

Peu de résultats sont disponibles au sujet des effets d'un entraînement olfactif sur le cerveau chez les patients atteints de troubles olfactifs.

Au niveau du bulbe olfactif (BO), tout comme chez les normosmiques, des corrélations positives ont été observées chez les patients entre volume de cette structure et capacités olfactives (Mueller *et al.*, 2005; Rombaux *et al.*, 2006a). Ainsi, dans le cas de troubles olfactifs post-viraux et posttraumatiques, le BO des anosmiques est plus petit que le BO des hyposmiques (Rombaux *et al.*, 2006a, 2006b). Lors d'un entraînement olfactif ou d'une guérison spontanée, l'amélioration de la performance olfactive s'accompagne d'une augmentation du volume du BO (Gudziol *et al.*, 2009; Haehner *et al.*, 2008).

Au niveau de la structure corticale, plusieurs études s'accordent sur le fait que les troubles olfactifs s'accompagnent d'une diminution du volume de matière grise dans des aires associées à l'olfaction telles que le cortex piriforme, le cortex orbitofrontal, le cortex cingulaire antérieur et le cortex insulaire (Bitter, Bruderle, *et al.*, 2010; Bitter, Gudziol, *et al.*, 2010; Peng *et al.*, 2013; Yao *et al.*, 2014). Aucun résultat quant aux effets de l'entraînement olfactif sur la structure du cortex chez les patients n'a été rapporté.

Au niveau fonctionnel, il a été montré que l'anosmie entraîne des changements : l'activité cérébrale est plus faible dans certaines régions tandis qu'elle est plus forte dans d'autres (Iannilli *et al.*, 2011). Les activations plus fortes découleraient de mécanismes de compensation : plus d'efforts sont requis pour détecter et analyser l'odeur (Reichert *et al.*, 2018). Une équipe s'est intéressée aux effets de l'entraînement olfactif et a observé, après entraînement, une augmentation du nombre de connexions fonctionnelles, permettant ainsi une meilleure connectivité entre les différentes régions

(Kollndorfer *et al.*, 2015). Cette équipe a également observé que, avant entraînement, un nombre excessif de connexions reliaient le cortex piriforme à des régions non-olfactives et que ces connexions excessives ont disparu après entraînement, ce qui suggère que l'entraînement a mené à une réorganisation fonctionnelle qui a permis d'éliminer les connexions aberrantes (Kollndorfer *et al.*, 2014).

L'entraînement olfactif mène éventuellement à l'expertise

Des experts de l'olfaction

L'olfaction est au cœur du métier de certains. En mobilisant constamment leur odorat, sommeliers et parfumeurs ont un nez expert des odeurs.

Les sommeliers sont des experts du vin. Pour eux, l'odorat est primordial car leur réussite repose sur l'acuité de leurs sens. Ils savent percevoir dans un vin de nombreuses nuances et en déduire ses propriétés. Ils arrivent à percevoir des odeurs relatives au vin à de plus faibles concentrations et savent distinguer des odeurs similaires plus facilement que les novices lorsque celles-ci sont liées au vin (Majid *et al.*, 2017). Leur capacité à identifier des odeurs est également supérieure, bien que cela ne soit pas généralisé à toutes les odeurs (Bende *et al.*, 1997). En plus de développer leur odorat et leur goût, les sommeliers accumulent au cours de leur formation de riches connaissances sur tout ce qui a trait au vin et aux vignobles du monde entier et, lors de l'analyse d'un vin, font appel à de nombreuses fonctions telles que la mémoire et d'autres sens comme par exemple la vue (Banks *et al.*, 2016).

Les parfumeurs sont également des experts de l'olfaction. Leur profession les mène à développer une capacité olfactive peu commune : celle d'imaginer une odeur. Pour les non-experts, il est difficile de se représenter mentalement une odeur et non pas la représentation visuelle d'une odeur : il est par exemple beaucoup plus facile d'imaginer l'image d'une fraise que d'imaginer son odeur. Les parfumeurs peuvent se représenter mentalement des odeurs et parviennent ainsi à créer des parfums (Plailly *et al.*, 2012).

Le cerveau des sommeliers

En sentant et dégustant de nombreux vins, les sommeliers mobilisent constamment leur odorat. Leur nez est expert des odeurs et cela s'accompagne, tout comme chez les parfumeurs (Delon-Martin *et al.*, 2013; Plailly *et al.*, 2012), de modifications dans le cerveau. En effet, des études de

neuroimagerie ont été menées chez les sommeliers, comparant ces experts de l'olfaction à des novices en la matière, et des différences ont été observées.

Au niveau structural, certaines régions olfactives sont plus volumineuses chez les sommeliers. C'est le cas pour le cortex piriforme, le cortex entorhinal, l'insula, ainsi que la région qui entoure le sillon olfactif. L'épaisseur du cortex augmente avec le nombre d'années d'expertise, alors que chez les novices, le cortex s'amincit avec l'âge (Banks *et al.*, 2016; Royet *et al.*, 2013).

Au niveau fonctionnel, les sommeliers réagissent plus vite aux odeurs de vin : une activation plus précoce du cortex olfactif primaire permet une analyse plus rapide de la familiarité et de l'agréabilité de l'odeur (Pazart *et al.*, 2014). De plus, les connexions entre les différentes régions olfactives sont renforcées chez les sommeliers, rendant ainsi le traitement de l'information olfactive plus efficace, ce qui leur permet par exemple d'identifier une odeur plus rapidement. L'activation est plus rapide et les connexions sont renforcées, mais l'activation de ces régions est cependant moins intense car, avec l'expérience, l'analyse de l'odeur requiert moins d'efforts (Royet *et al.*, 2013). En plus de ces différences visibles au sein du réseau olfactif, d'autres régions telles que le cortex préfrontal, le précunéus, le noyau caudé et le putamen sont considérablement mobilisées chez les sommeliers. Ces régions sont connues pour être impliquées dans des processus cognitifs de haut niveau comme la mémoire de travail, l'attention et l'imagerie mentale (Castriota-Scanderbeg *et al.*, 2005; Sreenivasan *et al.*, 2017). La mobilisation de fonctions de plus haut niveau permet d'approfondir l'analyse de l'odeur, fournissant ainsi aux sommeliers plus d'informations sur l'odeur perçue.

Neuroimagerie

Pouvoir observer le cerveau *in vivo*

Le développement de techniques de neuroimagerie a constitué une avancée spectaculaire dans les différents domaines qui s'intéressent au cerveau, que ce soit dans la recherche pour en comprendre sa structure et sa fonction, ou en clinique pour détecter des anomalies. Tandis qu'avant, les analyses post-mortem constituaient le principal moyen d'examiner le cerveau, la neuroimagerie a permis dans les dernières décennies une étude *in vivo* de celui-ci, donnant accès à des informations sur sa structure et sa fonction lorsqu'il est actif. La neuroimagerie donne par exemple la possibilité d'attribuer un rôle à une structure cérébrale en mettant en lien des données comportementales avec

une lésion observée, ou en analysant en temps réel les activations cérébrales qui ont lieu lorsque le participant effectue une certaine tâche.

La neuroimagerie permet également d'étudier la plasticité cérébrale : les changements résultant de la plasticité cérébrale peuvent être détectés grâce à la neuroimagerie, et mis en relation avec des données comportementales.

L'imagerie par résonance magnétique

Le but de l'imagerie par résonance magnétique (IRM) est d'obtenir une image tridimensionnelle du cerveau en générant un contraste entre les différents tissus qui le composent, permettant ainsi de différencier matière grise, matière blanche, et liquide céphalorachidien (LCR). Pour cela, le participant est placé dans un champ magnétique intense qui va être à l'origine du signal mesuré.

L'IRM repose sur le principe de la résonance magnétique nucléaire et s'appuie sur les propriétés physiques de l'atome d'hydrogène, dont le noyau est constitué d'un unique proton. Ce proton, chargé positivement et tournant sur lui-même, possède un moment magnétique de spin, c'est-à-dire qu'il agit tel un aimant et génère un faible champ magnétique. En temps normal, dans un tissu, les spins sont orientés de manière aléatoire, de telle sorte que l'aimantation résultante du tissu est nulle. Cependant, dans le cas de l'IRM, le champ magnétique appelé B0 mène les spins de tous les noyaux d'hydrogène à s'aligner dans la direction de ce champ. Lors de l'application d'un champ B1 perpendiculaire à B0, les spins vont basculer puis, lors de son interruption, les spins vont revenir à leur position de départ : c'est la relaxation. Le temps de relaxation – et donc l'aimantation résultante à un temps donné – dépend de la nature des tissus, et plus précisément de la quantité de noyaux d'hydrogène et donc de leur contenu en eau. C'est ainsi qu'en mesurant l'aimantation résultante en chaque point, une image tridimensionnelle du cerveau représentant les différents tissus peut être reconstituée (Hendee *et al.*, 1984).

En fonction des paramètres utilisés, différentes images peuvent être obtenues. Ainsi, une pondération dite en T1 favorise la visualisation de tissus tels que la matière grise et la matière blanche ; c'est la pondération souvent utilisée en IRM structurale. Une pondération en T2 ou T2*, quant à elle, permet de visualiser la magnétisation de l'hémoglobine et le signal BOLD (Blood Oxygen Level Dependent) ; c'est la pondération généralement utilisée en IRM fonctionnelle.

L'IRM permet donc d'obtenir, de manière non invasive, une image tridimensionnelle du cerveau avec une bonne résolution spatiale de l'ordre du millimètre.

Une fois l'acquisition faite, les données doivent être analysées. Ce processus requiert généralement un prétraitement afin que les images obtenues pour chaque participant puissent être comparées entre elles, puis une analyse statistique est effectuée.

Les études de neuroimagerie peuvent avoir des objectifs variés et viser à examiner différents aspects du cerveau : la structure ou la fonction, la matière grise ou la matière blanche ou l'ensemble du cerveau. Nous décrivons ici trois approches d'IRM communément utilisées : la mesure de l'épaisseur corticale qui permet de mettre en évidence des variations qui ont lieu au niveau du cortex, l'IRM de diffusion qui procure des informations sur la matière blanche, et l'IRM fonctionnelle qui apporte des données sur la fonction du cerveau.

IRM structurale et mesure de l'épaisseur corticale

L'IRM structurale permet entre autres la mesure de l'épaisseur corticale : à partir de l'image anatomique du cerveau, la distance entre les surfaces de matière blanche et de matière grise est déterminée, permettant ainsi de mesurer l'épaisseur du cortex à tout point du cerveau. Dans le cadre de la plasticité, différents mécanismes peuvent entraîner une évolution de l'épaisseur corticale : synaptogenèse, neurogenèse, pousse axonale, changements dans le nombre et la morphologie des cellules gliales, développement du système vasculaire (Zatorre *et al.*, 2012).

Le prétraitement des images avant analyse se fait en plusieurs étapes.

La normalisation spatiale permet qu'un voxel spécifique corresponde à une même localisation anatomique chez l'ensemble des participants. Parce qu'il existe une grande variabilité interindividuelle dans la morphologie cérébrale, les images obtenues doivent être modifiées pour ramener le cerveau à un espace anatomique standard. Pour cela, une transformation qui inclut translation, rotation, mise à l'échelle, et cisaillement, chacun étant réalisé sur les trois axes x, y et z, donc dépendant d'un total de 12 paramètres, est effectuée. Cette étape rend possible la comparaison des cerveaux des différents participants.

La segmentation permet une classification des tissus : parce que les différents tissus ont différentes propriétés, en fonction de l'intensité du signal, il est possible de savoir quel tissu se trouve à chaque voxel.

Après la segmentation, des modèles déformables sont utilisés pour créer, pour chaque participant, quatre surfaces : la surface de matière blanche (SB, entre matière blanche et cortex) et la surface de matière grise (SG, entre matière grise et liquide céphalo-rachidien) de chaque hémisphère. Ces surfaces ne sont pas constituées de voxels mais de vertex qui sont chacun caractérisés par des coordonnées X, Y, Z. Chaque surface est constituée d'environ 140 000 vertex. Les vertex de la SB et de la SG ont la même identité : à chaque vertex de la SB correspond un vertex de la SG. L'épaisseur corticale, qui correspond ainsi à la distance entre vertex de la SB et vertex de la SG correspondant, est déterminée au niveau de chaque vertex (http://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferWiki).

Le lissage moyenne la valeur associée à chaque vertex en fonction des vertex qui l'entourent. Cette étape a plusieurs intérêts. Premièrement, elle permet de normaliser les données, ce qui est nécessaire pour les tests paramétriques qui seront effectués lors de l'analyse statistique. Deuxièmement, elle permet de réduire les différences interindividuelles résiduelles. Enfin, le bruit est réduit.

L'analyse statistique constitue la dernière étape. Le même test (test t) est effectué à chaque vertex simultanément, l'objectif étant de voir s'il existe une différence significative entre les deux groupes au niveau de chaque vertex. Cette étape finale permet d'obtenir une carte du cerveau où sont mises en évidence les régions qui présentent des différences significatives au niveau de l'épaisseur corticale (Bermudez *et al.*, 2009; Frasnelli *et al.*, 2010; Frasnelli *et al.*, 2013; Kurth *et al.*, 2015).

IRM de diffusion et étude de la matière blanche

L'IRM de diffusion est une approche permettant l'étude de la connectivité structurale : l'image obtenue représente les fibres de matière blanche dans l'ensemble du cerveau. L'IRM de diffusion permet la mesure de l'anisotropie fractionnelle qui, dans le cadre de la plasticité, peut mettre en évidence des changements dans le nombre et le diamètre des axones, dans la densité des fibres nerveuses, dans la quantité de myéline, dans le nombre et la morphologie des cellules gliales ou encore dans le système vasculaire (Zatorre *et al.*, 2012).

Les fibres de matière blanche sont organisées en faisceaux qui forment un réseau au sein du cerveau. À l'intérieur d'un faisceau, la diffusion de l'eau est dépendante de la direction des fibres : tandis que dans les ventricules, les fluides circulent librement, la diffusion est beaucoup plus

restreinte au sein d'un faisceau, car myéline et axones constituent des barrières qui contraignent l'eau à se déplacer le long de la fibre. C'est sur ce principe que repose l'IRM de diffusion : le signal est sensibilisé à la diffusion des molécules d'eau. De multiples images du cerveau sont acquises avec, à chaque fois, une sensibilisation du signal à la diffusion dans une certaine direction. De multiples mesures sont donc obtenues pour chaque voxel. Un modèle peut alors être appliqué. Le modèle le plus courant est celui du tenseur de diffusion, où chaque voxel est représenté par une ellipsoïde dont les caractéristiques permettent d'estimer des paramètres tels que l'anisotropie fractionnelle. En fonction des contraintes de diffusion, l'ellipsoïde est plus ou moins sphérique : pour un voxel dans un milieu tel que l'intérieur d'un ventricule, par exemple, l'ellipsoïde sera plutôt sphérique car l'eau peut diffuser dans n'importe quelle direction, et l'anisotropie fractionnelle sera alors proche de 0. Pour un voxel situé à l'intérieur d'un faisceau de fibres, l'ellipsoïde sera plus allongée, décrivant les contraintes auxquelles la diffusion de l'eau est soumise, et l'anisotropie fractionnelle sera plus proche de 1.

Puisque l'anisotropie fractionnelle qui reflète les contraintes de diffusion est quantifiée pour chaque voxel, la localisation des faisceaux de fibres peut être déterminée. Ainsi, à partir de ces données, la tractographie permet de tracer les trajectoires suivies par les fibres de matière blanche et d'obtenir une carte tridimensionnelle représentant la connectivité structurale dans le cerveau (Johansen-Berg *et al.*, 2009; Zatorre *et al.*, 2012).

IRM fonctionnelle et activité cérébrale

L'IRM fonctionnelle (IRMf) permet de visualiser quelles régions cérébrales sont activées lors d'une certaine tâche. Dans le cadre de la plasticité, l'activation répétée d'une certaine région lors d'un entraînement peut par exemple stimuler des changements du système vasculaire afin d'optimiser l'oxygénation de cette région, ou encore des régions qui n'étaient pas activées avant l'entraînement peuvent être recrutées et inversement. Tous ces changements entraînent une variation du signal BOLD et peuvent donc être détectés avec l'IRMf.

Lorsqu'une région cérébrale est activée par une tâche, la consommation d'oxygène y augmente. L'oxyhémoglobine est alors transformée en désoxyhémoglobine. L'augmentation locale du débit sanguin permet un apport d'oxyhémoglobine. Cet apport fait plus que compenser la consommation d'oxygène, ce qui entraîne une diminution de la proportion de désoxyhémoglobine. Or, la

désoxyhémoglobine est paramagnétique : une diminution de sa concentration locale entraîne une augmentation du signal BOLD, qui peut être visualisé avec une pondération en T2*.

La séquence IRM utilisée est l'EPI (Echo-Planar Imaging), qui permet l'acquisition d'une image de faible résolution spatiale en environ 100 ms, ce qui permet d'obtenir l'acquisition des images du cerveau complet en 2 à 3 secondes. Le prétraitement inclut correction de la distorsion des images due aux inhomogénéités du champ magnétique, recalage de l'image anatomique sur l'image fonctionnelle moyenne, normalisation spatiale, segmentation, et lissage. Lors de l'analyse statistique, des contrastes sont utilisés pour comparer l'activation du cerveau dans différentes conditions, notamment lors de l'exécution d'une tâche et au repos, ce qui permet d'observer l'activation spécifique à la tâche effectuée. Ces contrastes permettent également de comparer deux groupes de participants (Gosseries *et al.*, 2008; Hertz-Pannier *et al.*, 2000).

Forces et faiblesses des différentes approches

Mesure de l'épaisseur corticale

La mesure de l'épaisseur corticale est spécifique à la matière grise. Elle est plus facilement interprétable d'un point de vue biologique que les mesures réalisées avec d'autres approches telles que la VBM (Voxel-Based Morphometry) qui peuvent être refléter à la fois des différences de taille, de position et de morphologie. Si sa spécificité peut être une force, elle peut également constituer une faiblesse car un processus ayant lieu dans la matière blanche, ou ayant lieu dans la matière grise mais ne modifiant pas l'épaisseur corticale, ne sera pas détecté, d'où l'intérêt de coupler cette approche à d'autres approches de neuroimagerie (Bermudez *et al.*, 2009).

Mesure de l'anisotropie fractionnelle

L'IRM de diffusion permet d'étudier la matière blanche. Puisque les réseaux fonctionnels s'appuient sur la connectivité structurale, avoir des données sur la provenance des informations qui parviennent à une région cérébrale et sur la destination des informations qui en sortent permet d'émettre des hypothèses sur la fonction de cette région. L'IRM de diffusion présente cependant quelques limites. Premièrement, l'anisotropie fractionnelle peut être modulée par différents facteurs ; à partir de cette mesure, il est difficile de tirer des conclusions sur les mécanismes sous-jacents avec certitude. L'interprétation biologique des résultats doit donc être réalisée avec précaution. Deuxièmement, l'anisotropie fractionnelle n'est qu'un paramètre estimé à partir d'un

modèle ; les images obtenues ne sont donc qu'une estimation de la trajectoire des fibres, ce qui peut donner lieu à des faux positifs et des faux négatifs. Enfin, certaines informations ne peuvent être obtenues avec l'IRM de diffusion. Les données se limitent à la matière blanche. La connectivité structurale issue d'estimations ne permet par exemple pas de conclure sur l'existence de synapses et donc d'une connectivité fonctionnelle entre deux régions du cerveau. Il est également impossible de déterminer si une connexion est antérograde ou rétrograde, ou d'observer la connectivité à l'échelle d'un axone individuel (Johansen-Berg *et al.*, 2009).

IRM fonctionnelle

Tandis que mesures de l'épaisseur corticale et de l'anisotropie fractionnelle nous renseignent sur l'anatomie du cerveau, l'IRMf nous renseigne sur son fonctionnement en nous permettant, grâce à sa haute résolution temporelle, d'observer quelles régions cérébrales sont activées au cours d'une tâche. Ainsi, il est possible d'établir des liens entre structure et fonction, et de définir les substrats anatomiques d'une fonction donnée. Tout comme toute autre approche, quelques limites sont associées à l'IRMf. Tout d'abord, il s'agit d'une mesure indirecte : ce n'est pas l'activation cérébrale qui est mesurée, mais le métabolisme qui en résulte. De plus, la résolution temporelle est améliorée au détriment de la résolution spatiale. Cependant, l'image fonctionnelle est alignée à une image anatomique de haute résolution, ce qui réduit donc l'impact de cette faiblesse. Un autre élément pouvant être considéré comme une limite est le fait qu'être allongé dans un scanner IRM réduit les possibilités de tâches qui peuvent être effectuées.

Combiner les approches

Ces approches se trouvent être complémentaires car, en les combinant, elles permettent d'étudier à la fois la structure du cortex, la structure de la matière blanche, et l'activité cérébrale.

Objectifs et hypothèses de recherche

L'objectif général de cette thèse était de mieux comprendre la capacité du cerveau à s'adapter à un environnement changeant. Plus spécifiquement, nous avons étudié la plasticité du système olfactif chez les spécialistes que sont les sommeliers.

Cette thèse s'est déroulée en plusieurs étapes.

Mise en place d'un outil de mesure

Cette première étude a été réalisée en préparation des études suivantes. Dans une étude ultérieure décrite ci-dessous, nous voulions examiner le lien entre performance olfactive et structure cérébrale en mesurant le volume de bulbes olfactifs gauche et droit séparément. Or, bulbes olfactifs gauche et droit reçoivent l'information issue respectivement des narines gauche et droite, mais peu de données étaient disponibles sur la performance olfactive de deux narines séparément. C'est pourquoi nous avons analysé les données issues de 278 participants avec un odorat fonctionnel et de 180 patients présentant des troubles olfactifs qui ont été testés pour les deux narines séparément avec le test des Sniffin' Sticks.

Cette étude a notamment montré l'intérêt de mesurer le seuil de détection pour les deux narines séparément. Les résultats de cette étude sont présentés dans l'article publié qui constitue le chapitre 2.

Mesure de la performance olfactive en début de formation

Nous avons testé des étudiants en sommellerie, futurs experts de l'olfaction, au début de leur formation.

Les étudiants en sommellerie testés lors de cette étude provenaient de deux établissements différents : le Centre de Formation Professionnelle Bel Avenir de Trois-Rivières, et l'Institut du Tourisme et d'Hôtellerie du Québec (ITHQ) à Montréal. Les étudiants en sommellerie ont été comparés à un groupe contrôle d'étudiants suivant une formation qui n'implique pas l'odorat. Ces étudiants ont été sélectionnés de manière à avoir la même répartition hommes/femmes et la même moyenne d'âge que dans le groupe d'étudiants en sommellerie, et provenaient principalement de l'Université du Québec à Trois-Rivières, de l'Université de Montréal, et de l'Université du Québec à Montréal.

Les capacités olfactives des étudiants ont été mesurées au cours des deux premiers mois de la formation en sommellerie à l'aide du test des Sniffin' Sticks. Cet outil nous a permis de réaliser différents tests :

- mesure du seuil de détection du n-butanol, pour les deux narines séparément
- test de discrimination, en utilisant la version étendue constituée de 32 triplets
- test d'identification en deux parties : identification libre puis identification avec indices
- test de mémoire olfactive

L'hypothèse principale est la suivante : des différences sont déjà visibles entre les deux groupes, en faveur des étudiants en sommellerie qui auraient donc déjà, au cours des deux premiers mois de formation, de meilleures capacités olfactives.

Les résultats de cette étude sont présentés dans l'article publié qui constitue le chapitre 3.

Évaluation des effets de la formation sur l'olfaction et le cerveau

Des études antérieures ont montré que le cerveau des sommeliers présentait des particularités dues à l'expérience tant au niveau structural qu'au niveau fonctionnel. La plupart de ces études sont transversales, réalisées chez des professionnels qui ont déjà acquis leur expertise. D'autres études, réalisées plutôt chez des patients souffrant de troubles olfactifs, montrent que l'entraînement olfactif peut être un moyen efficace d'améliorer les capacités olfactives, une amélioration qui est couplée à des modifications cérébrales. Ces études-là sont généralement longitudinales : les participants sont testés au début et à la fin d'un entraînement olfactif qui consiste souvent à sentir quatre odeurs de manière quotidienne pendant un certain nombre de semaines. Ces études montrent que l'entraînement olfactif peut aider à rétablir un odorat qui est au départ déficient. Dans le cadre de l'expertise, cependant, aucune étude longitudinale n'a été rapportée. Nous avons donc réalisé une étude longitudinale.

La formation des étudiants en sommellerie de l'ITHQ durait un an et demi. Au cours des deux premiers mois, en plus de la mesure de la performance olfactive décrite ci-dessous, les étudiants provenant de l'ITHQ ainsi que les participants contrôles correspondants ont aussi participé à une session d'IRM. Un an et demi plus tard, à la fin de la formation, les étudiants sont revenus participer aux mêmes tests olfactifs et une session d'IRM semblable.

La session d'IRM incluait :

- un scan anatomique de haute résolution, afin de mesurer l'épaisseur du cortex,
- une séquence permettant de visualiser le bulbe olfactif, pour mesurer le volume de cette structure,
- une séquence d'IRM de diffusion, afin de cartographier les fibres de matière blanche qui établissent les différentes connexions dans le cerveau,
- une séquence d'IRM fonctionnelle lors d'une tâche olfactive, pour observer l'activation cérébrale lors du traitement de l'information olfactive : des odeurs de vin blanc, vin rouge et jus leur étaient envoyées aux participants qui devaient déterminer dans une première

phase si l'odeur envoyée était du vin ou du jus, et dans une deuxième phase s'il s'agissait de vin blanc ou de vin rouge.

À partir de ces données, nous avons pu évaluer l'évolution des capacités olfactives et du cerveau au cours de la formation, dans le but d'évaluer les effets de la plasticité dans le cadre d'un entraînement à long terme.

Nos hypothèses principales sont les suivantes :

1. Les capacités olfactives des étudiants en sommellerie s'améliorent au cours de la formation.
2. Une évolution de la structure et de l'activité du cerveau est visible chez les étudiants en sommellerie.
3. Des corrélations sont visibles entre évolutions des capacités olfactives et du cerveau.

Cette étude a donné lieu à différents articles présentés dans les chapitres 4 et 5 ainsi qu'en annexe et traitant des capacités olfactives et du bulbe olfactif (chapitre 4), de l'épaisseur corticale et de l'IRM de diffusion (chapitre 5), et de l'IRM fonctionnelle (annexe 4).

Reproduction d'un entraînement olfactif en conditions expérimentales

Nous avons mis en place dans cette étude une tâche olfactive plus complexe et plus adaptée aux sommeliers que les Sniffin' Sticks qui ont été initialement conçus pour détecter les troubles olfactifs. Cette tâche consiste à identifier des odorants au sein d'un mélange.

Un groupe de participants naïfs dans le domaine de l'olfaction a été entraîné quotidiennement pendant une période de cinq jours sur une tâche de discrimination d'odeurs au sein d'un mélange : les participants devaient déterminer le nombre et identifier les composants de mélanges d'odeurs.

À la fin de cet entraînement, les participants ont été testés sur cette même tâche, et leurs performances ont comparées à celles de sommeliers expérimentés et de participants naïfs non entraînés qui ont testés sur cette même tâche.

Nos hypothèses principales sont les suivantes :

1. Les sommeliers sont plus performants que les deux autres groupes.
2. Un entraînement olfactif de seulement quelques jours est suffisant pour voir une évolution des performances.

Les résultats de cette étude sont présentés dans l'article publié qui constitue le chapitre 6.

Chapitre 2 – Nostril differences in the olfactory performance in health and disease

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Daphnée Poupon[1], Thomas Hummel[2], Antje Haehner[2], Antje Welge-Luessen[3], Johannes Frasnelli[1,4]

Affiliations: 1 Department of Anatomy, Université du Québec à Trois-Rivières, Canada

2 Department of ENT, Technische Universität Dresden, Germany

3 Department of Otorhinolaryngology, University Hospital Basel, Switzerland

4 Research Centre, Sacré Coeur Hospital, Montréal, Canada

Abstract

In the past few decades, several olfactory tests have been developed to assess olfactory performance and detect disorders. Contrary to other sensory systems, both nostrils are usually tested together; we hypothesized that monorhinal testing may reveal side differences in sensitivity which may be useful for the diagnosis of olfactory dysfunction. Using the "Sniffin' Sticks" test, we assessed olfactory function of 458 participants (278 healthy controls, 180 hyposmic patients), one nostril after the other, with three different tasks. For each participant and each task, we compared the scores obtained with both nostrils, and defined the best and worst nostrils. Thus we were able to establish normative data and to define cut-off values. Our results suggest that scores obtained with the worst nostril are the most efficient in detecting an olfactory disorder. This supports the importance of monorhinal testing, as it can allow an earlier and more accurate diagnosis than birhinal testing. This may be especially useful in the context of early detection of neurodegenerative diseases.

Keywords: olfaction, threshold, discrimination, identification, hyposmia.

Introduction

Compared to senses like vision or audition, validated clinical tests to assess olfactory sensitivity were introduced much later. Indeed, the olfactory tests which are now widely accepted such as the Sniffin' Sticks tests or the UPSIT (University of Pennsylvania Smell Identification Test) are only a few decades old (Doty *et al.*, 1984; Kobal *et al.*, 1996).

These tests are commonly used for both clinical and research purposes. Both tests are usually carried out by testing both nostrils together (birhinal testing). For this, normative data – describing the repartition of scores across the population – have been established, and cut-off values have been defined to facilitate the diagnosis of olfactory disorders (Doty *et al.*, 1984; Hummel *et al.*, 2001; Hummel *et al.*, 2007; Kobal *et al.*, 2000). From these and other studies, we further know that, when a gender difference is observed, women typically outperform men, and that olfactory performance improves during childhood and teenage years, and then decreases with aging (Bastos *et al.*, 2015; Doty *et al.*, 2014; Hummel *et al.*, 2001; Hummel *et al.*, 2007; Kobal *et al.*, 2000; Schriever *et al.*, 2014; Stevenson *et al.*, 2007).

In contrast to this, in vision and audition, both sides are usually tested independently, with one eye or ear tested after the other, because both eyes' or ears' sensibility may be independently affected by a condition. Similarly, separate tests for each nostril would make sense because there can be a difference of sensibility also between the nostrils (Gudziol *et al.*, 2007; Gudziol *et al.*, 2010). With birhinal testing however, this difference of sensibility can go unnoticed, since it is the most sensitive nostril's sensibility which determines both nostrils' joint sensibility (Frasnelli *et al.*, 2002). An olfactory loss affecting only one nostril is thus not perceived as long as the olfactory performance of the best nostril remains in the normal range (Gudziol *et al.*, 2010). In fact, over the last decade, side differences in olfactory performance have been reported in different conditions (Gudziol *et al.*, 2007; Gudziol *et al.*, 2010; Stamps *et al.*, 2013; Welge-Lussen *et al.*, 2010), raising the interest in separate testing of both nostrils. However, no normative data are yet available for monorhinal testing.

One particular advantage of monorhinal testing is that it allows to directly compare both nostrils with each other. This may be particularly useful in medical conditions suspected to affect both nostrils differently. Indeed, there is evidence of a lateralization – a functional asymmetry – in olfactory impairment associated with conditions such as Mild Cognitive Impairment,

Alzheimer's and Parkinson's diseases (Huart *et al.*, 2015; Negoias *et al.*, 2016; Stamps *et al.*, 2013; Zucco *et al.*, 2015). Functional asymmetry may be a marker of an early phase of these conditions (Sun *et al.*, 2012; Takeda, 2013) and therefore eventually serve as an instrument for screening and early diagnostics. It is important to note that, in this article, we refer to the functional asymmetry which directly depends on the difference of sensitivity between the nostrils, but we are not looking at the lateralization of cognitive functions (Broman *et al.*, 2001).

Using a widespread smell test (Sniffin' Sticks test), the first objective of this study was to examine the effects of gender and age on the three different olfactory tasks, and to determine the functional asymmetry (inter-nostril difference) between both nostrils. We hypothesized that monorhinal testing would show the same effects of gender and age as birhinal testing, that is to say a better performance in women, and lower scores in children and in older people. Concerning inter-nostril differences, our hypothesis was that the ratio of people showing differences between the nostrils (independent of left or right) would be greater than what would be expected by chance, thus hinting at the existence of functional asymmetry. The second objective of this study was to establish normative data for unilateral application of the three tasks performed with the Sniffin' Sticks test. As a third objective, we compared the performance of healthy participants and patients with different degrees of olfactory dysfunction. Since olfactory dysfunction can start on one side, thus lowering the olfactory abilities of one nostril, our hypothesis was that the scores obtained with the nostril defined as the worst nostril would be more efficient to discriminate between both groups than those obtained with the best nostril.

Materials and methods

This retrospective study was approved by the Ethics Committee of the Medical Faculty of the Technical University of Dresden, Germany, and complies with the Declaration of Helsinki for Medical Research involving Human Subjects.

Participants

Individuals from two distinct groups were included into the study. All of them gave an informed written consent to participate. The first group consisted of 278 healthy participants (138 women and 140 men). They were aged from 6 to 79 years with $M \pm SD = 31.1 \pm 18.2$; we divided them into four age groups (group 1, 6-15 years, $n = 43$; group 2, 16-35 years, $n = 146$; group 3, 36-55

years, n = 52; group 4, >55 years, n = 37). The second group consisted of 180 patients (95 women, 85 men) who consulted an ENT specialist for olfactory dysfunction. Patients were aged from 14 to 80 years, with M ± SD = 51.4 ± 14.6. However, as the majority was older than 35, we did not divide them into age groups. Instead, they were divided into four groups depending on the cause of hyposmia, i.e., sinunasal (N = 26), posttraumatic (N = 72), postinfectious (N = 61), and idiopathic (N = 21), as diagnosed by a specialist (TH, AH, AWL). Please see Table 1 for detailed information.

Table 1. Number of participants in both control and patient groups, repartition in age groups, and number of them to undergo each task (THR = threshold task, DIS = discrimination task, ID = identification task).

		Number			Age				Tests		
		Total	Female	Male	Min	Max	Mean	SD	THR	DIS	ID
Controls	Total	278	138	140	6	79	31.1	18.2	278	210	122
	Age group 1	43	16	27	6	15	11.0	3.1	43	37	43
	Age group 2	146	77	69	16	35	22.5	4.3	146	97	56
	Age group 3	52	28	24	36	55	47.7	5.3	52	42	7
	Age group 4	37	17	20	56	79	65.3	6.2	37	34	16
Patients		180	95	85	14	80	51.4	14.6	180	180	180

Olfactory testing

Olfactory performance was assessed using a monorhinal adaptation of the Sniffin' Sticks test (Hummel *et al.*, 1997). Sniffin' Sticks are felt-tip pens which are filled with odorants instead of ink. The experimenter presents the odorants to the participant by removing the cap and placing the pen's tip approximately 2 cm in front of both nostrils. In this study, we tested the two nostrils separately: the participant closed a given nostril with a finger during each odor presentation, and one nostril was tested after the other. The order of the nostrils was randomized.

We assessed the olfactory performance in (1) odor threshold, (2) odor discrimination, and (3) odor identification, in a random order, using the Sniffin' Sticks sets provided for each of these tests and following established procedures (for more details, see Hummel et al. 1997; Hummel et al. 2007).

In short, we assessed odor thresholds for phenylethyl alcohol (PEA) using a single staircase, three-alternative forced choice procedure: we presented participants with triplets of pens, one of them containing the odorant in a given concentration, the two other ones containing the solvent. Participants had to identify the pen containing the odorant. There were 16 different concentrations available. Triplets were presented starting with the lowest concentration, with a randomized order of the three pens; the concentration was increased upon an incorrect response and decreased when the odor was correctly identified in two successive trials. Threshold was defined as the average of the last four of seven staircase reversals. Scores ranged between 1 and 16.

To assess odor discrimination, 16 triplets of pens (two pens containing the same odorant and a third pen a different one) were presented. Participants had to identify the pen containing the different odorant. Scores ranged between 0 and 16.

In the third task, odor identification was assessed for 16 common odors. For each individual odor, a list of four descriptors was presented; participants had to identify the odorant by picking one of them. Scores ranged from 0 to 16.

Although we tested every patient on each test, it is important to point out that not all healthy participants of our control group underwent all tests (Table 1). All the tests were performed monorhinally: left and right nostrils were tested separately.

We therefore obtained a maximal total of six scores per participant, namely, scores for the right and the left nostrils separately, in the threshold, discrimination, and identification tasks. Those who didn't undergo all tests only had two or four scores. We further calculated, for each test and each participant, the absolute difference between the two nostrils; this difference reflects functional asymmetry. We further determined, for each test, best and worst nostrils. In summary, we obtained five variables for each test, namely (1) the score of the left nostril; (2) the score of the right nostril; (3) the score of the best nostril; (4) the score of the worst nostril; and (5) the absolute difference between both nostrils.

Data analysis

Data were analyzed using the software SPSS 22.0 for Windows. We ascertained normal distribution using the Kolmogorov-Smirnov test. For data with normal distribution, we subsequently used parametric tests; otherwise we applied appropriate non-parametric alternatives.

Post-hoc comparisons were Bonferroni corrected. Unless otherwise stated, results are reported as mean (±standard deviation).

Studying the effects of age, gender and side in controls

For data with normal distribution we computed a repeated-measures ANOVA (after a Levene's test to confirm the equality of variances) followed by pairwise comparisons and independent samples t-tests to examine the main effects of age group and gender, respectively. The variable "side" (right or left nostril) was used as a within-subjects factor to test whether one nostril was outperforming the other.

For data with non-normal distribution, we computed Kruskal-Wallis, Mann-Whitney U, and related samples Wilcoxon tests, to examine the effects of age group, gender, and side, respectively.

Functional asymmetry

We examined the difference between nostrils in each test. To be able to compare these differences between the different tests, we calculated Z-scores for each value and the difference between Z-scores obtained for best and worst nostrils in each test. We performed a Friedman's analysis on those.

We examined functional asymmetry by calculating the number of participants who had better scores in a given nostril for all three tasks. The hypothesis was that this proportion would be greater than the one expected if there was no such a phenomenon as functional asymmetry and it was all due to chance (which is 2*(0.5^3) = 25%, with 0.5^3 being the probability of getting one given nostril better in the three tests, which we multiply by two because it can be either the left or the right nostril). We used a binomial distribution to compare the obtained and expected proportions.

Normative data

We established normative data to describe across the population the scores obtained with the right and left nostrils, the best and worst nostrils, and the difference between nostrils across the population of healthy participants.

Comparing controls and patients

We used independent samples Mann-Whitney U tests to compare olfactory performance of controls and patients for the three tests. Further, we computed Kruskal-Wallis tests to examine the effect of the cause of the olfactory loss.

We also compared the functional asymmetry in the control and patient groups by using a chi-squared test.

We aimed at finding a way to diagnose olfactory losses by defining a cut-off value; a score lower than or equal to this cut-off value would indicate an olfactory loss, while a score higher than this value would indicate normosmia. Every diagnosis comes with an error risk as cut-off values are all associated with false negative (being considered as normosmic when there actually is an olfactory loss) and false positive (diagnosing an olfactory loss when there is not) rates. To assess the discriminative power of the different scores, and to define cut-off values, we used Receiver Operator Characteristic (ROC) curves on all data from participants aged above 16. Our aim was to determine which score was the most effective in discriminating someone affected by an olfactory disorder from someone who is not, that is to say a cut-off value with false negative and false positive rates as low as possible. ROC curves allow to do so, as these curves depict, for each possible score, the true positive rate ("sensitivity") in function of the false positive rate ("1-specificity"). To define the most accurate cut-off value for each test, we calculated, for each value, the Youden index (y = sensitivity + specificity - 1), and picked the value with the greatest Youden index (Bewick *et al.*, 2004; Youden, 1950). From the sensitivity and the specificity, we calculated the number of True Positives (TP), True Negatives (TN), False Positives (FP), and False Negatives (FN). We then determined the Positive Predictive Value (PPV = TP/ (TP + FP), the probability of truly having an olfactory disorder when the test is positive), the Negative Predictive Value (NPV = TN/ (TN + FN), the probability of truly not having an olfactory disorder when the test is negative). From these values, we calculated the accuracy (accuracy = 100*(PPV+NPV)/2) of the selected cut-off values for each of the tests (Florkowski, 2008).

In a ROC curve, the area under the curve (AUC) depicts how efficient the diagnostic tool is: the greater, the better. We used a Kruskal-Wallis test to examine whether the factor "nostril" (best nostril, worst nostril, difference between nostrils) had an effect on the AUC. We then tested the hypothesis that AUCs obtained with the score of the worst nostril were significantly greater than

AUCs obtained with the score of the best nostril by comparing, for each task, the AUCs corresponding to the scores of the best and the worst nostrils (Hanley *et al.*, 1982).

Results

Effects of age group, gender and side in controls

Odor threshold task

We found a main effect of gender and age group in the threshold task (ANOVA: $F(1;270) = 7.19$, $p = 0.008$, and $F(3;270) = 19.12$, $p < 0.001$, respectively). Women outperformed men (

Figure 1A). Age group 1 had the lowest scores (pairwise corrected comparisons age group 1 vs. all other age groups: all $p < 0.001$;

Figure 1B).

We did not observe any interaction between gender and age group ($F(3;270) = 0.17$, $p = 0.92$). There was not any main effect of side ($F(1;270) = 1.35$, $p = 0.25$), suggesting there was not a nostril outperforming the other one in the overall population (

Figure 1C), nor any interaction between side and age group ($F(3;270) = 1.327$, $p = 0.266$) or between side and gender ($F(1;270) = 1.509$, $p = 0.220$).

Odor discrimination task

Scores in the discrimination task were not normally distributed. There was no significant effect of gender in the discrimination task (Mann-Whitney, $p = 0.44$ for the right nostril, $p = 0.55$ for the left nostril).

For both nostrils, there were significant differences between age groups 1 and 2 (for both nostrils, Mann-Whitney, $p < 0.001$), 1 and 3 (Mann-Whitney, $p = 0.012$ for the left nostril, $p < 0.001$ for the right nostril), and 2 and 4 (for both nostrils, Mann-Whitney, $p < 0.001$), with group 1 scoring the lowest and group 2 scoring the highest (

Figure 1B).

There was no significant difference between left and right nostrils (Wilcoxon, $p = 0.21$).

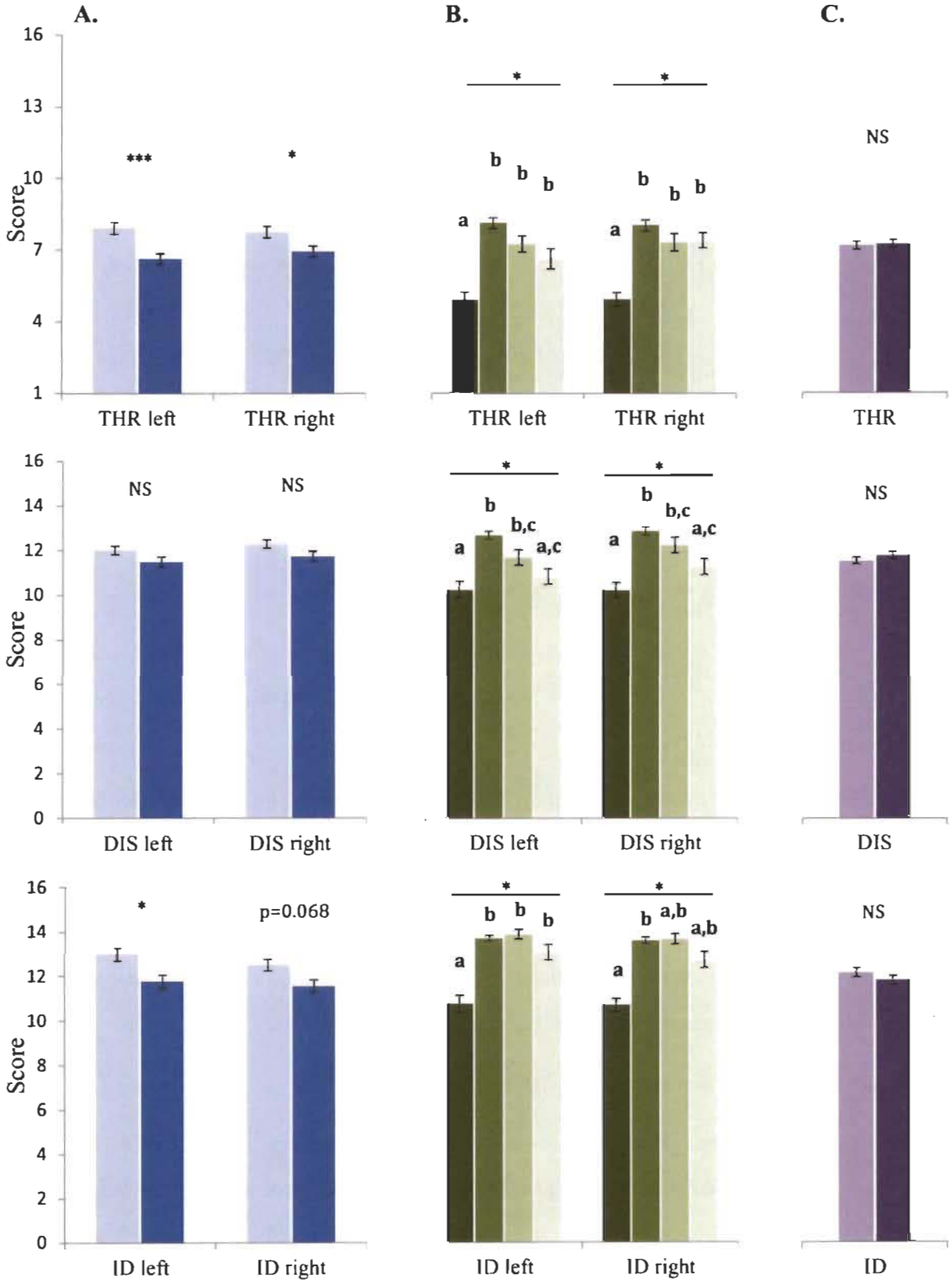
A.
B.
C.
Score
THR left
THR right
THR
DIS left
DIS right
DIS
ID left
ID right
ID
NS
p=0.068

Figure 1. Effects of gender, age and side on olfactory performance in controls.

*A. Women (red bars) performed better in threshold and identification tasks than men (blue bars). The difference was not significant in the discrimination task. B. Children aged 6-15 (dark green) performed lower than participants aged 16-35 (medium dark green), 36-55 (medium light green) and older than 56 (light green). The difference between age groups 2, 3 and 4 was significant only in the discrimination task. C. There was no significant between average scores (error bars: SEM) for left and right nostrils, indicating that there is no better nostril between left (light purple) and right (dark purple). Significant differences in post hoc comparisons between positions are indicated by different letters ($P < 0.05$). Columns indicate average scores, error bars indicate standard errors of mean (SEM). NS=Non Significant, * $p<0.05$, *** $p<0.001$. THR = threshold task, DIS = discrimination task, ID = identification task; left and right indicate nostril.*

Odor identification task

Scores in the identification task were not normally distributed. Women outperformed men with the left nostril (Mann-Whitney, $p = 0.016$). This difference was only a tendency with the right nostril after correction (Mann-Whitney, $p = 0.068$).

Age group 1 had significantly lower scores than all other groups for the left nostril (after Bonferroni adjustment, for the comparisons of age group 1 with age groups 2, 3, and 4 respectively, Mann-Whitney, $p < 0.001$, $p = 0.006$, and $p = 0.042$; comparisons between age groups 2, 3 and 4 were not significant). For the right nostril, age group 1 had significantly lower scores than age group 2 (Mann-Whitney, $p < 0.001$). Scores with the left nostril tended to be better than scores with the right nostril, but the difference was not significant (Wilcoxon, $p = 0.08$).

Difference between nostrils in the different tasks

The average absolute differences between nostrils, which are an indirect measure of functional asymmetry, were 1.59 (±1.58) points, 1.69 (±1.37) and 1.27 (±1.00) points, for threshold, discrimination and identification, respectively (Table 2). The difference between nostrils was not equivalent in all tasks, and the difference between associated Z-scores was significant (Friedman's analysis, $p < 0.001$), with the smallest inter-nostril difference in the identification

task (threshold vs discrimination, Wilcoxon, $p < 0.001$; threshold vs identification, Wilcoxon, $p=0.001$; discrimination vs identification, Wilcoxon, $p = 0.005$).

Functional asymmetry refers to the fact that one nostril is better than the other one; we assumed functional asymmetry if a participant had better scores in a given nostril for all three tasks. Although the probability to find such an asymmetry by chance is smaller than 25%, 36 of 93 (39%) healthy participants tested for all three tasks exhibited such a pattern (binomial, $p = 0.002$).

There was a main effect of age group on functional asymmetry in the threshold task (Kruskal-Wallis, $p = 0.006$), with the smallest difference in age group 1 (after Bonferroni adjustment, for the comparisons of age group 1 with age groups 2, 3, and 4 respectively, Mann-Whitney, $p < 0.001$, $p = 0.036$, and $p = 0.018$; comparisons between age groups 2, 3 and 4 were not significant). For discrimination and identification, we did not observe any significant effect of age group on functional asymmetry, although there was a statistical trend for an effect in the identification task, with age group 2 seemingly presenting a smaller difference between nostrils, but it became not significant after a Bonferroni adjustment (Kruskal-Wallis, $p = 0.060$).

There was no effect of gender on functional asymmetry in any of the three tasks.

Normative data

In Table 2, we present normative data on the scores obtained in the different tasks for the four age groups. Specifically, we highlight the scores obtained with the left, the right, the best, and the worst nostril, and the difference of scores between the two nostrils (functional asymmetry).

Table 2. Olfactory performance across the population in controls.

Descriptive statistics have been used to describe the repartition of the scores in the different group ages. Tasks: THR = threshold, DIS = discrimination, ID = identification. Nostrils: L = left, R = right, B = best, W = worst, Diff = difference between both.

		THR					DIS					ID				
		L	R	B	W	Diff	L	R	B	W	Diff	L	R	B	W	Diff
Age group 1 (6-15 years)																
Mean		4.89	4.92	5.33	4.48	0.84	10.22	10.19	10.92	9.49	1.43	10.74	10.67	11.42	10.00	1.42
Std. Deviation		2.00	1.82	1.93	1.80	0.90	2.23	2.00	2.01	1.97	0.65	2.41	1.90	1.99	2.10	0.76
Minimum		1.50	2.50	2.75	1.50	0.00	7	7	8	7	0	6	7	8	6	0
Maximum		11.25	11.75	11.75	11.25	4.75	15	14	15	14	3	16	15	16	15	3
Percentiles	5	2.50	2.55	2.80	2.50	0.25	7	7	8	7	1	7	8	8	7	0
	10	2.75	2.85	3.25	2.50	0.25	7	7	8	7	1	7	8	8	7	1
	25	3.50	3.75	4.00	3.25	0.25	9	8	9	8	1	9	10	10	8	1
	50	4.50	4.50	5.00	4.25	0.50	10	11	11	9	1	11	11	11	10	1
	75	6.00	6.25	6.25	5.00	0.75	12	12	13	11	2	13	12	13	12	2
	90	7.50	6.90	7.65	6.90	1.65	13	13	13	12	2	14	13	14	13	3
	95	9.80	8.55	9.95	7.90	3.45	14	13	14	13	3	15	14	15	14	3
Age group 2 (16-35 years)																
Mean		8.11	8.01	8.93	7.18	1.75	12.65	12.82	13.56	11.92	1.64	13.23	12.98	13.59	12.63	0.96
Std. Deviation		2.75	2.77	2.79	2.45	1.67	1.73	1.66	1.25	1.68	1.37	1.66	1.76	1.51	1.77	0.81
Minimum		1.00	1.25	2.00	1.00	0.00	8	8	11	8	0	9	7	9	7	0
Maximum		15.75	15.50	15.75	15.50	10.00	16	15	16	15	6	16	16	16	16	3
Percentiles	5	3.50	3.34	4.34	3.00	0.00	9	10	11	9	0	11	10	11	10	0
	10	5.00	4.25	5.50	3.93	0.25	10	11	12	10	0	11	10	12	10	0
	25	6.50	6.25	7.44	5.75	0.50	12	12	13	11	1	12	12	13	11	0
	50	7.75	7.88	8.75	7.25	1.25	13	13	14	12	1	13	13	14	13	1
	75	9.75	9.50	10.50	8.56	2.50	14	14	15	13	2	14	14	15	14	1
	90	11.50	12.08	12.58	10.50	3.83	15	15	15	14	4	16	15	16	14	2
	95	13.15	13.00	13.91	11.25	5.25	15	15	15	15	5	16	15	16	15	3
Age group 3 (36-55 years)																
Mean		7.22	7.29	8.07	6.43	1.64	11.64	12.19	12.74	11.10	1.64	13.86	12.29	13.86	12.29	1.57
Std. Deviation		2.48	2.71	2.41	2.52	1.45	2.22	2.21	1.82	2.29	1.59	1.57	1.70	1.57	1.70	1.51
Minimum		1.00	1.00	1.50	1.00	0.00	4	6	6	4	0	11	10	11	10	0
Maximum		11.50	14.00	14.00	11.50	5.25	14	16	16	14	6	16	15	16	15	4
Percentiles	5	2.31	2.81	3.99	1.65	0.00	5	6	9	5	0	11	10	11	10	0
	10	3.65	3.58	5.00	3.33	0.25	9	10	11	7	0	11	10	11	10	0
	25	5.31	5.63	6.50	4.06	0.50	11	11	12	10	1	13	11	13	11	0
	50	7.63	7.50	8.00	6.50	1.00	12	13	13	12	1	14	13	14	13	1
	75	9.00	9.19	9.50	8.44	2.75	13	14	14	13	2	15	13	15	13	3
	90	10.50	11.03	11.10	9.35	3.85	14	15	15	13	4					
	95	10.75	12.19	12.19	10.59	5.00	14	16	16	14	6					
Age group 4 (>55 years)																
Mean		6.60	7.38	7.89	6.09	1.80	10.79	11.24	12.09	9.94	2.15	12.69	11.88	13.19	11.38	1.81
Std. Deviation		2.54	1.97	2.20	2.04	1.75	2.01	2.05	1.64	1.81	1.60	2.44	2.39	2.04	2.47	1.56
Minimum		2.75	4.25	4.25	2.75	0.00	5	6	7	5	0	7	5	7	5	0
Maximum		14.75	11.50	14.75	11.50	6.25	14	15	15	13	5	15	15	15	15	5
Percentiles	5	3.20	4.48	5.15	3.20	0.00	7	7	9	6	0	7	5	7	5	0
	10	3.50	4.95	5.45	3.50	0.20	8	9	10	8	0	8	9	10	8	0

25	4.63	5.88	6.13	4.50	0.50	10	10	11	9	1	11	11	13	10	1
50	6.25	7.00	7.50	6.00	1.00	11	11	13	10	2	13	12	13	12	2
75	8.00	9.13	9.38	7.38	2.88	12	13	13	11	3	15	13	15	13	3
90	9.75	10.30	10.80	9.50	5.00	13	14	14	12	5	15	15	15	15	5
95	11.83	11.05	11.83	10.38	5.80	14	14	14	12	5					
All age groups															
Mean	7.24	7.31	8.07	6.48	1.59	11.72	11.98	12.69	11.00	1.69	12.32	11.98	12.79	11.52	1.27
Std. Deviation	2.80	2.75	2.81	2.50	1.58	2.18	2.14	1.86	2.12	1.37	2.36	2.14	2.03	2.30	1.00
Minimum	1.00	1.00	1.50	1.00	0.00	4	6	6	4	0	6	5	7	5	0
Maximum	15.75	15.50	15.75	15.50	10.00	16	16	16	15	6	16	16	16	16	5
Percentiles 5	3.00	3.24	3.50	2.74	0.00	8	8	9	7	0	8	8	9	7	0
10	3.50	3.75	4.48	3.25	0.25	9	9	10	8	0	9	9	10	8	0
25	5.25	5.50	6.19	4.50	0.50	10	11	12	10	1	11	10	11	10	1
50	7.25	7.25	8.00	6.50	1.00	12	12	13	11	1	13	12	13	12	1
75	9.00	9.25	9.75	8.06	2.25	13	14	14	13	2	14	14	14	13	2
90	10.75	10.78	11.75	9.50	3.75	14	15	15	13	4	15	15	15	14	3
95	11.53	12.50	13.01	10.76	5.00	15	15	15	14	5	16	15	16	15	3

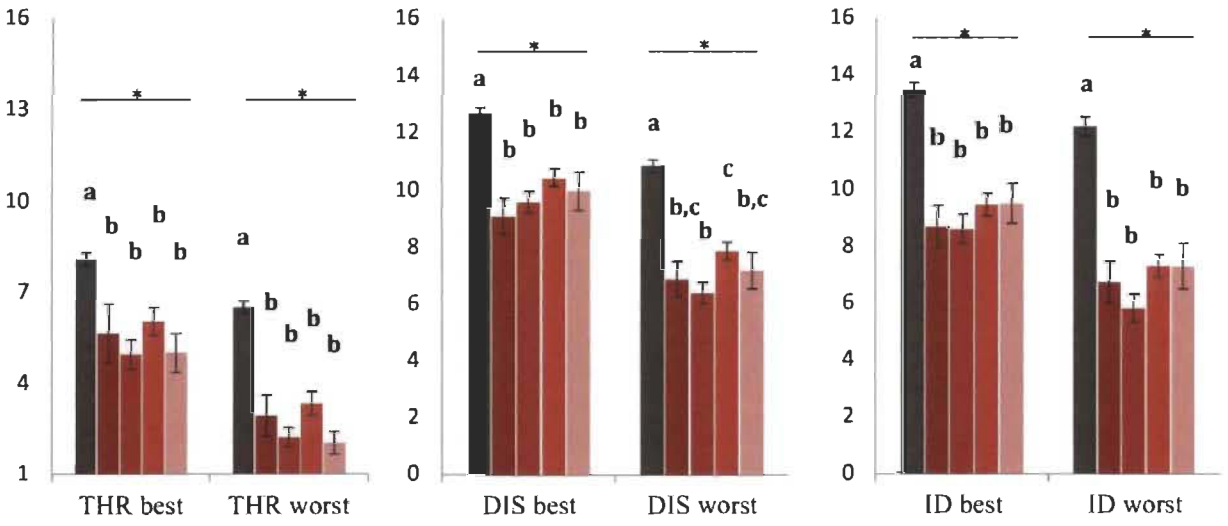

Figure 2. Olfactory performance in controls (dark grey) and patients with different causes of hyposmia: idiopathic (dark red), posttraumatic (medium dark red), postinfectious (medium light red), sinunasal (light red).

Average (error bars indicate SEM) scores for best and worst nostrils for three different tasks (THR = threshold task, DIS = discrimination task, ID = identification task). Significant differences in post hoc comparisons between positions are indicated by different letters ($P < 0.05$).

Comparisons between patients and controls

When comparing the overall group of controls with the group of patients, we observed that, in all tasks, patients scored significantly lower than controls, with $p < 0.001$ for each test (Figure 2). In addition, functional asymmetry was greater in patients than in controls, in all three tasks (threshold: $p = 0.013$; discrimination: $p < 0.001$; identification: $p < 0.001$; Figure 3). However, functional asymmetry was not more frequent in patients than in controls; the frequency of participants having one given nostril outperforming the other one in all three tasks was similar in controls (36 out of 93 participants; 38%) and in patients (60 out of 180 participants; 33%; $\chi^2(1) = 0.78$, $p = 0.38$).

Since there was an age difference between both groups, we selected controls so that the age matched between the two groups: while patients were aged from 14 to 80 years, with $M \pm SD = 51.4 \pm 14.6$, we selected, in the control group, $N = 110$ participants, aged from 14 to 79 years, with $M \pm SD = 49.5 \pm 14.9$. Patients still scored significantly lower than controls, with $p < 0.001$ for each test. With this age-matched control group, functional asymmetry was greater in patients than in controls for discrimination (Mann-Whitney, $p < 0.001$) and identification (Mann-Whitney, $p = 0.002$), but failed to reach significance for the threshold test (Mann-Whitney: $p = 0.070$).

For each task, we computed ROC curves for the scores obtained with the best nostril, the worst nostril, and the difference of scores between nostrils (Figure 4). The AUC, cut-off values with their associated specificity and sensitivity, PPV and NPV, and accuracy, were calculated for each test (Table 3). The most accurate cut-off value appeared to be the one associated with the threshold task, for the worst nostril: a score of 2.63 with an accuracy of 88.2 percent (sensitivity = 0.73, specificity = 0.97). There was a significant main effect of the nostril on the AUC (Kruskal-Wallis, $p = 0.039$) and the accuracy (Kruskal-Wallis, $p = 0.027$), with the accuracy for the score obtained with the worst nostril appearing to be the greatest. Comparing the AUCs two by two confirmed the finding that, in the threshold task, the score of the worst nostril was more efficient than the score of the best nostril to discriminate between those who have an olfactory disorder and those who have not ($z = -3.52$, $p < 0.001$). The difference was not significant in the discrimination task ($z = -1.28$, $p = 0.101$), and close to significance in the

identification task (z = -1.62, p = 0.053). These results show a better efficiency of the score of the worst nostril to detect an olfactory disorder.

As a final analysis, we compared patients with different causes of hyposmia (sinunasal, postinfectious, posttraumatic, idiopathic). Scores of the best nostril were not influenced by the different causes of hyposmia. However, there was a significant effect on scores of the worst nostril in the discrimination task, indicating that patients with posttraumatic hyposmia scored lower than patients with postinfectious hyposmia (p = 0.018). There was a tendency in the same direction in threshold and identification tasks; however, they failed to reach significance after correction (p = 0.054 and p = 0.066 respectively).

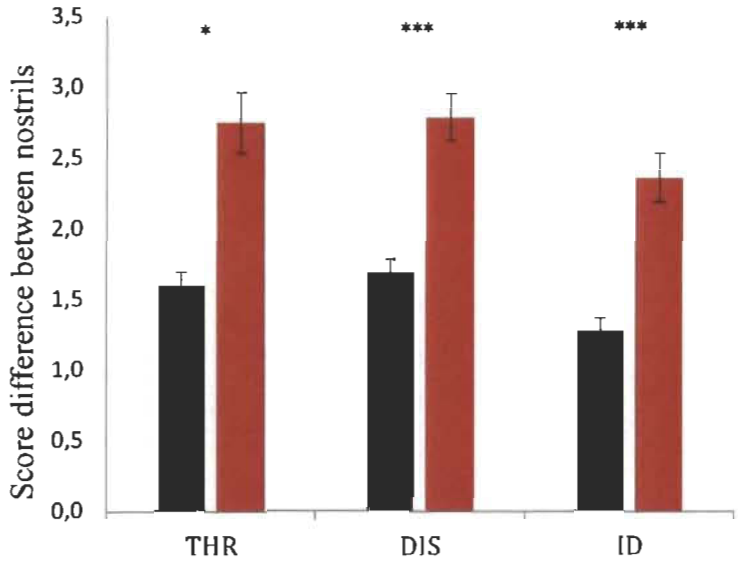

Figure 3. Functional asymmetry (absolute difference between scores for left and right nostril) in controls (dark grey) and patients (red).

*This asymmetry is more important in hyposmic patients than in controls. Columns represent average difference, error bars indicate SEM. * p<0.05, *** p<0.001. THR = threshold task, DIS = discrimination task, ID = identification task.*

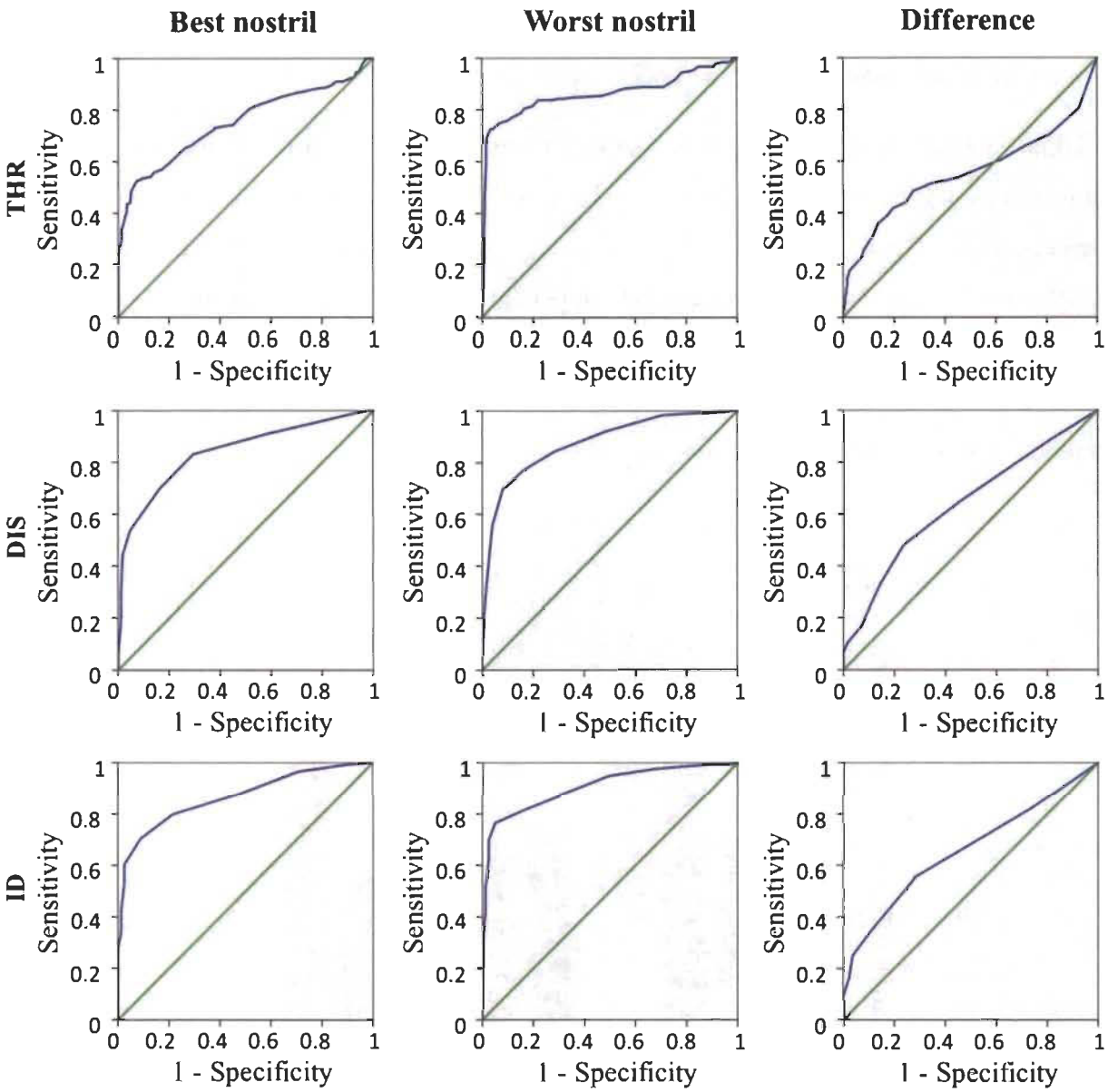

Figure 4. Receiver Operating Characteristics (ROC) curves.

ROC curves were computed for controls above 16 years old to compare the efficiency of the different scores to discriminate between controls and hyposmic patients for the best nostril, the worst nostril, and the absolute difference between both nostrils' scores, in three tasks (THR = threshold task, DIS = discrimination task, ID = identification task).

Table 3. Efficiency of the different tests as diagnostic tools to detect an olfactory disorder. The area under the curve (AUC) of the ROC curves was calculated. Positive and negative predictive values (PPV and NPV) allowed to define more precisely the accuracy of each test. The scores obtained with the worst nostril seemed to be the most efficient diagnostic tools. THR = threshold task, DIS = discrimination task, ID = identification task.

Task	Test	AUC	Cutoff value	Sensitivity	Specificity	Youden index	PPV	NPV	Accuracy
THR	Best	0.752	5.125	0.525	0.928	0.453	0.847	0.719	78.3
	Worst	0.862	2.625	0.726	0.966	0.692	0.942	0.822	88.2
	Difference	0.555	2.875	0.419	0.804	0.223	0.620	0.645	63.2
DIS	Best	0.842	12.5	0.832	0.705	0.538	0.682	0.846	76.4
	Worst	0.877	8.5	0.698	0.919	0.617	0.868	0.800	83.4
	Difference	0.639	2.5	0.480	0.763	0.243	0.607	0.658	63.2
ID	Best	0.867	11.5	0.704	0.911	0.615	0.858	0.802	83.0
	Worst	0.907	9.5	0.765	0.949	0.715	0.920	0.841	88.0
	Difference	0.658	1.25	0.553	0.722	0.275	0.602	0.680	64.1

Discussion

Here, we describe a study on monorhinal sensitivity. First, we established normative data for monorhinal testing. Second, we showed that detection threshold scores obtained from the worst nostril discriminate best between patients and healthy individuals. Third, we found that even healthy individuals exhibit side differences, which are most prominent for odor discrimination and identification. We further confirmed findings from birhinal testing, with healthy individuals aged 6-15 scoring lower than adults.

The normative data for unilateral Sniffin' Sticks tests that we established in this paper add to the ones available for bilateral testing (Hummel *et al.*, 2007). Overall, scores for the different tasks obtained in both studies are in the same range, at least for adults (age groups 2, 3, and 4), proving the reliability of the Sniffin' Sticks (Hummel *et al.*, 1997). We compared results obtained in our study (monorhinal) and the publication on birhinal normative data (Hummel *et al.*, 2007) for the reference age group 2 (16-35 years old): using Welch's t-test, we compared the scores of the best nostril and of the worst nostril with the scores of both nostrils, for the three tasks. Before we applied a Bonferroni correction, all comparisons between scores of both nostrils and worst nostril were indicating a significant difference, with our normative scores for the worst nostril being

significantly below the normative values for birhinal testing. This was not the case for scores of the best nostril. This additional analysis suggests that the scores from the best nostril correspond (roughly) to scores obtained in birhinal testing, which is coherent with the literature (Frasnelli *et al.*, 2002). It underlines, however, the notion that unilateral olfactory disorders could go unnoticed with birhinal testing, as the scores do not reflect those of the worst nostril.

For the detection of olfactory disorders, we defined cut-off values to help diagnose hyposmia. Such values had been defined for birhinal testing (Hummel *et al.*, 2001; Hummel *et al.*, 2007) but the interest of using monorhinal testing made it useful to define them unilaterally. A diagnosis is always associated with a risk of false positives and false negatives, and the aim is to define a cut-off value with the best accuracy. Our results show that the scores of the worst nostril are more efficient to detect an olfactory disorder than the scores of the best nostril or the differences of scores between nostrils. Above, we discussed the equivalence of scores obtained with bilateral testings and scores obtained with the best nostril, and the fact that unilateral olfactory disorders might go unnoticed with birhinal testing; this is confirmed here, as cut-off values defined from the scores of the worst nostril appear to be more accurate than those of the best nostril. This demonstrates that monorhinal testing provides more efficient diagnostic tools to detect olfactory disorders. The method we used to define cut-off values was not the same as the one used for birhinal testing (Hummel *et al.*, 2007). The latter consisted in picking the tenth percentile in the age group 2. With this method, we would have obtained a cut-off value of 3.93, which has a slightly higher sensitivity but a much lower specificity than the cut-off value 2.63 that we found (0.77 instead of 0.73 for the sensitivity, 0.88 instead of 0.97 for the specificity). It is not yet clear whether monorhinal testing could also be helpful for monitoring olfactory disorders, or to predict olfactory outcome at a follow up. Future longitudinal studies may address these issues.

Ideally, not all three tasks should be necessary to be able to diagnose an olfactory disorder, so that only one test could be performed, on both nostrils one after the other; if the lowest of the two scores happens to be lower than the cut-off value, an olfactory disorder can be diagnosed. If only one of them had to be chosen, it would be wise to pick the threshold task, as the cut-off value associated with this task is the one with the best accuracy (88.2 percent). The cut-off value associated with the identification task also has a high accuracy (88.0 percent), but this task can involve memory as, during the testing of the second nostril, the participant can remember the

odors they smelt during the testing of the first nostril. This transfer is inherent in the test and can bias the results, making it not ideal as a diagnostic tool.

In healthy participants, in all three tasks, there was no difference between left and right nostrils, but there was a significant difference between best and worst nostrils. One could think the tests may be unprecise, this result be due to a poor test-retest reliability, and therefore yield different results for each nostril, but the proportion of healthy participants showing side differences in favor of one given nostril, in all three tasks, is significantly greater than the proportion we would obtain by chance; this shows the differences between nostrils that we observe are not just an epiphenomenon of testing but truly reflect functional asymmetry. This functional asymmetry had been hinted by reports of side differences (Gudziol *et al.*, 2007; Gudziol *et al.*, 2010). It can be physiological, since differences between nostrils are observed in healthy participants. This result underlines the importance of monorhinal testing. The independence of the nostrils can be explained directly by the anatomy of the olfactory system. Indeed, each nostril has its own olfactory epithelium with olfactory receptor nerves sending the information to its own olfactory bulb; just like visual and auditory information, olfactory information follows two separate paths into the brain, leading to possible differences of sensibility between the two nostrils. Differences between nostrils have been showed to increase with age (Gudziol *et al.*, 2007). In our study, age group 4 had a tendency to display the greatest side differences. The increase of these differences with aging is coherent with the fact that olfactory abilities decrease with age. Indeed, we can hypothesize that this olfactory loss is asymmetric, affecting one nostril more than the other, or both nostrils at different points in time, thus increasing the difference between nostrils. Another explanation of the increase of differences between nostrils with aging would be the decrease of attentional and other cognitive resources needed for a high test-retest reliability. Functional asymmetry can be physiological, but the degree of lateralization can be an indicator of future bilateral olfactory loss (Gudziol *et al.*, 2010), and this is one more reason to use monorhinal testing, because it could help to detect olfactory disorders which are still unilateral before they become bilateral. Our results, presented above, contrast with the idea that the differences between nostrils that we measured could be only due to the tests being unprecise, because the proportion of participants presenting a functional asymmetry was too high to be explained by chance. However, even though different previous studies show that the test-retest reliability of the Sniffin' Sticks is high when the tests are performed several times on the same participant (r =

0.80 for odor discrimination, r = 0.88 for odor identification, and r = 0.92 for odor threshold; Albrecht *et al.*, 2008; Haehner *et al.*, 2009; Hummel *et al.*, 2001), it is still difficult to completely dismiss the possibility of an effect of a lack of test-retest reliability between nostrils. Another explanation to these differences between nostrils could be the nasal cycle; indeed, the sensitivity of the nostrils is fluctuating (Sobel *et al.*, 1999), and one nostril could be weaker at the time of testing, and this could be reversed a few hours later. Longitudinal studies would be needed to determine if this is the case, but earlier studies did not find any correlation between nasal patency and olfactory function, making this explanation somewhat unlikely (Frasnelli *et al.*, 2002).

The effect of gender on the olfactory performance is disputed; though some studies suggest that women significantly outperform men (Hummel *et al.*, 2007), other studies show no significant effect of gender (Hummel *et al.*, 2001; Kobal *et al.*, 2000). In our study, women, on average, seemed to score higher than men in all three tasks, but the difference was significant only in the threshold task and for the right nostril in the identification task. Our results therefore support the notion that, if gender differences are found, women typically outperform men (Doty *et al.*, 2009).

All the studies seem to agree on a decrease of the olfactory performance with age (Hummel *et al.*, 2001; Kobal *et al.*, 2000), a decrease which would be due to functional and pathological changes occurring in the olfactory system with age (Doty *et al.*, 2014). Although, on average, age group 4 scored lower than age groups 2 and 3, there was no significant difference between those three groups in the threshold and identification tasks. The discrimination task was the only one showing a significant difference between age groups 2 and 4. The absence of more significant differences could be the repartition into age groups itself: all individuals aged more than 55 were in group 4, but the decrease of the olfactory abilities could happen later than this, and therefore be undetectable with such age groups.

There are a couple of limitations to this study. First, since this is a retrospective study, our different groups are not equivalent: participants in the control group do not perfectly match patients in term of age or gender. While patients are mostly above 36, the most represented age group in controls is the age group 2 (16-35 years). Since not all tests have been carried out in controls, this leads to sometimes very low numbers of participants: that is for example the case of age group 3, in which the identification task was performed on only 7 participants. It is however important to point out that, in line with a previous study (Hummel *et al.*, 2007), the reference

group is age group 2, in which we have a large sample size. We also performed the analyses on a selected group of controls whose age matched the patient group, and the observations remained the same, with significant differences between both groups. Second, as mentioned above, the side differences that we observed could be partly due to a lack of test-retest reliability, and it is difficult to assess how much. Third, as discussed above, monorhinal testing leads to a transfer problem in the identification task which can bias the results, as memory can be involved. A solution could be to use different odors for each nostril, but this would not be ideal either, as all odors are not equally difficult to identify; thus, we would be unable to compare both nostrils accurately. One has to take this into consideration when evaluating identification scores.

In conclusion, age and gender affect results in monorhinal olfactory testing. Even if there is not a nostril better than the other one between left and right, we observe functional asymmetry in both healthy participants and patients. Such functional asymmetry requires monorhinal testing to be detected. We provide normative data and, in addition, cut-off values to discriminate between olfactory dysfunction and normal function. The detection threshold obtained on the weaker nostril discriminates best between both groups. These findings may be useful in the diagnosis of olfactory dysfunction and hence in the early detection of several medical conditions such as Parkinson's and Alzheimer's diseases.

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Chapitre 3 – Sommelier students display superior abilities to identify but not to detect or discriminate odors early in their training

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Daphnée Poupon[1], Pauline Fernandez[2], Johannes Frasnelli[1,3]

Affiliations: 1 Department of Anatomy, Université du Québec à Trois-Rivières, Canada

2 Institut du Tourisme et d'Hôtellerie du Québec, Montréal, Canada

3 Research Centre, Sacré Coeur Hospital, Montréal, Canada

Abstract

Introduction: Experts acquire superior abilities in their specific domains by training. Sommelier students, who are future olfaction experts, could be an excellent model to study the effects of olfactory training.

Methods: We tested whether sommelier students display superior olfactory abilities early in their education: within the first two months of education, we examined the olfactory function, i.e., discrimination and identification of odors as well as olfactory threshold and olfactory memory, of n = 25 sommelier students and compared them to n = 29 control students. We also tested episodic and working memory.

Results: We found that sommelier students outperformed controls in free and cued identification, but we did not observe any difference in discrimination or threshold tasks. There was also no difference in memory tasks.

Conclusions: Early in their education, sommelier students appear to be better at identifying odors, but do not display other superior olfactory abilities.

Implications: Results suggest that sommeliers are better at identifying odors than the average person, either because they enter into training with superior identifications skills or are able to learn to identify odors at a very fast rate.

Keywords: olfaction; expertise; sommeliers; training; memory

Introduction

An important interindividual variability exists when it comes to olfactory performance: while a part of the population suffers from olfactory losses due to different possible causes, another portion is expert in this domain, e.g. sommeliers and perfumers (Dileo *et al.*, 1994; Majid *et al.*, 2017; Royet *et al.*, 2013). Olfactory performance is thus variable across individuals, but also in time, since the sense of smell can be trained. Olfactory training is actually considered a way for patients with olfactory dysfunction to improve olfactory abilities: smelling four odors twice a day for a variable period of time from three to nine months led to an improvement of their sense of smell, which was tested before and after training (Altundag *et al.*, 2015; Damm *et al.*, 2014; Fleiner *et al.*, 2012; Geissler *et al.*, 2014; Haehner *et al.*, 2013; Hummel *et al.*, 2009; Kollndorfer *et al.*, 2014; Kollndorfer *et al.*, 2015; Konstantinidis *et al.*, 2013; Pekala *et al.*, 2016). Training is effective even in individuals with a normal sense of smell, increasing the sensitivity to specific odors (Dalton *et al.*, 2002; Livermore *et al.*, 2004; Rabin *et al.*, 1986), even when participants had specific anosmia and could not, at the start of the experiment, perceive the specific odorant that was used (Croy *et al.*, 2015; Moller *et al.*, 1999; Wang *et al.*, 2004). Finally, training allowed experts to become professionals: during their education, future sommeliers learn to taste wine, therefore substantially using their taste and olfaction. Their expertise gives them the ability to describe wines more precisely and using less subjective factors than novices (Parr *et al.*, 2011).

Good models are needed to study the effects of olfactory training, and we suggest that sommelier students could be an excellent one. A first reason to think so is that their training takes place in more ecological conditions than an experimental one, with a possibly stronger motivation: while the sole purpose of an experimental olfactory training is to complete a therapy or a scientific study, sommelier students train with the aim to become professionals. Another reason why they could be a good model is that training may quickly impact olfactory performance and, therefore, differences

in olfactory performance between these future experts who receive a training and novices who are not trained may appear early, meaning that there is no need to wait for a long time before testing them. Durations of the olfactory trainings reported in the literature are variable. When it consisted of smelling four odors twice a day, which was mostly used in patients with olfactory dysfunction, it lasted from three to nine months (Altundag *et al.*, 2015; Damm *et al.*, 2014; Fleiner *et al.*, 2012; Geissler *et al.*, 2014; Haehner *et al.*, 2013; Hummel *et al.*, 2009; Kollndorfer *et al.*, 2014; Kollndorfer *et al.*, 2015; Konstantinidis *et al.*, 2013; Negoias *et al.*, 2016). In other studies, olfactory training using only one odor lasted two or three months (Croy *et al.*, 2015; Dalton *et al.*, 2002). However, there have also been reports of training-induced effects visible after just three weeks (Wang *et al.*, 2004), one week (Livermore *et al.*, 2004), or even after repeating a test three times in a row (Rabin *et al.*, 1986). This is congruent with what can be found in other domains such as juggling: a three-month training was shown to lead to changes visible even in brain anatomy (Draganski *et al.*, 2004) before another study reported that a week was enough for changes to appear (Driemeyer *et al.*, 2008). All of this shows that a few weeks of training can be long enough for the olfactory performance to improve, and therefore supports the idea that differences between sommelier students and novices can appear early. Our first hypothesis was that, early during their education, future experts already outperform non-experts, which would support the idea that sommelier students constitute a good model to study the effects of olfactory training. To test this hypothesis, we assessed olfactory function of sommelier students on four olfactory tasks within the first two months of their education and compared them to a control group of students whose classes did not include olfactory training. Literature suggests that some olfactory tasks involve more cognitive factors than others (Hedner *et al.*, 2010), with identification and olfactory memory tasks being more "cognitive" than discrimination and threshold tasks which are more "perceptual". While more perceptual tasks depend on what we refer to as basic olfactory processing, more cognitive tasks rely on what we refer to as higher order olfactory processing.

Olfactory processing involves areas such as the hippocampus which is involved in memory, and more specifically in episodic memory (Corkin *et al.*, 1997; Eichenbaum *et al.*, 2007). Performances in olfactory and cognitive tasks are related (Hedner *et al.*, 2010), and some brain regions involved in memory networks are larger or thicker in sommeliers (Banks *et al.*, 2016; Pazart *et al.*, 2014). Therefore, there are reasons to think olfaction and memory are related and could affect each other. Because part of sommelier education involves learning about and memorising the different grape

varieties and the different compounds that can be found in wine, potential superior olfactory performance in sommelier students could be due not just to potential superior olfactory abilities but also to a potential superior memory. We tested this hypothesis by comparing sommelier students and controls on episodic memory measures. We also tested working memory, which is less likely to be influenced by olfaction as no anatomical relation has been shown between networks involved in these functions. Thus, we had data on two forms of memory: a form of memory likely to be related to olfaction (episodic memory, which is the memory of past personal experiences), and one that is not (working memory, which temporarily holds information available for processing).

Materials and methods

This study was approved by the Ethics Committee of the University of Quebec in Trois-Rivières, Canada.

Participants

All participants gave informed written consent to participate.

The experimental group consisted of 25 sommelier students (13 women). They were aged from 18 to 35 years (M ± SD = 24.8 ± 4.4). 8 of them came from the Centre de Formation Professionnelle Bel-Avenir in Trois-Rivieres, 17 came from the Institut de Tourisme et d'Hôtellerie du Québec in Montreal. They were all tested within the first two months of their education. More specifically, we tested them between 3 and 9 weeks after the start of education. During these first weeks, their classes included six hours a week of sommellerie in which they started to train their olfactory skills and learn how to discriminate and identify odors in wine. Beside this class, they also trained to serve in a restaurant and started to acquire general knowledge about wine and the profession of sommelier.

The control group consisted of 29 students (16 women). They were aged from 20 to 36 years (M ± SD = 24.6 ± 4.3). They were taking courses that did not involve any olfactory training at University of Quebec and University of Montreal.

Olfactory testing

Olfactory performance was assessed using an extended version of the Sniffin' Sticks test (Frasnelli *et al.*, 2010; Hummel *et al.*, 1997; Hummel *et al.*, 2007). Sniffin' Sticks are felt-tip pens which are filled with odorants instead of ink. The experimenter presents the odorants to the participant by removing the cap and placing the pen's tip approximately 2 cm in front of both nostrils. Different tasks were completed.

We assessed olfactory performance by measuring (1) odor threshold, (2) odor discrimination, (3) odor identification, and (4) olfactory memory, using the Sniffin' Sticks sets provided for each of these tests and following established procedures (for details, see Frasnelli *et al.*, 2010; Hummel *et al.*, 1997; Hummel *et al.*, 2007). It has been shown that olfactory performance assessment is more complete when it is performed unirhinally, i.e., when nostrils are tested separately, especially for the threshold task (Poupon *et al.*, 2017). Therefore, we tested nostrils separately for the odor threshold task, which is the most perceptual task, i.e., this task does not involve as many cognitive factors as the other tasks (Hedner *et al.*, 2010).

Odor detection threshold

We assessed odor thresholds for phenylethyl alcohol (PEA) using a single staircase, three-alternative forced choice procedure: we presented participants with triplets of pens, one of them containing the odorant in a given concentration, the two other ones containing the solvent. We used a 16-step geometric series starting from a 4% phenylethyl alcohol solution (dilution ratio 1:2 in deionized aqua conservata as diluent). Triplets were presented at intervals of approximately 20 seconds. Participants had to identify the pen containing the odorant. In this task, we tested the two nostrils separately: the participant closed a given nostril with a finger during each odor presentation, and one nostril was tested after the other. The order of the nostrils was randomized. There were 16 different concentrations available. For each nostril, triplets were presented starting with the lowest concentration, with a randomized order of the three pens; reversal of the staircase was triggered when the odor was correctly identified in two successive trials with a subsequent reversal of the staircase when participants failed to correctly identify the odor. Threshold was defined as the mean of the last four of seven staircase reversal points (Hummel *et al.*, 1997). Scores ranged between 1 and 16, and this for each nostril.

Odor discrimination

To assess odor discrimination, 32 triplets of pens (two pens containing the same odorant, and a third pen containing a different one) were presented. Participants had to identify the target pen, i.e. the pen containing the different odorant. The 32-triplet discrimination test is an extended version of the commercially available 16-triplet test (Frasnelli *et al.*, 2010). The set that is used is actually the one used for the 16-triplet discrimination test, but triplets are mixed: there are 16 triplets labelled from 1 to 16, each triplet being composed of one target pen and two paired pens. In the 32-triplet version, after testing the 16 triplets, 16 additional triplets are created by combining the target pen of triplet 1 with the paired pens of triplet 16, etc. Odors are listed in Hummel *et al.*, 1997. Scores ranged from 0 to 32.

Odor identification

Odor identification was assessed for 16 common odors: orange, leather, cinnamon, peppermint, banana, lemon, liquorice, turpentine, garlic, coffee, apple, cloves, pineapple, rose, anise, fish. All of these odors can be found in wine. Each odorant was presented a first time and participants had to identify it without any cue (free identification). The second time, for each individual odor, a list of four descriptors was presented; participants had to identify the odorant by picking one of them (cued identification). Lists of descriptors for each odorant have been established by the creators of the Sniffin' Sticks test, and were therefore the same for all participants. We obtained two scores: free and cued identification, each ranging from 0 to 16 and corresponding to the number of odors that were correctly identified. In the free identification task, participants scored only if the identification was fully correct, e.g. naming lemon or leather for orange would both count zero point.

Olfactory memory

We assessed olfactory memory by using two sets of 16 pens with the identification set used in the previous task, and another set of 16 pens containing different odorants. Only 8 pens from each set were used for this task; half of the participants were tested with pens labelled with even numbers, the other half with odd numbers. The order of pens was randomized. Participants had to tell whether the odorant was part of the identification task. This task took place about 40 minutes after the identification task.

The score for this task consisted in the sensitivity index d' that we calculated using the signal detection theory (MacMillan *et al.*, 2005): we calculated the numbers of hits (i.e. the participant said an odor was present in the identification task and that odor was indeed present) and of false alarms (i.e. the participant said an odor was present in the identification task but it was not). From that, we calculated sensitivity index d':

$$d' = z(hit\ rate) - z(false\ alarm\ rate)$$

The sensitivity index d' indicates the ability to detect whether odors were present in the identification task: d' = 1 roughly corresponds to 69% of correct answers (hits and correct rejections), d' = 2 roughly corresponds to 95% of correct answers.

We therefore obtained a total of six scores per participant in the olfactory tasks: two scores in the threshold task (right and left nostrils), one score in the discrimination task, two scores in the identification tasks (free and cued), one score in the olfactory memory task.

When reported, results are noted as Mean ± SD.

Memory testing

We tested two types of memory: episodic memory and working memory.

We used the Rey Auditory Verbal Learning Test (RAVLT) to test episodic memory (Rey, 1958). The experimenter read out loud a list of 15 words. Immediately after, participants had to give as many words as they could remember. This was repeated 5 times. After that, a distractor list of 15 other words was read out loud and participants had to repeat as many of them as they could remember, then were asked to repeat the words they could remember from the first list. The number of words recalled at this moment constituted a first score, ranging from 0 to 15 and called t = 0. Half an hour later, without rereading, they were asked to repeat the words of the first list. The number of words recalled then constituted a second score, also ranging from 0 to 15 and called t = 30.

To test working memory, we used the n-back test (Cohen *et al.*, 1994). Letters appeared on the computer screen one after the other and, for each letter, participants had to press 1 if the letter was the target, 2 if it was not. The test was divided into four tasks. In the first one, named 0-back, the target is the letter X. In the next tasks, namely 1-back, 2-back, and 3-back, a letter is the target if it

is the same as the letter that appeared 1, 2, or 3 ranks earlier, respectively. The participant is thus required to actively memorize the sequence. Scores consisted of the hit rate obtained in each task: we obtained three scores ranging from 0 to 1 and corresponding to the hit rate obtained in 1-back, 2-back and 3-back. 0-back was a task whose purpose was to get familiar with how the test works; no score was obtained from this task.

Procedure

Testing took two hours. Tests were organised in two blocks. The first block of tests consisted of the identification task followed by the threshold task and ending with the olfactory memory task, the second block of tests consisted of the RAVLT, the n-back test, and the discrimination task. Usually, the first block was done before the second one but, due to organisational aspects, for 10 participants, the second block was done before the first one. The discrimination task, which contains many odors, could have then interfered with the olfactory memory task, but a Mann-Whitney U test showed that the order of blocks had no significant effect on the performance in the olfactory memory task ($p=0.811$).

Data analysis

Data were analyzed using the software SPSS 23.0 for Windows.

We performed a factor analysis (Principal Component Analysis or PCA) following standard procedures. Specifically, we used a PCA in 25 iterations with Kaiser normalization and varimax rotation on our 6 olfactory variables. We extracted two components loading higher than the mean eigenvalue. Independent samples t-tests on the regression factor scores allowed us to compare sommeliers and controls. To go more into details, independent samples Mann-Whitney U tests were used to compare both groups for each of our 6 olfactory variables, which were not normally distributed. Because testing required several weeks and students were therefore tested at different times following the start of their education, we further calculated, for each sommelier student, the number of days between the start of education and the testing, and used Spearman correlations to examine whether olfactory performance and number of days into the training were related. To assess whether there were differences between groups in non-olfactory tasks, we used independent samples Mann-Whitney U tests. Spearman correlations allowed us to look for possible correlations

between the different olfactory and memory tasks. Alpha was set at 0.05, and we used Bonferroni-Holm correction for multiple comparisons.

Results

Olfactory performance

Principal component analysis revealed two factors with Component 1 accounting for 30.4% and Component 2 accounting for 21.1% of the total explained variance. Together, the two components explained 51.5% of the total variance in the data. The first component depended mostly on identification and olfactory memory tasks, the second one on threshold and discrimination tasks (see

Table 4). We therefore labelled Component 1 as "higher order olfactory processing" because identification and olfactory memory involve more cognitive factors than the other tasks, and Component 2 as "basic olfactory processing" because discrimination and threshold tasks are more perceptual tasks. T-tests performed on regression factor scores showed that there was a significant difference between sommelier students and controls for the first component ($t(52) = 3.895$, $p < 0.001$), but not for the second one ($t(52) = 1.357$, $p = 0.181$), indicating that there was a difference between groups in the most cognitive olfactory tasks (higher order olfactory processing) but not in the most perceptual ones (basic olfactory processing; see Figure 5).

We looked more into details and examined each olfactory task separately to see if there was any difference between sommelier students and controls. Scores obtained in each task are depicted in Figure 6. After correction for multiple comparisons, the difference was significant for cued identification ($p < 0.001$) and for free identification ($p = 0.048$), with sommelier students performing better than controls. There was no significant difference in the olfactory memory task (sommeliers: 1.73 ± 0.590; controls: 1.42 ± 0.696) or in the discrimination task (sommelier students: 25.52 ± 3.07; controls: 25.03 ± 3.23).

We found no correlation between the number of days into training at the time of testing and any of the olfactory tasks (see Table 5).

Table 4. Scores in olfactory tasks expressed in component loadings.

After performing a factor analysis (Principal Component Analysis) on 6 olfactory variables, two components were extracted (both loading higher than the mean eigenvalue). Gray shading represents the groups of tasks revealed by the PCA, with a threshold fixed at 0.5: olfactory memory, free and cued identification tasks loaded high on Component 1, while threshold for left and right nostrils and discrimination loaded high on Component 2.

	Component 1 (30.4%)	Component 2 (21.1%)
Threshold left	-0.16	0.80
Threshold right	0.33	0.54
Discrimination	0.07	0.65
Free identification	0.55	0.37
Cued identification	0.73	-0.06
Olfactory memory	0.79	0.03

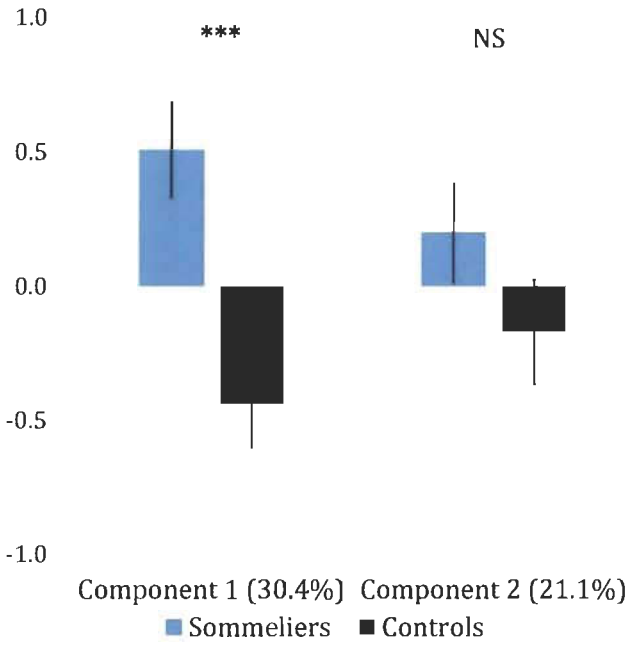

Figure 5. Regression factor scores for each component in sommeliers (light) and controls (dark).

Component 1 (30.4%), which we labelled as "higher order olfactory processing," depends mostly on olfactory memory, free and cued identification tasks, while Component 2

*(21.1%), labelled as "basic olfactory processing," depends mostly on threshold for the left and right nostrils and discrimination. Columns indicate average scores, error bars indicate standard errors of mean (SEM). NS = Non Significant, *** $p < 0.001$.*

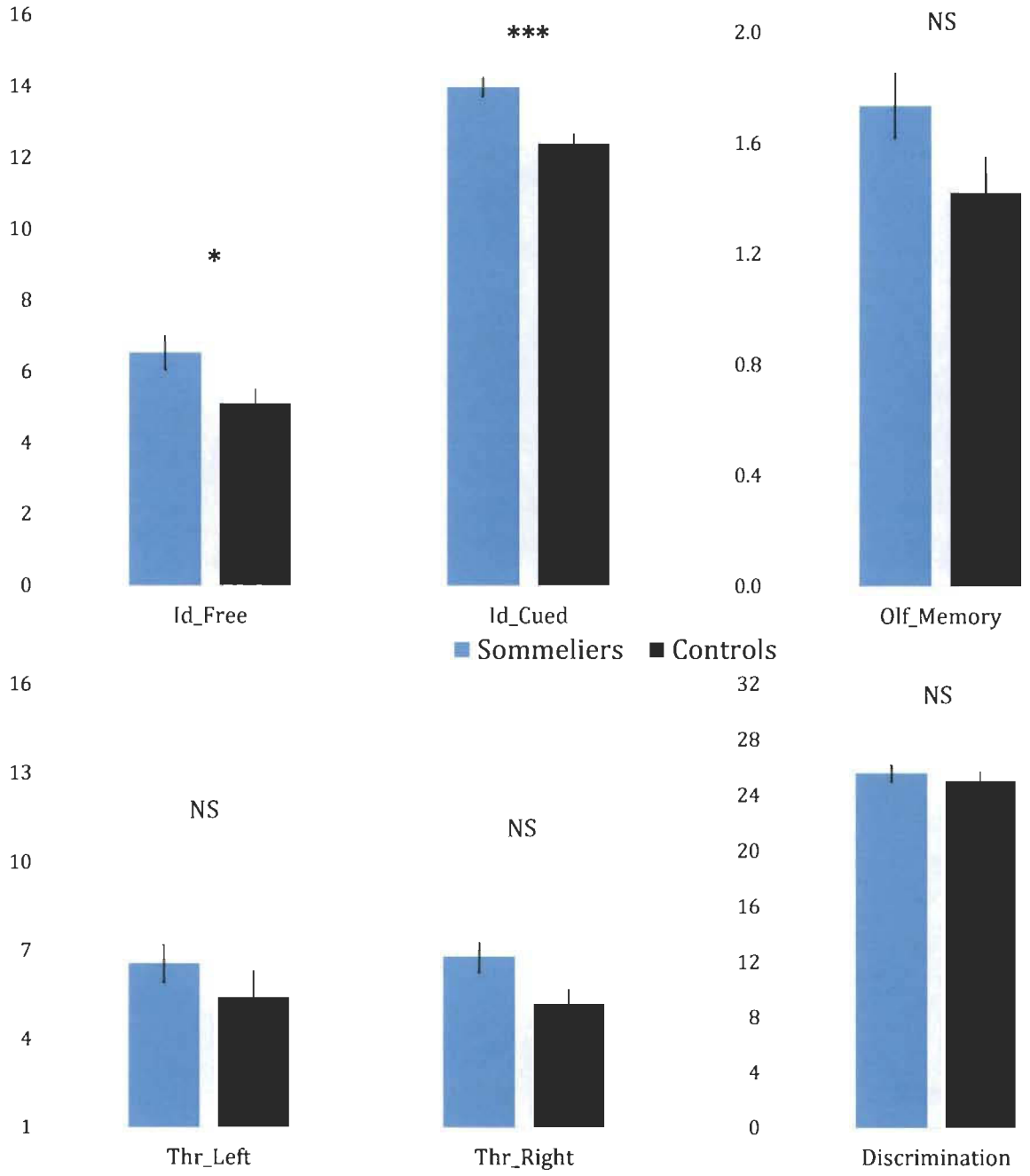

Figure 6. Scores in olfactory tasks in sommeliers (light) and controls (dark).

Scores are depicted for our different olfactory tasks: free and cued identification (labelled Id_Free and Id_Cued respectively), olfactory memory (Olf_Memory), discrimination

*(Discrimination), threshold for left, right, best and worst nostrils (Thr_Left, Thr_Right). Columns indicate average scores, error bars indicate standard errors of mean (SEM). NS = Non Significant, * $p < 0.05$, *** $p < 0.001$.*

Memory tasks

We tested whether one group performed better than the other in memory tasks (RAVLT and n-back). There was no significant difference to be found in any of them (see Figure 7).

There was also no correlation between memory and olfactory tasks (see Table 5).

Table 5. Spearman correlations between olfactory tasks and the number of days of training at the time of testing, and between olfactory and memory tasks.
Nb_Days = number of days of training; RAVLT_T0 and RAVLT_T30 = scores at 0 and 30 minutes in the RAVLT task; 1-back, 2-back and 3-back = scores in the n-back test; Id_Free and Id_Cued = free and cued identification task; Thr_Left and Thr_Right = threshold score for the left and right nostrils; Dis = discrimination; Olf_Mem = olfactory memory.

		Id_Free	Id_Cued	Thr_Left	Thr_Right	Dis	Olf_Mem
Nb_Days	Corr. coef.	0.105	-0.174	0.182	0.159	0.357	-0.063
	p-value	**0.619**	**0.404**	**0.385**	**0.447**	**0.080**	**0.765**
RAVLT_T0	Corr. coef.	0.185	-0.016	0.188	0.009	0.125	0.034
	p-value	**0.181**	**0.909**	**0.174**	**0.948**	**0.367**	**0.808**
RAVLT_T30	Corr. coef.	0.064	-0.130	0.078	-0.038	0.057	0.067
	p-value	**0.658**	**0.362**	**0.586**	**0.793**	**0.693**	**0.638**
1-back	Corr. coef.	0.013	-0.065	-0.044	0.022	-0.070	-0.088
	p-value	**0.928**	**0.660**	**0.769**	**0.884**	**0.639**	**0.552**
2-back	Corr. coef.	-0.032	-0.136	-0.007	-0.085	0.083	0.071
	p-value	**0.828**	**0.358**	**0.960**	**0.567**	**0.574**	**0.633**
3-back	Corr. coef.	0.125	-0.022	-0.106	0.055	0.214	0.051
	p-value	**0.396**	**0.883**	**0.473**	**0.711**	**0.145**	**0.731**

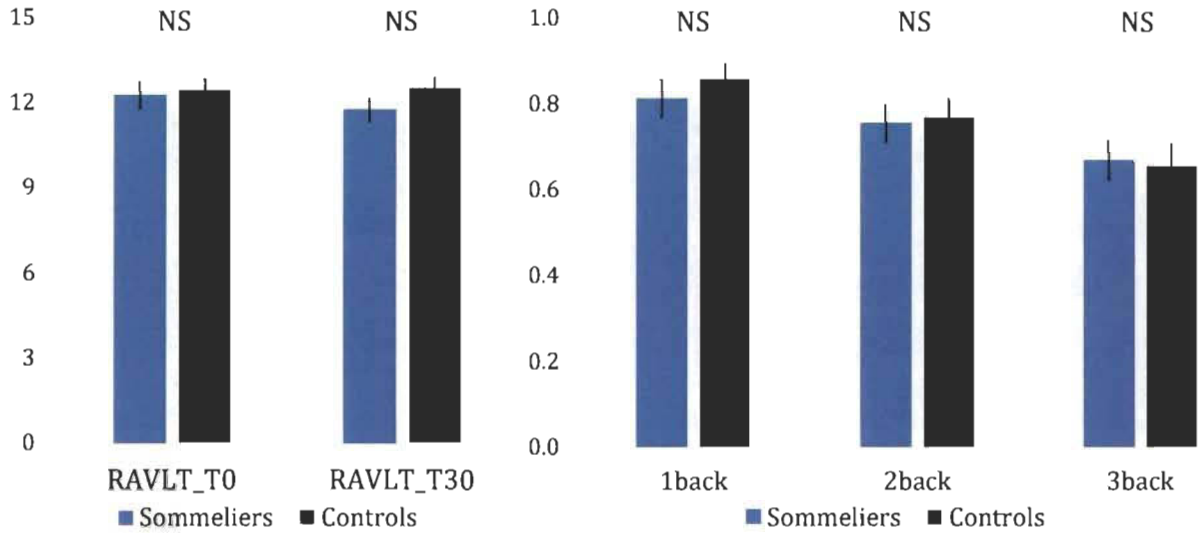

Figure 7. Scores in memory tasks in sommeliers (light) and controls (dark).

A. Episodic memory (RAVLT). Number of words recalled immediately after the learning phase (RAVLT_T0) and 30 minutes later (RAVLT_T30). B. Working memory (n-back test). Accuracy of responses obtained in the different tasks: 1-back, 2-back and 3-back. Columns indicate average scores, error bars indicate standard errors of mean (SEM). NS = Non Significant.

Discussion

The main finding in this study was that, within the first two months of their education, sommelier students already identify odors more accurately than control students. They do not outperform them in other olfactory tasks, and there was no significant difference between the two groups in memory tasks.

There was a significant difference between sommelier and control students in cued and free identification tasks, but not in the olfactory memory, discrimination and detection threshold tasks. These results are rather congruent with some of the literature, as higher order olfactory processing seems to be modulated more easily than basic olfactory function: studies comparing experts and novices showed that wine experts had superior abilities in discriminating, recognising and identifying odors, but were not better than novices at detecting n-butanol odor, an odor other than phenylethanol which is also commonly used to measure detection thresholds (Bende *et al.*, 1997; Parr *et al.*, 2002). Also, within the scope of an olfactory training, for example, cognitive functions of olfactory processing seem more likely to be affected than its perceptual aspect (Pekala *et al.*,

2016). Indeed, several studies reported an effect of olfactory training on the identification task (Altundag *et al.*, 2015; Fleiner *et al.*, 2012; Haehner *et al.*, 2013; Konstantinidis *et al.*, 2013) or on the discrimination task (Geissler *et al.*, 2014), which have both been shown to involve more cognitive factors than other tasks (Hedner *et al.*, 2010). When effects on thresholds were reported, enhanced thresholds were specific to the odors used during the training (Croy *et al.*, 2015; Dalton *et al.*, 2002; Haehner *et al.*, 2013; Hummel *et al.*, 2009; Kollndorfer *et al.*, 2014; Kollndorfer *et al.*, 2015; Moller *et al.*, 1999; Rabin *et al.*, 1986; Wang *et al.*, 2004), implying that this is not a generalized effect, and therefore suggesting that basic olfactory function is indeed less easily modulated than higher order olfactory processing. In our case, similar mechanisms could be involved, explaining why sommelier students display greater abilities in more cognitive olfactory tasks early in their education.

The PCA revealed two principal components, which we interpreted as representing basic olfactory processing, and higher order olfactory processing. These two components account for 51.5% of the variation in the data; they are the two components which describe best our six variables. The remaining 48.5% can be attributed to other components of lesser impact which can be interpreted as interindividual differences or any factor that might have impacted the testing. Interindividual differences can result in differences both in the olfactory performance at the start of training, and in the evolution of olfactory performance due to training; indeed, because of innate abilities that vary from one to another and because of the different experiences we go through, we perform differently in the different tasks and have variable learning rates.

We did not find any difference between sommelier students and controls in memory tasks, or any relation between olfactory and memory tasks. Therefore, we can assume that sommelier students outperforming control students in odor identification was independent from unspecific memory effects. However, other types of memory may be involved for which we did not test, such as semantic memory; therefore, one has to be careful when interpreting this result.

The main limitation of this study is that our approach is not completely experimental as the two groups of students were already distinct at the time of recruitment; ideally, participants would have been randomly split into two groups after being recruited. Here, we could imagine that being interested in sommellerie has led them to use odors more than students in the control group, and that this could be enough to improve their olfactory function prior to starting their education.

Another possibility could be that, because they noticed they had superior olfactory abilities, they decided to train to become sommeliers. On the other hand, we can also imagine that participants from the control group had wine tasting as a hobby or had any other experience in their past that might have impacted their sense of smell. Our inclusion criteria to recruit controls were already numerous (they had to correspond to sommelier students in age and gender, had to be students, and had to have a normal sense of smell); we did not match them depending on their olfactory background and assumed they mostly were, like most of the population, novices. To evaluate olfactory function, we used a test which was designed to separate individuals with reduced olfactory function from individuals with a normal sense of smell. Such tests may not be optimal for distinguishing between individuals with normal and those with superior olfactory function. For example, sommeliers may be particular good at detecting specific odors, such as cork odor. Future studies should evaluate whether the types of odorants chosen in the study could impact the results.

The present study does not yet allow us to disentangle whether the few differences we observed between sommelier students and controls result from the first weeks of education or were visible already before the beginning of education. Two scenarios can explain the absence of correlation between olfactory performance and number of days into training at the time of testing. A first possibility would be that the few differences we observed do not result from the first weeks of training but were already present before the start of training. A second possibility would be that sommelier students quickly improved during the first three weeks of training, before we started to test them. To clear up this question with more certitude, future studies should test them before they start education. However, this does not affect the findings of our study because our aim was not to precisely determine when differences appear, but to assess whether differences are already visible early in the education.

We suggested that sommelier students could be a good model to study the effects of olfactory training because 1) they are motivated by the prospect of becoming sommeliers, which makes their training take place in more ecological conditions than an experimental one, and 2) because differences in olfactory performance might be visible early. Our study aimed at examining this second point and results show that they indeed already display greater abilities to identify odors during the first weeks. Because sommelier students tested after nine weeks of training are not significantly better than those tested after three weeks, we suggest that these differences are either

already present before the start of training, or either the result of a quick improvement happening during the first three weeks of training. Either way, differences are visible early and, by testing them at different times during their training, we can keep track of the evolution of their abilities. Thus, this supports the idea that sommelier students constitute a good model to study the effects of olfactory training. Besides being a good model for that purpose, they could also be a good model to study brain plasticity. Indeed, brain plasticity appears to be an underlying mechanism related to the enhancement of olfactory performance in experts: correlations have been found between olfactory performance and characteristics such as density or thickness of some olfactory brain regions, in both sommeliers (Banks *et al.*, 2016; Sreenivasan *et al.*, 2017) and perfumers (Delon-Martin *et al.*, 2013; Plailly *et al.*, 2012). Most of these differences in brain anatomy are correlated with years of expertise (Banks *et al.*, 2016; Delon-Martin *et al.*, 2013), suggesting that training results in neuroanatomical changes which would allow a more efficient processing of the olfactory stimulus, thus enhancing the performance. Therefore, sommelier students constitute an excellent model.

Conclusion

Sommelier students constitute a good model to study the effects of olfactory training. During the first two months of education, they are already better at identifying odors, but show no superior ability in olfactory memory, discrimination or detection threshold tasks, or in memory tasks. Sommelier students may also be a good model to study training-related brain plasticity; further studies on sommelier students could shed light on the mechanisms involved in becoming an expert in olfaction, and on the role brain plasticity plays in the enhancement of olfactory performance.

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Chapitre 4 – Olfactory bulb volume increases during sommelier training

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Daphnée Poupon[1], Pauline Fernandez[2], Johannes Frasnelli[1,3]

Affiliations: 1 Department of Anatomy, Université du Québec à Trois-Rivières, Canada

2 Institut du Tourisme et d'Hôtellerie du Québec, Montréal, Canada

3 Research Centre, Sacré Coeur Hospital, Montréal, Canada

Abstract

Brain plasticity is essential for experts to acquire the abilities they need. Sommeliers, who are olfaction experts, display differences in olfactory brain areas that correlate with greater olfactory abilities. The volume of olfactory bulb, which plays a crucial role in olfactory processing, is positively correlated with olfactory performance. However, this structure has never been examined in sommeliers. We conducted a longitudinal study and tested 17 sommelier students at the start and at the end of their year-and-a-half-long training and compared them to 17 control students. We measured olfactory bulb volumes and used the Sniffin' Sticks test to evaluate olfactory performance. During training, olfactory bulb volume increased in sommelier students while there was no significant evolution in controls. Our olfactory tests did not reveal any significant improvement of the olfactory function in sommelier students. Changes in olfactory bulb volume are an effect of training-related brain plasticity.

Keywords: plasticity, brain, olfaction, olfactory bulb, training, sommelier

Introduction

Brain plasticity is known to be a crucial mechanism during childhood, but it remains essential in adulthood: while it was assumed for a long time that the brain was adjustable only during youth, numerous reports have shown that brain plasticity also happens in adults. Experiences shape the brain, regardless of age. This mechanism allows professionals to acquire the skills they need: training leads to improved capacities and changes in brain structure. Sommeliers and perfumers are a great example: their profession requires these olfaction experts to train their noses on a daily basis, and their refined sense of smell comes along with differences in the brain. Structural changes such as an increase of cortical thickness in olfactory brain areas, e.g. the piriform cortex, the entorhinal cortex or the insula, have been observed in sommeliers. This increase positively correlates with the number of years of experience (Banks *et al.*, 2016; Royet *et al.*, 2013). Functional differences have also been reported: greater connectivity between and earlier activation of olfactory brain areas contribute to making processing of olfactory stimuli more efficient (Pazart *et al.*, 2014; Sreenivasan *et al.*, 2017).

The olfactory bulb (OB) is an ovoid structure located under the frontal lobe of the brain. It constitutes the first relay of olfactory processing as it receives input directly from the olfactory epithelium: olfactory receptor neurons project to the ipsilateral OB and synapse with second-order neurons known as mitral and tufted cells that will convey the information deeper into the brain (Gottfried, 2010; Huart *et al.*, 2013; Mori *et al.*, 1999). In healthy people with a normal sense of smell, significant positive correlations between OB volumes and olfactory performance have been reported: a bigger OB is associated with better olfactory abilities (Buschhuter *et al.*, 2008; Seubert *et al.*, 2013). Similar correlations have been observed in patients with olfactory dysfunction: the more impaired is the olfactory function, the smaller is the OB (Liu *et al.*, 2017; Rombaux *et al.*, 2006a). Causes of olfactory dysfunctions can be various. They are congenital in some rare cases, but mostly happen later in life and are thus acquired; olfactory function evolves over time, and so does OB volume. Patients with a unilateral complete nasal obstruction were subject to a decrease of the ipsilateral OB volume: the absence of olfactory input on one side during a few months resulted in a smaller ipsilateral OB, while the contralateral OB was not affected (Askar *et al.*, 2015). OB volume changes are not only caused by olfactory dysfunction: age, for example, is another factor that can impact OB volume. Indeed, just like olfactory performance, OB volume decreases with age (Hang *et al.*, 2015). On the contrary, olfactory training can increase OB volume. Olfactory

training typically consists of smelling a few odors every day during a few months, and is a potential way for patients to recover from olfactory dysfunction: olfactory training can improve olfactory performance, and these changes correlate with an increase of OB volume (Haehner *et al.*, 2008; Rombaux *et al.*, 2009). Olfactory training can have the same effect in people with a normal sense of smell: participants were tested at the start and at the end of a four-month olfactory training. The specificity of this olfactory training was that only one nostril was trained. The OB on the side of the trained nostril became bigger, and so did the contralateral OB, which confirms the relation between olfactory function and OB volume, and also indicates that the underlying mechanism is complex and involves some top-down process allowing an OB that is not stimulated to grow along with a stimulated OB (Negoias *et al.*, 2017). Indeed, besides receiving input from the olfactory epithelium, OB neuronal activity is modulated by some centrifugal input from cerebral structures such as the primary olfactory cortex, the amygdala, the hippocampus, the locus coeruleus and the raphe nuclei (Lazarini *et al.*, 2011). Changes in OB volume would be due to neurogenesis that still happens in the adult olfactory system, and to synaptogenesis between olfactory receptor neurons and mitral cells in the OB (Curtis *et al.*, 2007; Eavri *et al.*, 2013; Lotsch *et al.*, 2014) (for a review, see (Huart *et al.*, 2013, 2019; Zatorre *et al.*, 2012)).

Though the OB has been the subject of many studies, there has been no report of OB volume in olfactory specialists. Most studies in sommeliers and perfumers also happen to be cross-sectional, which is efficient to compare them with controls, but does not allow to see an evolution over time. We therefore aimed at examining the effects of sommelier training on OB volume and olfactory function. To do that, we tested sommelier students a first time at the start of their training and once again a year and a half later, at the end of their training. We compared sommelier students with a group of students whose training does not involve the sense of smell. We used Magnetic Resonance Imaging (MRI) to measure OB volume and tested olfactory performance by measuring olfactory detection threshold. We hypothesized that (1) OB volume increases during sommelier training; (2) olfactory performance increases during sommelier training; and (3) OB volume and olfactory performance correlate positively.

Materials and methods

Participants

17 sommelier students enrolled at the Institut de Tourisme et d'Hôtellerie du Québec in Montreal took part in this study. The group was composed of 7 women aged 26.1 ± 4.7 years at the time of the first visit, and of 10 men aged 25.7 ± 5.0 years. The control group consisted of 17 students from the University of Montreal or the University of Quebec in Montreal. These participants were chosen to match sommelier students in age and gender and was therefore composed of 7 women aged 26.6 ± 4.3 years, and of 10 men aged 25.6 ± 5.7 years. One sommelier student was excluded from the study because of pregnancy, defined by the Ethics Committee as a contraindication for the MRI scan.

A year and a half later, at the end of sommelier students' professional training, 12 sommelier students and 13 control participants returned for the second part of the study.

Sommelier training

The participants in the sommelier group underwent the International Service and Sommelier Training of the Institut de Tourisme et d'Hôtellerie du Québec in Montreal (https://www.ithq.qc.ca/en/school/future-students/programs/program/international-service-and-sommelier-training/), which is the prerequisite for becoming professional sommeliers. The training consists of 1200 hours of classes; olfactory training takes place in most of these classes as only 45 hours do not involve any sensory analysis. Besides these 1200 hours of classes, there is also a minimum of 905 hours of work experience obtained during different compulsory internships that include 4 months in an English-speaking establishment outside Quebec, 3 months at a Michelin-starred or Relais & Châteaux restaurant in France, and a month at a vineyard in France.

Students in the control group came from different fields of study, e.g. administration, psychology, life sciences, economics, humanities, which did not involve any practical training of the sense of smell.

Olfactory bulb volume

MRI images were acquired at the Unité de Neuroimagerie Fonctionnelle (UNF) at the IUGM. The UNF provides access to a Prisma Fit 3 Tesla MRI scanner from Siemens.

To measure OB volume, we used a standard protocol resulting in 2-mm-thick T2-weighted images in Turbo Spin Echo (TSE) mode. Images were obtained in the coronal plane and there was no gap between the 2-mm-thick slices, with voxel size: 0.16 x 0.16 x 2 mm^3. This method has been described as the most suitable method for OB volumetry (Huart *et al.*, 2013; Seubert *et al.*, 2013).

We used the MIPAV (Medical Image Processing, Analysis, and Visualization) application to measure the OB volume by manually contouring the OB surface on each coronal slice, from anterior to posterior, with pixel size 0.16 x 0.16 mm^2. The first slice (most anterior one) to be considered is the one on which the OB first becomes visible. A sudden decrease in the diameter of the OB marks the posterior end of the OB and allows to identify the last slice to be used in the measurement. Once OB surfaces are delineated on each slice, all surfaces are added up, and multiplied by the slice thickness (2 mm) to obtain the OB volume in mm^3. This approach is commonly used in studies examining OB volumes, and has proven to be reliable and accurate (Huart *et al.*, 2013; Seubert *et al.*, 2013; Yousem *et al.*, 1998).

To obtain a volume of the whole brain, we also acquired a T1-weighted structural volume using an MPRAGE sequence. This sequence provides 176 contiguous sagittal slices with an isotropic spatial resolution of 1 mm^3 (repetition time 2300 ms, echo time 2.26 ms, flip angle 8°, in-plane field of view 256 mm). An automated reconstitution of a tridimensional image of the brain was performed using Freesurfer 6.0 for Linux (http://surfer.nmr.mgh.harvard.edu), which provided us with the volume of the whole brain.

Olfactory performance

Olfactory performance was assessed using an extended version of the Sniffin' Sticks test (Frasnelli *et al.*, 2010; Hummel *et al.*, 1997; Hummel *et al.*, 2007). Sniffin' Sticks are felt-tip pens which are filled with odorants instead of ink. The experimenter presents the odorants to the participant by removing the cap and placing the pen's tip approximately 2 cm in front of both nostrils. Different tasks were completed.

We assessed olfactory performance by measuring (1) odor threshold, (2) odor discrimination, (3) odor identification, and (4) olfactory memory, using the Sniffin' Sticks sets provided for each of these tests and following established procedures (for details, see Frasnelli et al., 2010; Hummel et al., 1997; Hummel et al., 2007). In most studies, the Sniffin' Sticks test is performed birhinally, i.e., both nostrils are tested simultaneously, but it has been shown that olfactory performance assessment is more complete when it is performed unirhinally, i.e., when nostrils are tested separately, especially for the threshold task (Poupon *et al.*, 2017). Therefore, we tested nostrils separately for the odor threshold task, which is the most perceptual task, i.e., this task does not involve as many cognitive factors as the other tasks (Hedner *et al.*, 2010). To do so, the participant closed a given nostril with a finger during each odor presentation, and one nostril was tested after the other. The order of the nostrils was randomized. We therefore obtained a threshold estimate for each nostril.

Odor detection threshold

We assessed odor thresholds for phenylethyl alcohol (PEA) using a single staircase, three-alternative forced choice procedure: we presented participants with triplets of pens, one of them containing the odorant in a given concentration, the two other ones containing the solvent. We used a 16-step geometric series starting from a 4% phenylethyl alcohol solution (dilution ratio 1:2 in deionized aqua conservata as diluent). Triplets were presented at intervals of approximately 20 seconds. Participants had to identify the pen containing the odorant. In this task, we tested the two nostrils separately: the participant closed a given nostril with a finger during each odor presentation, and one nostril was tested after the other. The order of the nostrils was randomized. There were 16 different concentrations available. For each nostril, triplets were presented starting with the lowest concentration, with a randomized order of the three pens; reversal of the staircase was triggered when the odor was correctly identified in two successive trials with a subsequent reversal of the staircase when participants failed to correctly identify the odor. Threshold was defined as the mean of the last four of seven staircase reversal points (Hummel *et al.*, 1997). Scores ranged between 1 and 16, and this for each nostril.

Odor discrimination

To assess odor discrimination, 32 triplets of pens (two pens containing the same odorant, and a third pen containing a different one) were presented. Participants had to identify the target pen, i.e.

the pen containing the different odorant. The 32-triplet discrimination test is an extended version of the commercially available 16-triplets test (Frasnelli *et al.*, 2010; Haehner, Mayer, *et al.*, 2009). The set that is used is actually the one used for the 16-triplet discrimination test, but triplets are mixed: there are 16 triplets labelled from 1 to 16, each triplet being composed of one target pen and two paired pens. In the 32-triplet version, after testing the 16 triplets, 16 additional triplets are created by combining the target pen of triplet 1 with the paired pens of triplet 16, etc. Odors are listed in Hummel et al., 1997. Scores ranged from 0 to 32.

Odor identification

Odor identification was assessed for 16 common odors. Two sets of Sniffin' Sticks are available for this task. The first one that we used at the start of training is composed of the following odors: orange, leather, cinnamon, peppermint, banana, lemon, licorice, turpentine, garlic, coffee, apple, cloves, pineapple, rose, anise, fish. The second one that we used at the end of the training is composed of 16 other common odors: pear, coke, lilac, grapefruit, grass, raspberry, honey, ginger, coconut, lavender, melon, peach, mushrooms, smoked meat, chocolate, onion (Haehner, Mayer, *et al.*, 2009). Notes corresponding to all of these odors can be perceived in wine. Each odorant was presented a first time and participants had to identify it without any cue (free identification). The second time, for each individual odor, a list of four descriptors was presented; participants had to identify the odorant by picking one of them (cued identification). Lists of descriptors for each odorant have been established by the creators of the Sniffin' Sticks test, and were therefore the same for all participants. We obtained two scores: free and cued identification, each ranging from 0 to 16 and corresponding to the number of odors that were correctly identified. In the free identification task, participants scored only if the identification was fully correct, e.g. naming lemon or leather for orange would both count zero point.

Olfactory memory

We assessed olfactory memory by using the two sets of 16 pens designed for the identification task, knowing that at each session, only one set was used for the identification task. Only 8 pens from each set were used for this task; half of the participants were tested with pens labelled with even numbers, the other half with odd numbers. The order of pens was randomized. Participants had to tell whether they had smelled the odorant during the identification task. This task took place about 40 minutes after the identification task.

The score for this task consisted in the sensitivity index d' that we calculated using the signal detection theory (MacMillan *et al.*, 2005): we calculated the numbers of hits (i.e. the participant said an odor was present in the identification task and that odor was indeed present) and of false alarms (i.e. the participant said an odor was present in the identification task but it was not). From that, we calculated sensitivity index d':

$$d' = z(hit\ rate) - z(false\ alarm\ rate)$$

The sensitivity index d' indicates the ability to detect whether odors were present in the identification task: d' = 1 roughly corresponds to 69% of correct answers (hits and correct rejections), d' = 2 roughly corresponds to 95% of correct answers.

We therefore obtained a total of six scores per participant in the olfactory tasks: two scores in the threshold task (right and left nostrils), one score in the discrimination task, two scores in the identification tasks (free and cued), one score in the olfactory memory task.

Analysis

Data were analyzed using the software SPSS 23.0 for Windows.

We performed two analyses. Since we measured left and right OB volumes and tested nostrils separately in the threshold task, we first analyzed these variables on each side. We included the other olfactory tasks in the second analysis where we compared bilateral olfactory performance and total OB volume (left + right OB volumes).

Alpha was set at 0.05 and we used Bonferroni-Holm corrections for multiple comparisons.

Unilateral OB volume and olfactory threshold

OB volume and olfactory threshold were our two dependent variables. For each of them, we performed a repeated measures ANOVA with two within-subject factors: *time* (2 levels: start of training "T1", and end of training a year and a half later "T2"), and *side* (2 levels: left and right). *Group* (2 levels: sommelier students and control participants) was defined as between-subject factor. We also used *whole brain volume* as covariate in the repeated measures ANOVA we performed for OB volume, and performed Pearson correlations to investigate the potential correlations between OB volume and whole brain volume.

Then, we performed post-hoc repeated measures ANOVAs in sommeliers and controls separately, with *time* and *side* as within-subject factors, to investigate if there was a group-specific evolution of OB volume or olfactory threshold between T1 and T2.

Finally, we calculated evolutions ΔT2-T1 for each dependent variable and performed Pearson correlations to examine if there was any correlation between olfactory thresholds and OB volume.

Overall OB volume and olfactory tasks

This analysis included more olfactory scores. Because there were strong correlations between left and right thresholds and between free and cued identification scores, we only kept for this analysis the better threshold, which reflects the score obtained when both nostrils are tested simultaneously (Frasnelli *et al.*, 2002), and the score obtained in the cued identification task, which is most commonly used in studies with the Sniffin' Sticks. We therefore performed a repeated measures ANOVA with two within-subject factors: *time* (2 levels: start of training "T1", and end of training a year and a half later "T2"), and *test* (4 levels: better threshold, discrimination, cued identification, olfactory memory). *Group* (2 levels: sommelier students and control participants) was defined as between-subject factor.

Then, we performed Pearson correlations to examine if there was any correlation between olfactory performance and total OB volume.

Results

Olfactory bulb volume

Figure 8 depicts a coronal slice of a brain zoomed in on the OB, as seen on the MIPAV software. OB volumes are represented on Figure 9.

We found a significant interaction *time*group* ($F_{1,22} = 16.246$, $p = 0.001$) and a significant effect of *whole brain volume* ($F_{1,22} = 16.842$, $p < 0.001$) on OB volumes. There was no significant main effect of *time* ($F_{1,22} = 2.339$, $p = 0.140$), *group* ($F_{1,22} = 1.015$, $p = 0.325$), *side* ($F_{1,22} = 0.007$, $p = 0.934$) or any interaction.

To disentangle the interaction, we compared OB volume at T1 and T2 in each group separately. In the sommelier group, we observed a significant main effect of *time* with OB volume being bigger at T2 than at T1 ($F_{1,11} = 12.028$, $p = 0.005$). In contrast, there was no significant effect of *time* in

controls ($F_{1,12}$ = 0.474, p = 0.504). In other words, the evolution of OB volume over time was significant in sommeliers, but not in controls (Figure 9).

We then investigated the effect of *whole brain volume*; this variable correlated with OB volume (left OB at T1: r = 0.456, p = 0.016; right OB at T1: r = 0.371, p = 0.033; left OB at T2: r = 0.578, p = 0.006; right OB at T2: r = 0.622, p = 0.004). There was no effect of group on whole brain volume ($F_{1,33}$ = 0.939, p = 0.340).

Olfactory performance

Olfactory scores are depicted on Figure 10.

In our first analysis, we found a significant interaction *time*group* ($F_{1,23}$ = 8.951, p = 0.007) and a significant effect of *time* with thresholds at T2 better than at T1 ($F_{1,23}$ = 7.510, p = 0.012), but no effect of *group* ($F_{1,23}$ = 1.435, p = 0.243), *side* ($F_{1,23}$ = 0.241, p = 0.628) or any interaction.

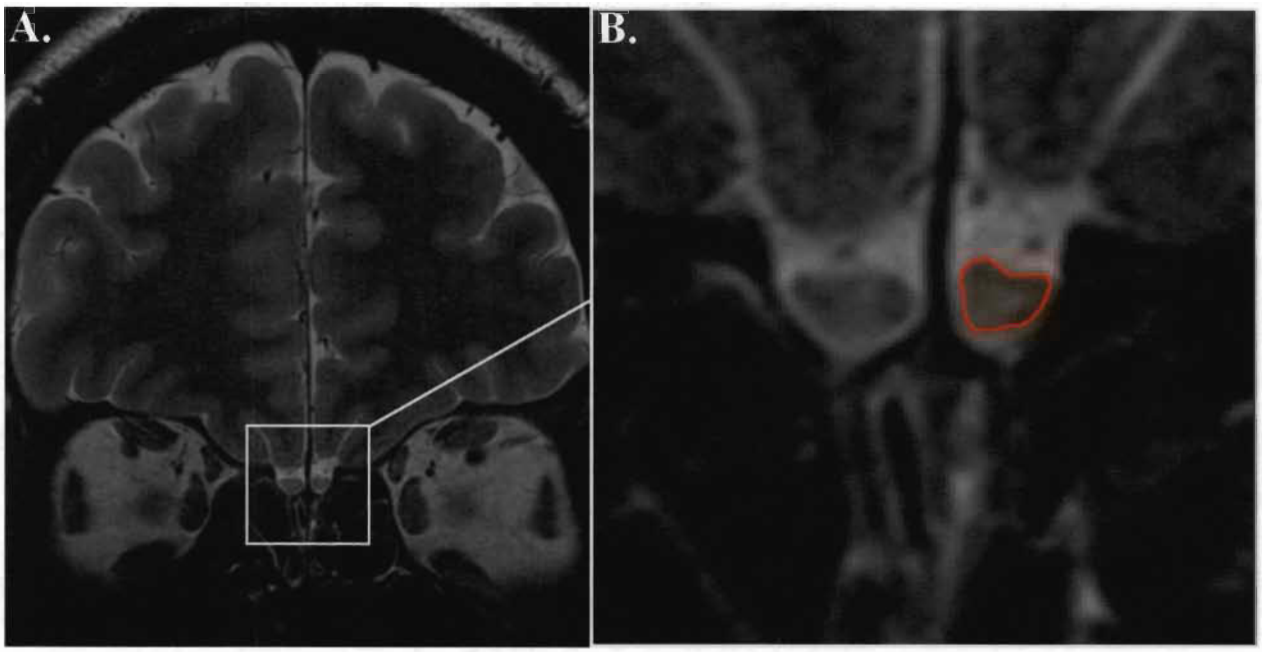

Figure 8. Coronal slice of the brain as seen on MIPAV software.

A. zoomed out, B. zoomed in on the olfactory bulbs. The red line delineates the left olfactory bulb.

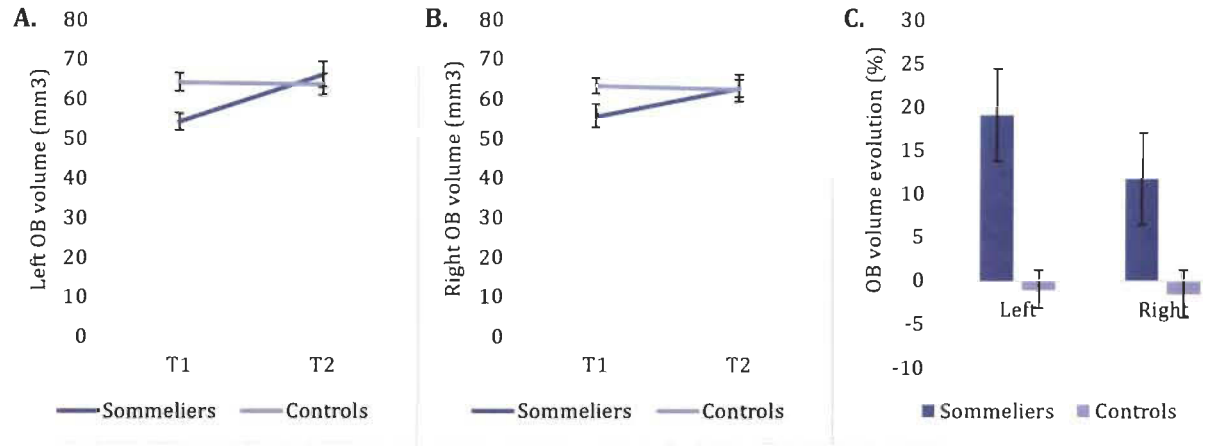

Figure 9. OB volume during sommelier training.

A. and B. Left and right olfactory bulb volume (in mm^3) at the start of training (T1) and at the end of training (T2) in sommelier students (dark) and controls (light). C. Evolution of OB volumes during training (in %) in sommelier students (dark) and controls (light).

To disentangle the interaction, we compared olfactory thresholds at T1 and T2 in each group separately. In sommeliers, there was no main effect of *time* ($F_{1,11} = 0.033$, $p = 0.860$) or any other variable and interaction. In contrast, in controls there was a significant main effect of *time* with olfactory thresholds being better at T2 than at T1 ($F_{1,12} = 29.775$, $p < 0.001$; Figure 10).

In our second analysis, for other olfactory tasks, we found no significant of *group* ($F_{1,23} = 0.176$, $p = 0.678$) or *time* ($F_{1,23} = 0.906$, $p = 0.351$), nor interaction *time*group* ($F_{1,23} = 3.591$, $p = 0.071$), meaning that olfactory scores in general did not significantly evolve between T1 and T2 and there was no overall significant difference between groups.

Correlations between OB volume and olfactory performance

There was no correlation between OB volume and olfactory threshold at T1 and T2 (left: T1: $r = -0.241$, $p = 0.176$; T2: $r = -0.111$, $p = 0.598$; right: T1: $r = -0.012$, $p = 0.947$; T2: $r = 0.084$, $p = 0.690$).

We also found no correlation between evolutions of OB volume and olfactory threshold (left: sommeliers: $r = 0.259$, $p = 0.416$; controls: $r = -0.234$, $p = 0.442$; right: sommeliers: $r = -0.315$, $p = 0.319$; controls: $r = -0.443$, $p = 0.129$).

There was also no significant correlations between OB volumes and other olfactory tests.

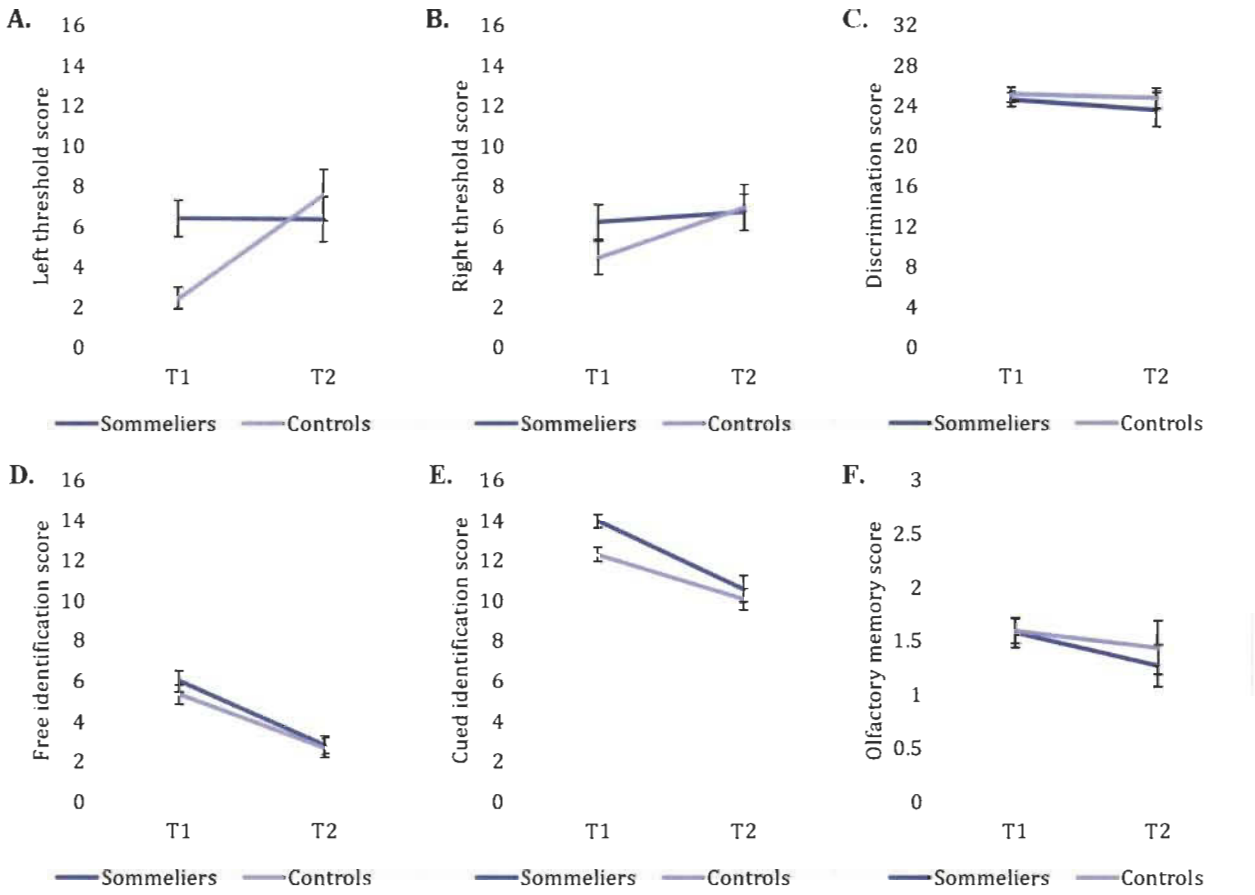

Figure 10. Olfactory performance at the start of training (T1) and at the end of training (T2) in sommelier students (dark) and controls (light).

Scores obtained in the detection threshold task with the left nostril (A.) and the right nostril (B.), in the discrimination task (C.), the free identification task (D.), the cued identification task (E.) and the olfactory memory task (F.).

Discussion

The main result of our study was that OB volume increased during sommelier training, but not in a control group. However, our olfactory tests did not bring to light any improvement of the olfactory function; thus, change in OB volume was not correlated with enhanced scores on the Sniffin' Sticks test.

We report data about OB volume in experts measured twice during a longitudinal study that is conducted in sommelier students. This allowed us to examine the effect of training on OB volume. The increase of OB volume as sommelier students become olfaction experts is a great example of

training-related brain plasticity, and fits with what can be found in literature about OB volume in patients and healthy people with a normal sense of smell: since a greater OB volume is associated with a better sense of smell (Buschhuter *et al.*, 2008; Liu *et al.*, 2017; Rombaux *et al.*, 2006a; Seubert *et al.*, 2013), and that olfactory training results in an increase of OB volume (Haehner *et al.*, 2008; Negoias *et al.*, 2017; Rombaux *et al.*, 2009), it makes sense that becoming a professional leads to an increase of OB volume.

We showed that training led to an increase of OB volume. Four mechanisms have been proposed to explain OB plasticity, i.e. how the OB can grow with training. Synaptogenesis is a first possible mechanism: activity modulates the connections between neurons, i.e. in this case, the synapses between olfactory receptor neurons and mitral cells, and lead to an increase or decrease of the number of synapses, thus modulating the size of the structure (Eavri *et al.*, 2013; Zatorre *et al.*, 2012). Other mechanisms consist in an increase of the number of neurons. Neurogenesis is a remarkable ability of the olfactory system. Continuous neurogenesis occurring at the level of the olfactory epithelium is a second proposed mechanism. Indeed, the olfactory epithelium contains stem cells that can differentiate into new olfactory receptor neurons (Schwob *et al.*, 2010). This regeneration is essential to maintain a functional sense of smell since olfactory receptor neurons are directly exposed to the environment and thus can be damaged. By repeatedly activating the olfactory system, one can hypothesize that olfactory training stimulates neurogenesis in the olfactory epithelium. Olfactory receptor neurons then grow axons which synapse with mitral cells in the OB, which could explain why OB volume increases. A third possible mechanism relies on neurogenesis in the supraventricular zone of the lateral ventricle: neural stem cells produce neuroblasts which migrate toward the OB and differentiate into olfactory interneurons in the OB. These additional cells could explain OB growth. However, this mechanism which has first been demonstrated in adult rodents and monkeys (Kornack *et al.*, 2001; Lois *et al.*, 1996; Ming *et al.*, 2011) is still debated in adult humans. In fact, even if neural stem cells have been observed along the lateral ventricle in humans (Johansson *et al.*, 1999; Sanai *et al.*, 2004), their ability to produce neuroblasts which migrate to the OB is still a matter of debate (Curtis *et al.*, 2007; Sanai *et al.*, 2007; Sanai *et al.*, 2011). Finally, intrinsic bulbar plasticity constitutes a fourth possible mechanism underlying OB plasticity: neural stem cells are present in the adult human OB and it was hypothesized they could be responsible for an increase of the number of cells, and thus OB growth

(Pagano *et al.*, 2000). However, while the study of functional genomics suggests neurogenesis in the OB (Lotsch *et al.*, 2014), no OB neurogenesis has been detected (Bergmann *et al.*, 2015).

While we expected olfactory performance to improve in sommelier students, scores obtained by sommelier students in olfactory tasks were not significantly better at T2 than at T1. Because OB volume increased during sommelier training but their olfactory scores did not evolve, we found no correlation between evolutions of OB volume and olfactory performance. In the control group, there was no significant differences between T1 and T2 for most tests, except in the threshold task in which we observed a surprising improvement of the performance. The improvement of olfactory abilities during an olfactory training cannot always be revealed with the Sniffin' Sticks: studies showed that smelling four odors every day for a few months led to an improvement of the olfactory sensibility but this effect was specific to the odors used during training and could not be detected with the Sniffin' Sticks threshold task (Dalton *et al.*, 2002; Mori *et al.*, 2015). This kind of training also led to an improvement of the ability to identify the four odors but the effect was not generalized and thus undetectable with the Sniffin' Sticks identification task (Mori *et al.*, 2015). It is interesting to mention that these are the results observed in participants with a normal sense of smell, but olfactory training is also used in patients with olfactory dysfunction and it has been reported multiple times that smelling four odors every day during a few months leads to an improvement that is not specific to the odors that were used: scores in the Sniffin' Sticks test improved with training, especially in the discrimination and identification tasks (Altundag *et al.*, 2015; Damm *et al.*, 2014; Fleiner *et al.*, 2012; Geissler *et al.*, 2014; Haehner *et al.*, 2013; Hummel *et al.*, 2009; Konstantinidis *et al.*, 2013). However, in participants with a normal sense of smell, a generalized effect of training was also observed, in a study where olfactory training consisted in tasks that were more complex than just passively smelling odors on a daily basis: the effect of training was measurable with the Sniffin' Sticks test, mostly with the identification task (Al Ain *et al.*, 2019). In our study, since sommelier training involves various complex exercises, we could have expected similar results with greater scores in the identification task, especially because it has also been shown that wine experts perform better than novices in high-order olfactory tasks such as identification, olfactory memory or discrimination of odorants within mixtures, but not in more basic tasks such as olfactory detection thresholds (Parr *et al.*, 2002; Poupon *et al.*, 2018; Poupon *et al.*, 2019). The absence of a significant improvement in the olfactory tasks may be due to the fact that, combined with a number of participants that is not very high, the Sniffin' Sticks test was

initially designed to detect olfactory dysfunction, and might therefore not be suited for accurately discriminating between people with a normal sense of smell and olfaction experts. Indeed, while early during their training, sommelier students outperformed the control group in the identification task (Poupon *et al.*, 2019), they did not improve and even have a tendency to perform less well at the end of their training. When we tested them at the end of their training, we noticed that they had a more analytic approach: during the identification task, they took more time to answer. In the free identification task, while participants from the control group usually gave one answer, sommelier students used different descriptors as they could smell hints of different odors in each pen. Even when they were faced with a list of four descriptors in the cued identification task, they were more hesitant than the control group as they perceived notes corresponding to several of the four descriptors, which mostly happened with the chocolate odor, for which the list of descriptors also included vanilla and biscuit. In other cases, mostly for fruity odors, it was common to hear them say that none of the four descriptors fitted as the odor was too intense and not natural enough, especially compared to the refined nuances they are used to smelling in wine. However, the Sniffin' Sticks test and its procedure did not allow us to measure this feature.

In conclusion, this study aims at examining effects of training-related brain plasticity on the OB. Unlike other studies in which olfactory training consists of smelling a few odors every day during several weeks, the olfactory training we evaluated here is not as experimental since it is a sommelier training leading students to become professionals. OB volume increased during their training, which constitute the first data we have about OBs in olfaction experts. Further studies should examine OB volume in sommeliers with more or less experience to test, for example, whether the OB keeps getting bigger over years, or if at some point a limit is reached and the OB stops growing.

Ethical statement

This study was approved by the Ethics Committee of the Institut Universitaire de Gériatrie de Montréal (IUGM) research center, the Ethics Committee of the Institut de Tourisme et d'Hôtellerie du Québec in Montreal, and the Ethics Committee of the University of Quebec in Trois-Rivières, Canada. All participants gave informed written consent to participate. There was no conflict of interest.

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Chapitre 5 – Sommelier training leads to both increases and decreases of cortical thickness

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Daphnée Poupon[1], Sarah Banks[2], Pauline Fernandez[3], Johannes Frasnelli[1,4]

Affiliations: 1 Department of Anatomy, Université du Québec à Trois-Rivières, Canada

2 University of California, San Diego, United States

3 Institut du Tourisme et d'Hôtellerie du Québec, Montréal, Canada

4 Research Centre, Sacré Coeur Hospital, Montréal, Canada

Abstract

Sommeliers' brains display structural differences due to their expertise. While most studies on this topic are cross-sectional, we used a longitudinal design to test sommelier students at the start and at the end of their training that we compared to a control group to study the effects of training-related brain plasticity. More specifically, we used magnetic resonance imaging (MRI) to measure cortical thickness and diffusion-weighted imaging (DWI) to measure fractional anisotropy. Combining these two methods allowed us to explore both the cortex and the white matter. We found that, during sommelier training, thickness of the right entorhinal cortex significantly increased, but also that cortical thickness decreased in other brain areas. We did not observe any significant changes in the white matter. Our findings about cortical thickness changes support the "overproduction-pruning" model of brain plasticity, according to which the effects of training-related plasticity are nonlinear and simultaneously involve different processes.

Keywords: plasticity, sommeliers, training, cortical thickness, diffusion, magnetic resonance imaging

Introduction

Brain plasticity allows experts to acquire the skills they need: through training, they acquire and refine abilities that come with changes in brain structure and function. Effects of training-related brain plasticity can be observed in top athletes, musicians, or in professionals whose jobs require specific skills. For example, structural differences were observed in the hippocampus of London taxi drivers: the posterior hippocampus is larger while the anterior hippocampus is smaller than average. This makes sense since the hippocampus is involved in spatial memory. More precisely, the anterior hippocampus is involved in encoding new spatial information while the posterior hippocampus is involved when previously encoded spatial information is used; with years of experience, taxi drivers know London better and better and thus need less and less encoding of new spatial information and more and more using of their mental map of the town (Maguire *et al.*, 2000). Likewise, brain changes facilitate visuo-spatial processing and coordination in professional badminton players (Di *et al.*, 2012), grant musicians refined hand motor skills (Amunts *et al.*, 1997), and allow radiologists to process and interpret radiographs more effectively (Harley *et al.*, 2009).

Training-related changes in the brain can be structural or functional and can occur in both the cortex and the white matter. These changes can be detected with neuroimaging techniques such as magnetic resonance imaging (MRI) which allows to study different aspects of the brain. Measuring cortical thickness is efficient to evaluate changes in the grey matter. Numerous studies reported that training and expertise in different domains can impact cortical thickness. Auditory cortex as well as frontal regions involved in high cognitive function were reported to be thicker in musicians (Bermudez *et al.*, 2009). In another study, a nine-month training of social skills resulted in cortical thickness changes in well-known socio-affective and socio-cognitive brain networks (Valk *et al.*, 2017). The ability to perceive and identify odors is also related to brain anatomy: correlations were found between olfactory performance and cortical thickness in olfactory regions but also in some non-olfactory regions (Frasnelli *et al.*, 2010). Another study targeting sommeliers reported that these experts in olfaction display a thicker entorhinal cortex, which plays a key role in olfactory processing (Banks *et al.*, 2016). Being an expert is not needed to observe the effects of training-

related brain plasticity: training novices for six weeks in different olfactory tasks and testing them before and after training showed that cortical thickness increased in regions such as the right entorhinal cortex, the right inferior frontal gyrus and the bilateral fusiform gyrus (Al Ain *et al.*, 2019).

Diffusion-weighted imaging (DWI) is efficient to examine changes in white matter. This MRI method is based on water diffusion through the brain. Diffusion is directionally more or less constrained in different parts of the brain: in ventricles, fluids are free to diffuse in any direction whereas in a nerve tract, axons and myelin constitute barriers that constrain water to diffuse along nerve fibers. DWI allows to measure fractional anisotropy (FA), which indicates at each voxel how constrained water diffusion is: FA ranges from 0, which would indicate fluids can diffuse in any direction, to 1, in regions where diffusion is limited to only one direction. This measure is sensitive to different properties of nerve tracts such as axon diameter and packing density, quantity of myelin, axon permeability and fiber geometry (Zatorre *et al.*, 2012). Therefore, measuring FA can allow to track white matter changes. Training-related brain plasticity can lead to such changes: DWI was for example used to show that a two-month working memory training led to local increases of FA which would be due to myelination (Takeuchi *et al.*, 2010).

Many studies about brain plasticity and expertise are cross-sectional. In the field of olfaction, only a few have used a longitudinal design to examine the effects of training-related brain plasticity using MR imaging. The main study doing that consisted in testing adults with a normal sense of smell before and after a six-week-long olfactory training (Al Ain *et al.*, 2019). In our study, we were interested in the effects of a long-term olfactory training: we tested sommelier students at the start and at the end of their year-and-a-half-long training. Our aim was to evaluate how the brains of these future experts evolved during training. Our hypothesis was that training-related brain changes would include local variations of cortical thickness and fractional anisotropy.

Materials and methods

Participants

17 sommelier students enrolled at the Institut de Tourisme et d'Hôtellerie du Québec in Montreal took part in this study. The group was composed of 7 women aged 26.1 ± 4.7 years at the time of the first visit, and of 10 men aged 25.7 ± 5.0 years. This first visit took place between 3 and 9 weeks

after the start of their training. The control group consisted of 17 students from the University of Montreal or the University of Quebec in Montreal. These participants were chosen to match sommelier students in age and gender and was therefore composed of 7 women aged 26.6 ± 4.3 years, and of 10 men aged 25.6 ± 5.7 years. One sommelier student was excluded from the study because of pregnancy, defined by the Ethics Committee as a contraindication for the MRI scan.

A year and a half later, at the end of sommelier students' professional training, 12 sommelier students and 13 control participants returned for the second part of the study.

Sommelier training

The participants in the sommelier group underwent the International Service and Sommelier Training of the Institut de Tourisme et d'Hôtellerie du Québec in Montreal (https://www.ithq.qc.ca/en/school/future-students/programs/program/international-service-and-sommelier-training/), which is the prerequisite for becoming professional sommeliers. The training consists of 1200 hours of classes; olfactory training takes place in most of these classes as only 45 hours do not involve any sensory analysis. Besides these 1200 hours of classes, there is also a minimum of 905 hours of work experience obtained during different compulsory internships that include 4 months in an English-speaking establishment outside Quebec, 3 months at a Michelin-starred or Relais & Châteaux restaurant in France, and a month at a vineyard in France.

Students in the control group came from different fields of study, e.g. administration, psychology, life sciences, economics, humanities, which did not involve any practical training of the sense of smell.

Brain imaging

MRI images were acquired at the Unité de Neuroimagerie Fonctionnelle (UNF) at the IUGM. The UNF provides access to a Prisma Fit 3 Tesla MRI scanner from Siemens.

T1-weighted MRI

To measure cortical thickness, we acquired a T1-weighted structural volume using an MPRAGE sequence. This sequence provides 176 contiguous sagittal slices with an isotropic spatial resolution of 1 mm3 (repetition time = 2300 ms, echo time = 2.26 ms, flip angle = 8°, in-plane field of view = 256 mm).

Diffusion-weighted MRI

To analyze fractional anisotropy in different parts of the white matter, we used a sequence defined by the following parameters: repetition time = 3000 ms, echo time = 68 ms, flip angle = 90°, in-plane field of view = 220 mm, voxel size = 2x2x2 mm^3, 66 slices. The diffusion weighting was isotropically distributed along 64 directions for b-values of 1000 s/mm^2, 2000 s/mm^2 and 3000 s/mm^2. Additionally, 5 images with no diffusion weighting (b-value = 0 s/mm^2) were acquired, leading to a total of 197 images for each participant.

Analysis

The analysis was performed with FreeSurfer 6.0 for Linux (http://surfer.nmr.mgh.harvard.edu).

Cortical thickness

Measuring cortical thickness consists in reconstituting a tridimensional image of the brain, modelling white surface (at the limit between white and grey matter) and pial surface (between grey matter and cerebrospinal fluid), and measuring the distance between these two surfaces.

The automated reconstitution of a tridimensional image of the brain performed by FreeSurfer involves skull stripping, volumetric labeling, intensity normalization, white matter segmentation, surface extraction and gyral labeling. For each hemisphere, each surface is made of about 140,000 vertices each defined by X, Y and Z coordinates. Vertices of the white surface and the pial surface have the same identity: each vertex of the white surface has a corresponding vertex in the pial surface, which allows to calculate the distance between the two surfaces, that is to say the cortical thickness.

Because we have longitudinal data, we used FreeSurfer's longitudinal stream which consists of three preprocessing steps. The first step is a cross-sectional processing corresponding to the reconstitution of a tridimensional image of the brain as described in the previous paragraph, independently for each time point. The output is then used in the second step to create a within-subject template corresponding to the average anatomy of the participant across time. The third step uses this within-subject template to create, for each time point, final results that are more accurate and reliable than the independent cross-sectional runs. Once this preprocessing was done for each participant, we computed longitudinal data from cortical thickness measures at T1 (first

time point, at the start of training) and T2 (second time point, at the end of training). These longitudinal data included:

- the average thickness across time: $avg = (thickness\ T1 + thickness\ T2) / 2$
- the rate of change in mm/year: $rate = (thickness\ T2 - thickness\ T1) / (T2 - T1)$
- the symmetrized percent change: $SPC = 100 * rate / avg$

Additional postprocessing steps include smoothing using a 5 FWHM kernel and resampling onto FreeSurfer average subject FSaverage.

Finally, a group analysis was performed using a general linear model (GLM) with *SPC* as our dependent variable and *group* as our between-subject factor. A correction for multiple comparisons can be done by Monte Carlo cluster-wise simulation. Results were thresholded at $p < 0.05$ when corrected for multiple comparisons, or at $p < 0.0001$ for predicted regions. FreeSurfer stores significance as -log10(p-value); a significance of 4 and more corresponds to $p < 0.0001$ uncorrected.

For more details, see https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/LongitudinalTutorial.

Fractional anisotropy

Reconstruction of the diffusion data from the collected images involves registering all diffusion volumes to a low-b target (an image with no diffusion weighting, b-value = 0 s/mm^2, whose contrast is better), so that all diffusion-weighted images are aligned with one another. In addition to that, FreeSurfer uses the anatomical reconstruction to register, for each participant, the diffusion data with the anatomical data. This automated reconstruction results in a volume depicting fractional anisotropy (FA) throughout the brain. FA values range from 0 (isotropic, water molecules can move in any direction) to 1 (highly anisotropic, water molecules diffusion is highly constrained). The next step consists in resampling the white-matter parcellation and subcortical segmentation from the anatomical analysis into the diffusion space so that FA values in regions of interest (ROIs) can be extracted. The group analysis uses a general linear model (GLM) with *FA* as our dependent variable, *time* as our within-subject factor and *group* as our between-subject factor.

For more details, see https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/DiffusionV6.0.

Results

Cortical thickness

When applying a correction for multiple comparisons, we found no significant cluster. When we lowered the threshold to $p < 0.0001$ uncorrected, we observed that sommelier training had a significant effect on cortical thickness in several clusters: in sommelier students, there was an increase of cortical thickness in the right entorhinal cortex, and a decrease of cortical thickness in the left inferior temporal gyrus, the triangular portion of the right inferior frontal gyrus (pars triangularis), the left superior parietal and superior frontal gyri (for more details, see Table 6 and Figure 11).

Table 6. Significant effects of sommelier training on cortical thickness.

Effect of group on the following structures was significant at a $p < 0.0001$ uncorrected level. Coordinates (x, y, z) are in the MNI space. The size corresponds to the number of vertices where a significant difference was observed. Sig = -log10(p-value); a significance of 4 and more corresponds to $p < 0.0001$, a positive significance indicates an increase of cortical thickness in sommeliers compared to controls while a negative significance indicates a decrease.

Region	Coordinates			Size	Sig.
	x	y	z		
L inferior temporal gyrus	-52.7	-61.9	-3.9	28	-5.71
R entorhinal cortex	28.8	-7.9	-32.8	16	5.38
R pars triangularis	52.8	28	3.3	3	-4.23
L superior parietal gyrus	-29.7	-52.6	51.7	8	-4.23
L superior frontal gyrus	-21.2	23.9	47.5	2	-4.12

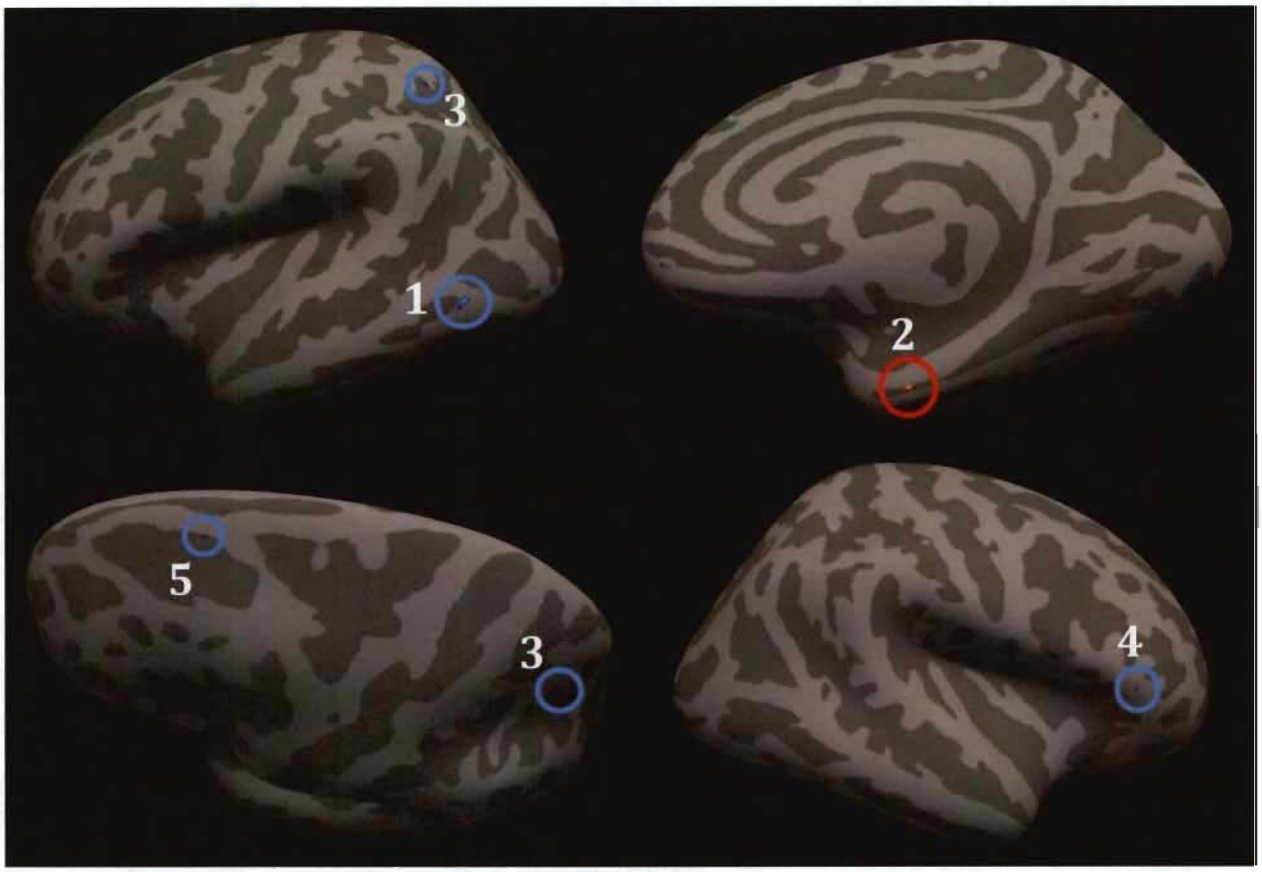

Figure 11. Effect of sommelier training on cortical thickness.

Comparison of symmetrized percent change (SPC) over training between sommelier and control students: blue clusters indicate that cortical thickness in sommelier students decreased during training while the red and yellow cluster indicates an increase of cortical thickness. A. Lateral view of the left hemisphere. B. Inferomedial view of the right hemisphere. C. Superolateral view of the left hemisphere. D. Lateral view of the right hemisphere. $p < 0.0001$ uncorrected: 1. Inferior temporal gyrus, 2. Entorhinal cortex, 3. Superior parietal gyrus, 4. Pars triangularis of the inferior frontal gyrus, 5. Superior frontal gyrus.

White matter fractional anisotropy

We found no significant effect of *group* on fractional anisotropy ($F(1;23) = 0.052$, $p = 0.822$). There was a significant effect of *time* ($F(1;23) = 12.110$, $p = 0.002$) but since there was no

significant interaction *group*time* ($F(1;23) = 2.197$, $p = 0.152$), the effect of *time* is not due to sommelier training.

Discussion

We found that sommelier training led to changes in cortical thickness in five different regions. We did not observe any significant effect of sommelier training on fractional anisotropy.

In sommelier students, cortical thickness increased in the right entorhinal cortex. A similar observation was reported following a six-week-long olfactory training (Al Ain *et al.*, 2019). This result makes sense as the entorhinal cortex is one of the primary olfactory regions (Patel *et al.*, 2014) and its volume is greater in sommeliers, with its cortical thickness positively correlated to years of experience (Banks *et al.*, 2016).

We also observed in sommelier students a decrease of cortical thickness in the left inferior temporal gyrus (ITG), the triangular portion of the right inferior frontal gyrus (tIFG), the left superior parietal gyrus (SPG) and the left superior frontal gyrus (SFG). We did not expect cortical thickness to decrease because greater olfactory abilities are usually associated with larger brain structures and thicker cortices, as several studies reported (Al Ain *et al.*, 2019; Banks *et al.*, 2016; Buschhuter *et al.*, 2008; Frasnelli *et al.*, 2010; Hummel *et al.*, 2003; Seubert *et al.*, 2013). Most of these studies are cross-sectional and compare two groups of individuals instead of examining the evolution of cortical thickness like we did, but the longitudinal study (Al Ain *et al.*, 2019) also seemed to validate the idea that cortical thickness increases with training: all the changes they observed after their six-week-long olfactory training were local increases of cortical thickness, including in two brain regions where we observed a decrease namely the left ITG and the right tIFG. However, in fields other than olfaction, there have been reports of learning-dependent decreases of cortical thickness, for example following a nine-month training of social skills (Valk *et al.*, 2017) or after a week-long training aiming at improving processing speed (Takeuchi *et al.*, 2011), which suggest that cortical thinning could have a role in learning. Another idea is that the progression of learning-dependent changes is nonlinear: this was the theory supported by a team who observed that, over a seven-week-long training during which right-handed participants practiced writing and drawing with the left hand, cortical thickness increased in the first four weeks but then decreased again despite continued practice and increasing task proficiency (Wenger *et al.*, 2017). This led to the 'overproduction – pruning' model of plasticity according to which, first, the number of synapses

increases greatly at the beginning and, then, behaviorally-relevant connections are stabilized while connections that prove to be functionally irrelevant are eliminated by pruning (Lindenberger *et al.*, 2017). This model is supported by evidence provided by two-photon microscopy in mice during motor training: rapid formation of new dendritic spines was followed by a slower process of spine elimination while newly-formed and retained dendritic spines are stabilized and probably function as the physiological substrate for skill acquisition and improvement (Xu *et al.*, 2009). This model is in line with previous findings supporting the idea that changes in the brain appear quickly: in a study where participants were tested several times during a five-week-long juggling training, increases of cortical thickness were visible after only a week, leading the authors to suggest that learning a new task has more impact on brain structure than continued training of an already-learned task (Driemeyer *et al.*, 2008). Finally, this model would explain why we found that cortical thickness decreased in brain regions where Al Ain et al. observed an increase: in Al Ain et al.'s study, olfactory training lasted only six weeks and participants were tested before it started while, in our study, training lasted a year and a half and we tested sommelier students when their training had already started, mostly during the second month. Therefore, because timing was different, it is possible that Al Ain et al. observed the increase of cortical thickness that happens at the start of training during a first phase of overproduction, while we first tested our participants when they were possibly already near the end of this first phase and thus, we observed a decrease due to a second phase during which more synapses would be eliminated by pruning than newly formed. Those dynamic changes over time support the idea that training-related brain plasticity has complex nonlinear effects that involves several processes.

Apart from the entorhinal cortex, which is known as an olfactory processing area, the brain regions where we observed changes in cortical thickness are not typically associated with olfaction, but previous studies still provide explanations. The tIFG, or pars triangularis, is involved in higher order processing of olfactory function: in participants with a normal sense of smell, this region is activated when judging the familiarity of odors (Plailly *et al.*, 2005). It is also activated in response to olfactory stimulation in patients with Parkinson's disease whose olfactory abilities are untypically well-preserved (Hummel *et al.*, 2010; Welge-Lussen *et al.*, 2009; Westermann *et al.*, 2008), and a positive correlation between density of this region and the ability to identify odors has also been reported in patients with corticobasal syndrome (Pardini *et al.*, 2009). In the ITG and the SFG, two other regions where we observed changes in cortical thickness, grey matter volumes have

been reported to be reduced in anosmic patients, i.e. patients with a complete loss of smell (Peng *et al.*, 2013). The SFG is involved in higher cognitive functions and especially working memory (du Boisgueheneuc *et al.*, 2006; Klingberg, 2006). The ITG is an area involved in visual processing as part of the ventral stream, which plays a role in object recognition (Kupers *et al.*, 2011; Kupers *et al.*, 2014; Mishkin *et al.*, 1983; Ungerleider *et al.*, 1994). The SPG, finally, also responds to visual stimulation as part of the dorsal stream (Stickel *et al.*, 2019). Visual areas have been reported to be activated during purely olfactory tasks (Dade *et al.*, 2002; Zatorre *et al.*, 2000), and there was evidence that stimulating the visual cortex by transcranial magnetic stimulation improves performance in odor quality discrimination (Jadauji *et al.*, 2012), which explains why areas involved in visual processing would be impacted by olfactory training.

Maybe because our number of participants was rather small, our study did not allow us to observe any effect of sommelier training on fractional anisotropy. Therefore, we do not have any conclusive results to report about changes due to training-related plasticity in the white matter.

The main findings of our study support the overproduction-pruning model of plasticity. According to this model, changes in the brain are nonlinear as several processes are involved. During a first phase, by repeatedly recruiting the same neural networks, training would stimulate an overproduction of synapses that would result in an increase of cortical thickness. During a second phase, while synaptogenesis would slow down, synaptic pruning would eliminate all behaviorally-irrelevant synapses, leading to a decrease in the number of connections and thus a decrease in cortical thickness. One can assume that there could be a third phase during which continued practice would have little effect on the brain and the number of synapses would be mostly stabilized, or slightly increase with time which would explain reports of positive correlations between cortical thickness and years of experience (Banks *et al.*, 2016). Probably depending on factors such as inherent plasticity and relevance for the trained task, it would make sense that synaptogenesis and synaptic pruning happen at different rates and different degrees in different regions of the brain; the dynamic of cortical thickness changes would vary from one brain region to another, which would explain why we found that cortical thickness increased in a brain region while it decreased in others. Future longitudinal studies should be designed while keeping in mind the idea that the effects of training-related plasticity are not linear: having more than two time points would be ideal to fully observe the effects of training-related brain plasticity.

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Chapitre 6 – Can the identification of odorants within a mixture be trained?

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Daphnée Poupon[1], Pauline Fernandez[2], Salomé Archambault Boisvert[1], Chloé Migneault-Bouchard[1], Johannes Frasnelli[1,3]

Affiliations: 1 Department of Anatomy, Université du Québec à Trois-Rivières, Canada

2 Institut du Tourisme et d'Hôtellerie du Québec, Montréal, Canada

3 Research Centre, Sacré Coeur Hospital, Montréal, Canada

Abstract

Identifying odors within mixtures is a difficult task: humans are able to recognise only up to four odors within a mixture. We wanted to test the effects of olfactory training on this ability. We used seven odorants to create 35 olfactory stimuli of one, two, three, four or five odorants. The task consisted in identifying the odorants present within the mixture. We trained novices on this task for five days: they came to the lab to perform the task once a day before coming back for the final testing. Then, we compared them to sommeliers, thus olfaction experts, and untrained novices. Results showed that sommeliers outperformed the other groups with mixtures of up to four odorants but not with mixtures of five odorants. The short olfactory training allowed trained participants to perform as well as sommeliers when it came to identifying single odorants, but was not enough to improve their performance when stimuli were mixtures of two or more odorants. This study supports the idea that the number of odors we can recognise within a mixture is limited but suggests training can improve the performance: a short olfactory training is enough to enhance the ability to identify single odorants, while expertise refines identification ability of mixtures of up to four odorants.

Keywords: olfaction, mixtures, identification, expertise, sommeliers, training

Introduction

The sense of smell provides us with knowledge of our chemical environment. While most of the odors surrounding us are mixtures, it is rather uncommon to encounter a monomolecular odorant, and identifying single odor components within a mixture is therefore difficult. This implies that, in an environment where numerous odors surround us, some of them might be masked by others and consequently go unnoticed, thus making us miss the important information they might be carrying. For example, spoiled food will release an odor that will warn us about a potential danger and stop us from eating it. We are able to recognize this smell even in the presence of many other food odors that can be found in the kitchen. Being unable to identify an individual odor among all of them would make us miss the warning of spoiled food.

Different research teams developed olfactory tasks in which diverse odorants were combined into various mixtures. They concluded that humans are able to identify up to four components within a mixture (Jinks *et al.*, 1999; Livermore *et al.*, 1998a). This limit appears to be independent of odor types and of cognitive factors, and rather seems to reflect a physiological inability to process an important amount of information about odors perceived simultaneously (Laing *et al.*, 1989; Livermore *et al.*, 1998b; Thomas-Danguin *et al.*, 2014). In mixtures, some odorants can blend to form a new odor that keeps only few characteristics of the initial odorants, thus making recognition of the components difficult (Barkat *et al.*, 2012). Being exposed to the individual components before smelling the mixture enhances the perception of the components, but the task still remains challenging (Le Berre *et al.*, 2010).

Sommeliers are olfaction experts. Extensive training and professional practice provide them with greater olfactory abilities: they can detect wine-related odors at lower concentrations and discriminate between similar wine-related odors better than novices (Majid *et al.*, 2017). They also score better at identification tasks, even if this enhanced ability is not generalised to all odors (Bende *et al.*, 1997). Their profession requires the ability to detect and identify the different aromas that can be found in wine. One may think that years of experience increase the number of odorants that can be identified within a mixture, but it has been reported that the limit also applies to experts: for mixtures of four or more odorants, neither experts nor novices are able to recognise the components. However, experts process mixtures of two or three compounds more efficiently as

they can recognise a particular odorant with a higher accuracy than novices (Livermore *et al.*, 1996).

Sommeliers acquire their expertise by yearlong training. Their training includes learning how to smell and taste wine, as well as acquiring general knowledge about wine and different vineyards around the world. Once they are professionals, they practice on a daily basis. Their training is different from and more diversified than olfactory training as it is usually used in scientific studies, which consists of repeating the same task every day for a certain amount of time: in the past decade, olfactory training has been used to improve olfactory function of patients suffering from hyposmia or anosmia, i.e. partial or complete loss of the sense of smell (Altundag *et al.*, 2015; Damm *et al.*, 2014; Fleiner *et al.*, 2012; Geissler *et al.*, 2014; Haehner *et al.*, 2013; Hummel *et al.*, 2009; Kollndorfer *et al.*, 2014; Kollndorfer *et al.*, 2015; Konstantinidis *et al.*, 2013). Olfactory training has also proven to be effective in individuals with a normal sense of smell, for example by increasing their sensitivity to specific odors (Dalton *et al.*, 2002; Livermore *et al.*, 2004; Rabin *et al.*, 1986). Effects of olfactory training can appear early: while it can last a few months when it is designed to help patients recover their sense of smell, other studies reported effects after only three weeks (Wang *et al.*, 2004), one week (Livermore *et al.*, 2004), or even after repeating a test three times in a row (Rabin *et al.*, 1986). Usually, olfactory training effects are measured with commercially available tests such as the Sniffin' Sticks test, a test with established procedures that can be used to examine different aspects of the sense of smell such as the capacity to detect, discriminate, and identify odors, or the UPSIT, which provides another way to evaluate the ability to identify odors (Doty *et al.*, 1984). However, these tests do not provide any information about the effects of olfactory training on odor mixtures perception; while studies have reported the effect of expertise on the ability to identify odorants within a mixture, we do not have data about those of an olfactory training performed in experimental conditions.

We aimed at examining the ability to recognise odorants within a mixture in sommeliers, trained and untrained novices. To do that, we trained a group of novices for five days on a task in which they were asked to identify the components of different odor mixtures, then tested them on this task and compared them to a group of sommeliers and a group of untrained novices. This design allowed us to test the effects of two types of training. Our hypotheses were that 1) sommeliers perform better than novices, 2) trained novices perform better than untrained novices.

Materials and methods

This study was approved by the Ethics Committee of the University of Quebec in Trois-Rivieres, Canada.

Participants

All participants gave informed written consent to participate.

Participants were distributed into three groups. The first group was constituted of professional sommeliers from the Institut de Tourisme et d'Hôtellerie du Québec (N=10, 7 women, age 35.6 ± 6.6). The second group was constituted of trained novices (N=18, 13 women, age 24.3 ± 4.5). Before being tested on the task described below, this group of novices was trained daily for five days on this same task. The third group was constituted of untrained novices (N=19, 10 women, age 24.2 ± 3.6).

We made sure participants did not have olfactory disorders with an identification task performed using the Sniffin' Sticks test and following the established procedure (Hummel *et al.*, 1997). In this task, 16 odorants were presented in a 4-alternative forced-choice paradigm and participants had to identify them. Scores ranged from 0 to 16. A score below or equal to 11 indicates hyposmia (Hummel *et al.*, 2007). At first, we had 20 trained novices and 20 untrained novices, but we excluded three participants because of their low score at the task.

Table 7: Odorants used to prepare mixtures. Note that odors were not diluted.

Odorant	Odor description	Initial concentration (%)	Quantity (mg)
Acetic acid	Vinegar	≥99	40
Benzaldehyde	Almond	≥99	50
L-carvone	Mint	99	100
Ethyl octanoate	Liquorice	≥99	240
Eugenol	Clove	99	50
Limonene	Citrus	93	330
α-pinene	Pine	98	530

Olfactory stimuli

We used seven odorants, their common description is given between brackets: limonene (citrus), L-carvone (mint), α-pinene (pine), eugenol (clove), ethyl octanoate (liquorice), acetic acid (vinegar), benzaldehyde (almond; see Table 7).

A set of 35 olfactory stimuli was used for the task, and included seven monomolecular stimuli, seven mixtures of 2 odorants, seven mixtures of 3 odorants, seven mixtures of 4 odorants, and seven mixtures of 5 odorants.

We had three different sets constituted of different combinations of odorants (see Table 8). This allowed to present mixtures in different orders and avoid effects that could be due to a specific series of olfactory stimuli. The odorants we chose have been used in previous studies and shown not to mask each other when combined in a bimolecular mixture (Laing *et al.*, 1989). Their relative concentration in the mixtures was selected so that they were isointense. To do that, we tested different concentrations in a pilot study and, in a first phase, asked volunteers to evaluate relative intensities of the different odorants. In a second phase, we asked professionals from the Institut de Tourisme et d'Hôtellerie du Québec (sommeliers, chefs, maîtres d'hôtel) to match different odorants depending on their intensity. We also asked these professionals to name the odorants and used their answers to define odorants' odor descriptions (e.g. almond, clove, pine). These professionals were not those who participated to the main study. Mixtures could only be smelled, not tasted.

Following the compositions described in Table 8, mixtures were prepared in 30 mL glass bottles with 3mL syringes: a makeup sponge was placed at the bottom of each bottle, odorants were dropped directly on the sponge. They were prepared every day and kept at room temperature.

Olfactory tasks

The task consisted in determining which odorants were present within the mixtures. One of the three sets was randomly attributed to each participant. To familiarise themselves with the odorants, participants were allowed to smell the seven individual odorants as long as they wanted before the beginning of the test. Then, bottles were presented one after the other to the participant. A 30-second period was given to smell the bottle. Then, the participant had 35 seconds to identify the odorants present within the mixture by selecting them from the list of seven odorants. A feedback

was provided: after each trial, the participant was told how many and which odorants were present within the mixture. After seven bottles, a 5-minute pause was taken to avoid olfactory fatigue. Following this procedure, the 35 bottles of the set were presented to the participant. For each mixture, we recorded hits (i.e. the participant indicated the presence of an odorant that was present in the mixture) and false alarms (i.e. the participant indicated the presence of an odorant that was not present in the mixture).

Table 8: Combinations of odorants in mixtures of the three different sets A, B and C. Odorants are 1) acetic acid, 2) benzaldehyde, 3) L-carvone, 4) ethyl octanoate, 5) eugenol, 6) limonene, 7) α-pinene.

	Set A							Set B							Set C						
Odorants	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1 odorant	x							x							x						
		x							x							x					
			x							x							x				
				x							x							x			
					x							x							x		
						x							x							x	
							x							x							x
2 odorants	x					x		x	x						x				x		
		x	x					x		x					x						x
		x		x				x			x					x			x		
		x					x		x				x				x	x			
			x				x			x		x					x			x		
				x	x						x			x				x		x	
						x	x						x	x					x		
3 odorants	x	x	x					x	x		x				x		x	x			
	x	x			x			x		x				x	x		x		x		
	x			x		x		x				x		x	x					x	x
		x			x	x			x	x		x				x	x			x	
			x	x			x		x				x	x		x		x	x		
			x		x		x			x	x		x			x		x			x
				x		x	x				x	x	x						x	x	x
4 odorants	x	x	x	x				x	x	x				x	x	x	x			x	
	x	x			x	x		x	x		x			x	x	x		x	x		
	x		x			x	x	x			x	x		x	x	x			x		x
	x			x	x	x			x	x	x		x		x		x		x	x	
			x	x		x	x		x	x		x	x		x		x		x		x
			x		x	x	x		x		x	x	x			x	x	x			x
				x	x	x	x			x	x	x	x	x		x		x	x	x	
5 odorants	x	x	x			x	x	x	x	x		x	x		x	x	x	x	x		
	x		x	x	x		x	x	x	x		x		x	x	x	x	x		x	
	x			x	x	x	x	x	x		x	x	x		x	x	x	x			x
		x	x	x	x	x		x	x		x		x	x	x	x		x	x		x
		x	x	x		x	x	x	x			x	x	x	x		x	x	x	x	
		x	x		x	x	x	x		x	x		x	x	x		x		x	x	x
		x		x	x	x	x			x	x	x	x	x		x	x	x	x		x

Olfactory training

Participants from the trained novices group underwent a five-day olfactory training prior testing: every day, they came to the lab and were asked to determine the compositions of 35 mixtures

following the procedure described above. Like during the final task, a feedback was provided after each trial. The set used for final testing was different from those used during training, e.g., participants trained with sets A and B were tested with set C.

Analysis

Data were analysed with SPSS 23.0.

We used the signal detection theory to analyse data from the task with odor mixtures (MacMillan *et al.*, 2005). Our data consisted of the numbers of hits and of false alarms for each participant and each mixture. From that, we calculated the average numbers of hits and false alarms for a given number of odorants; therefore, we had for each participant the numbers of hits and false alarms for 1, 2, 3, 4, and 5 odorants. We could then calculate sensitivity index d':

$$d' = z(hit\ rate) - z(false\ alarm\ rate)$$

The sensitivity index d' follows a normal distribution and indicates the ability to detect an odorant within the mixture: d' = 1 roughly corresponds to 69% of correct answers (hits and correct rejections), d' = 2 roughly corresponds to 95% of correct answers. We further calculated the response bias c:

$$c = 0.5 \times (z(hit\ rate) + z(false\ alarm\ rate))$$

A negative bias indicates a tendency to report more odorants than there actually are, while a positive bias indicates a tendency to report less. Thus, for each participant, we had 10 scores: d' and c for stimuli of 1, 2, 3, 4 and 5 odorants.

Sensitivity index and response bias constituted our two dependent variables. For each of them, we performed a repeated measures ANOVA with *number of odorants* (five levels: stimuli of 1, 2, 3, 4 or 5 odorants) as within-subject factor, *group* (three levels: sommeliers, trained novices, untrained novices) as between-subject factor, and *age* and *gender* as covariates. Pairwise comparisons allowed us to know between which groups the difference was, when it was significant. We then performed post-hoc univariate ANOVAs with *group* as between-subject factor and *age* as covariate, which allowed us to see, for each number of odorants, whether there was a significant difference between groups and, if so, which group stuck out.

Results

Sensitivity index

We found significant main effects of *group* ($F_{2,42} = 3.606$, $p = 0.036$), age ($F_{1,42} = 4.447$, $p = 0.041$) and *number of odorants* ($F_{4,168} = 4.097$, $p = 0.003$) on the sensitivity index. There was also a significant interaction between *number of odorants* and *group* ($F_{8,168} = 2.267$, $p = 0.025$). There was no effect of *gender* ($F_{1,42}=0.930$, $p=0.340$). Pairwise comparisons revealed that the main effect of *group* was mostly due to sommeliers: significant differences were observed between sommeliers and trained novices ($p = 0.015$), and between sommeliers and untrained novices ($p = 0.010$), but not between trained and untrained novices ($p = 0.813$).

To disentangle the interaction, we carried out univariate ANOVAs for each number of odorants: we found a main effect of *group* for mixtures of 3 and 4 odorants ($F_{2,46} = 3.501$, $p = 0.039$ and $F_{2,46} = 4.835$, $p = 0.013$, respectively), with sommeliers having a better sensitivity index than participants from the two other groups (Figure 12). The main effect of *group* was almost significant for monomolecular and bimolecular stimuli ($F_{2,46} = 2.814$, $p = 0.071$ and $F_{2,46} = 2.727$, $p = 0.077$, respectively). For bimolecular stimuli, sommeliers also outperformed trained and untrained novices ($p = 0.028$ and $p = 0.039$, respectively). For monomolecular stimuli, however, it was between trained and untrained novices that the difference was significant ($p = 0.029$), with trained participants performing better than untrained novices and having a sensitivity index similar to that of sommeliers (Figure 12). There was no significant difference for mixtures of 5 odorants.

Response bias

We found a significant main effect of group ($F_{2,42} = 5.046$, $p = 0.011$) on response bias. The main effect of the number of odorants ($F_{4,168} = 1.879$, $p = 0.116$) and the interaction between group and number of odorants ($F_{8,168} = 1.810$, $p = 0.078$) failed to reach significance. Pairwise comparisons yielded a significant difference between trained and untrained novices ($p = 0.003$). More precisely, there was a significant difference for mixtures of 4 and 5 odorants ($F_{2,46} = 4.920$, $p = 0.012$ and $F_{2,46} = 8.370$, $p = 0.001$, respectively), with a response bias significantly lower in trained participants than in untrained novices ($p = 0.003$ and $p < 0.001$ for 4 and 5 odorants, respectively; Figure 13). This indicates that, for mixtures of 4 and 5 odorants, trained participants tended to select more odorants than untrained novices. There was no significant difference between

sommeliers and trained participants ($p = 0.671$) or between sommeliers and untrained novices ($p = 0.135$).

Discussion

The main findings of this study were that 1) sommeliers recognised odorants within a mixture of up to four components better than novices, but with five components there was no difference. 2) Olfactory training enhanced the ability to identify monomolecular stimuli to such an extent that there was no difference between trained novices and sommeliers, but it was not enough to improve the capacity to recognise odorants within mixtures.

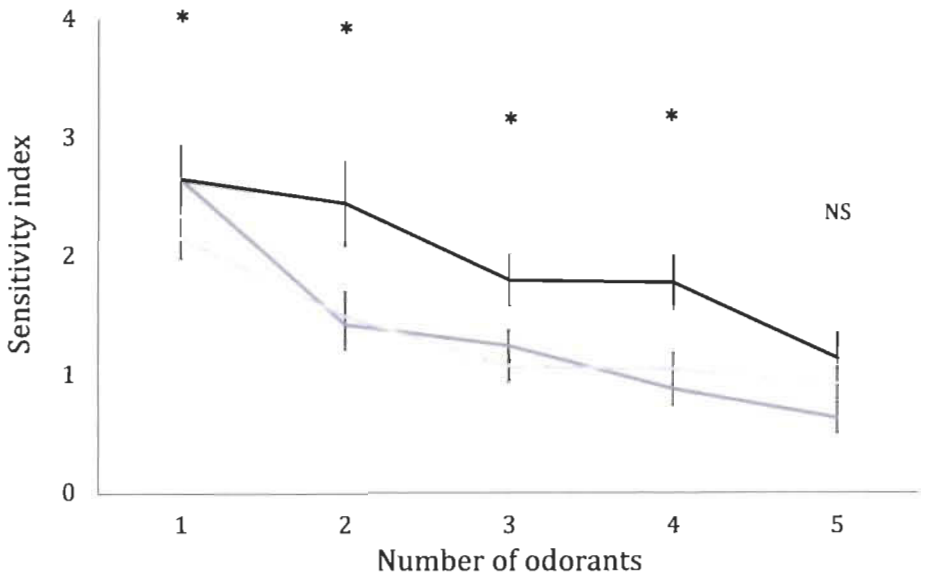

*Figure 12. Sensitivity index for olfactory stimuli of 1, 2, 3, 4 and 5 odorants in sommeliers (black line), trained (dark line) and untrained novices (light line). NS = Non Significant, * $p<0.05$*

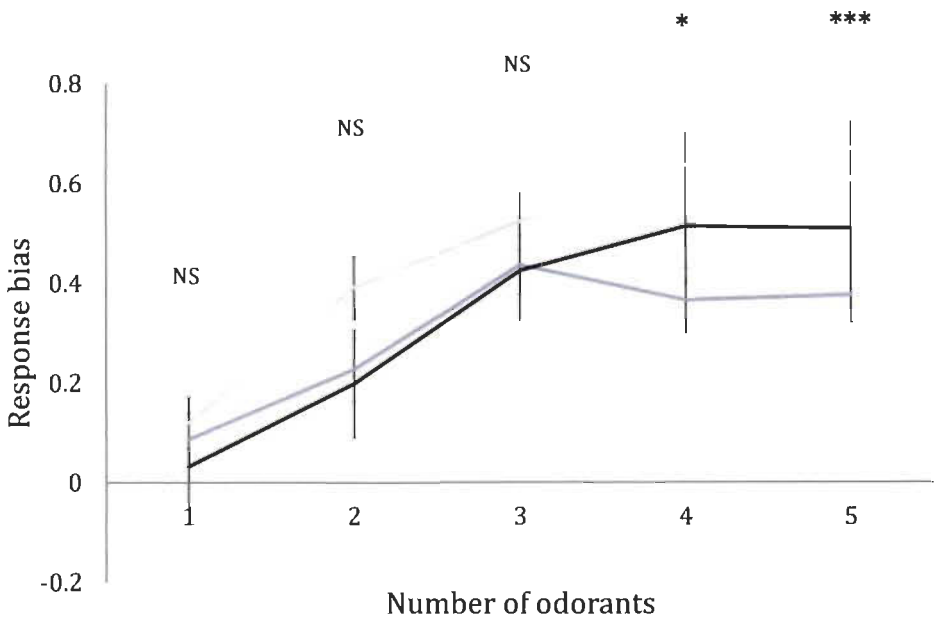

*Figure 13. Response bias for olfactory stimuli of 1, 2, 3, 4 and 5 odorants in sommeliers (black line), trained (dark line) and untrained novices (light line). NS = Non Significant, * $p<0.05$, *** $p<0.001$.*

Sommeliers were better at identifying odorants within mixtures of up to four components, but their sensitivity index decreased for five-component odor mixtures and was then not significantly different from novices'. These results align with that of Livermore et *al.* (1996) who found that experts could identify odorants of two- and three-component odor mixtures with a higher accuracy, and that expertise was not sufficient to overcome the limit of four odorants: for five-component odor mixtures, the task appears to be challenging for both groups. A possible explanation could be that it is physiologically impossible for humans to process information stemming from more than four odorants smelled simultaneously (Laing *et al.*, 1989). For example, it has been proposed that competitive mechanisms between odorants result in an inhibition of olfactory receptors (Jinks *et al.*, 1999), which could explain why olfactory information is lost. Another hypothesis could be that odorants activate their respective receptors but, during processing, spatial maps of the different odorants overlap and identifying them individually therefore becomes challenging. Thus, there is an upper limit to improvement when it comes to the number of odorants that can be recognised within a mixture. However, expertise enhances the perception of mixtures containing few odorants. While the configural perception of a mixture (i.e. perception of the mixture as a whole) is favored

in novices, it has been shown that expertise favors elemental perception (i.e. perception of the components within the mixture; Barkat *et al.*, 2012). This might be due to the type of task they are required to perform on a daily basis as sommeliers: when evaluating a wine, they need to perceive its components and not only the wine as a whole. This could explain how their expertise enhances their sensitivity index in our task.

A five-day olfactory training was enough for trained novices to learn how to identify the single odorants that were used and to perform as well as sommeliers. This result shows that effects of olfactory training can appear early, which has already been reported by previous studies: besides the study which found that repeating the same test three times led to an enhancement of performance (Rabin *et al.*, 1986), another one found that an 11-hour olfactory training based on beer was enough to modulate the participants' ability to sort beers (Chollet *et al.*, 2001). The effect we observed was however limited to monomolecular stimuli, as there was no difference between trained and untrained novices for mixtures of two components and more. Their training was short; results obtained from sommeliers may lead to speculate that a longer olfactory training might improve the ability to identify odor components within mixtures, and not only monomolecular stimuli.

Olfactory training also had an effect on the response bias: for mixtures of four and five odorants, trained participants had a lower response bias, indicating that they tended to select more odorants than sommeliers and untrained participants. A possible explanation could be that, since they trained on the task and a feedback was provided, they knew there were mixtures with four and five odorants, which might have made them less hesitant to select more odorants.

It would have been interesting to see the progression of trained novices during the five days of training but, unfortunately, we did not record their performance during training. Future studies should include such record to evaluate progress throughout training.

There was an age difference between groups, with sommeliers being older than trained and untrained novices, and we observed a significant effect of age on the sensitivity index. This is not surprising as it has been reported that olfactory performance decreases with age after peaking between 20 and 30 (Hummel *et al.*, 1997; Hummel *et al.*, 2001; Hummel *et al.*, 2007; Kobal *et al.*, 2000). In our study, the mean age of sommeliers is 35 while it is 24 in both trained and untrained novices, but we corrected the effect of age by defining it as covariate.

Identifying odorants within a mixture is a complex task. Complexity is an advantage since we are comparing participants who have a normal sense of smell with experts whose sense of smell is likely to be more acute; we can expect them to perform well, which would confront us with ceiling effects if the task was too easy. Our task is also more similar to the tasks that sommeliers are trained for, since their profession requires them to distinguish and evaluate the different compounds found in wine odors. This task is therefore more appropriate to examine sommeliers' abilities. However, identifying odor compounds within a mixture remains challenging even for experts. A short olfactory training allows to become as good as sommeliers to recognise monomolecular stimuli but is not enough to enhance the ability to identify odorants within mixtures. This study sheds light on how training can improve our performance in this complex task: humans seem to be unable to distinguish more than four odorants within a mixture, but a short olfactory training can improve identification of a single odorant, while a longer training leading to expertise refines identification ability of odors within mixtures. Our study suggests that olfactory training helps identifying specific target odors, individually, or within binary, tertiary or quaternary mixtures consisting of known components. This is not the same task as the identification of the presence of a specific odor (e.g., cork taint off-flavor) in a highly variable background (e.g., several types of wine), which is a typical task for the sommelier profession. In future studies it would therefore be interesting to verify if a few specific odors are more readily identified.

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Chapitre 7 – Discussion

Les principaux résultats des études réalisées au cours du doctorat concernent les effets d'un entraînement olfactif à long terme sur le cerveau : au cours de la formation en sommellerie, le volume du bulbe olfactif (BO) a augmenté tandis que l'épaisseur corticale a évolué dans différentes régions cérébrales. Le test des Sniffin' Sticks ne nous a pas permis d'observer une amélioration de la performance olfactive et donc d'établir des corrélations entre évolution de la performance olfactive et évolution de la structure du cerveau. Cependant, la tâche plus complexe d'identification d'odorants au sein de mélanges nous a permis de mettre en évidence les capacités olfactives supérieures des sommeliers.

Augmentation du volume du bulbe olfactif

Le volume du BO des étudiants en sommellerie a augmenté au cours de leur formation, ce qui représente un bel exemple de plasticité cérébrale liée à l'entraînement.

Corrélations et effets d'un entraînement olfactif

L'augmentation du volume du BO des étudiants en sommellerie au cours de leur formation est en accord avec ce qui a été rapporté dans de précédentes études. En effet, des études réalisées chez des participants normosmiques ont montré qu'un plus grand volume du BO est associé à de meilleures capacités olfactives (Buschhuter *et al.*, 2008; Seubert *et al.*, 2013) et qu'un entraînement olfactif mène à une augmentation du volume du BO (Negoias *et al.*, 2017). Des observations semblables ont été faites chez des patients souffrant de troubles olfactifs : une sensibilité olfactive déficiente est associée à un moindre volume du BO (Liu *et al.*, 2017; Rombaux *et al.*, 2006a), et un entraînement olfactif permet de restaurer le volume de cette structure (Haehner *et al.*, 2008; Rombaux *et al.*, 2009).

Modulation de l'activité du bulbe olfactif

Le BO, qui constitue un relais entre système olfactif périphérique et système olfactif central, serait modulé de différentes manières. Puisqu'il traite l'information olfactive qui provient de l'épithélium olfactif, il semble intuitif que le BO soit soumis à une modulation ascendante. Cependant, ce n'est pas tout : un entraînement latéralisé, c'est-à-dire l'entraînement d'une seule narine, induit une

augmentation significative du volume non seulement du BO ipsilatéral mais également du BO controlatéral (Negoias *et al.*, 2017). Le fait que le BO se situant du côté de la narine non entraînée augmente également en volume suggère que, en plus d'une modulation ascendante depuis l'épithélium olfactif, le BO est aussi soumis à une modulation descendante.

Modulation ascendante

La modulation ascendante correspond à la modulation du BO par le système olfactif périphérique, c'est-à-dire de l'épithélium olfactif.

L'existence d'une modulation ascendante est soutenue par de nombreuses études montrant qu'une diminution du volume du BO survient à la suite de la diminution de l'influx de l'information olfactive. C'est ce qui a été observé dans le cas de troubles olfactifs causés par exemple par une perte d'odorat post-infectieuse (Mueller *et al.*, 2005; Rombaux *et al.*, 2006b), une inflammation des sinus (Rombaux *et al.*, 2008), une laryngectomie (Veyseller *et al.*, 2011) ou encore une obstruction nasale unilatérale (Altundag *et al.*, 2014; Askar *et al.*, 2015). Ces troubles olfactifs ne venant pas du BO, il est donc clair que la diminution du volume du BO est une conséquence de la diminution de l'influx de l'information olfactive, ce qui confirme que le BO est soumis à une modulation ascendante.

Un autre argument repose sur le fait que l'amélioration du seuil de détection à la suite d'un entraînement olfactif est corrélée à l'augmentation du volume du BO (Gudziol *et al.*, 2009; Haehner *et al.*, 2008). Le seuil de détection dépendant surtout du système olfactif périphérique (Moberg *et al.*, 1997), cette observation suggère que le BO est régulé par la périphérie.

Enfin, une autre étude a rapporté que les différences entre volume du BO gauche et volume du BO droit correspondaient aux différences entre sensibilité olfactive de la narine gauche et sensibilité olfactive de la narine droite ; par exemple, si la narine droite est plus performante que la narine gauche, le BO droit sera plus volumineux que le BO gauche (Hummel, Haehner, *et al.*, 2013). Cela semble également indiquer que le volume du BO dépend de l'information olfactive en provenance de la périphérie.

Modulation descendante

En plus de la modulation ascendante, le BO reçoit des informations de structures cérébrales supérieures et est donc aussi soumis à une modulation descendante (Ennis *et al.*, 2015). Lorsque

les fibres nerveuses proviennent du cortex olfactif primaire, la modulation consiste en un rétrocontrôle négatif du BO : grâce à des connexions GABAergiques, les cellules mitrales sont inhibées et l'activité du BO est ainsi modulée. Lorsque les fibres nerveuses proviennent d'autres structures, la modulation intervient dans différents contextes et jouent un rôle par exemple dans l'habituation aux odeurs ou dans l'apprentissage conditionné (Huart *et al.*, 2019).

Plusieurs arguments soutiennent l'existence d'une modulation descendante. Premièrement, les patients souffrant de troubles neurologiques et psychologiques tels que la maladie d'Alzheimer (Thomann *et al.*, 2009), la sclérose en plaques (Goktas *et al.*, 2010; Yaldizli *et al.*, 2016), l'épilepsie (Hummel, Henkel, *et al.*, 2013), la dépression (Negoias *et al.*, 2010; Rottstadt *et al.*, 2018), ou la schizophrénie (Nguyen *et al.*, 2011) ont des BO moins volumineux, ce qui montre que des évènements ayant lieu dans le cerveau ont un effet sur le BO. Chez les personnes dépressives, le volume du BO est ainsi négativement corrélé à la gravité de la dépression (Negoias *et al.*, 2010). Deuxièmement, il a été montré que les aveugles précoces ont des capacités olfactives supérieures et un BO plus volumineux. Cela suggère que la plasticité du BO fait partie des mécanismes mis en jeu pour compenser le déficit visuel (Rombaux *et al.*, 2010).

L'activité du BO serait donc régulée par des modulations ascendante et descendante. Dans le cas des étudiants en sommellerie, puisque l'augmentation du volume du BO résulte d'un entraînement et donc d'une stimulation soutenue du système olfactif périphérique, il est intuitif de penser qu'une modulation ascendante était impliquée, mais cela n'exclue pas la possibilité de rétrocontrôles et d'autres mécanismes correspondant à une modulation descendante.

Mécanismes sous-jacents

Les études de neuroimagerie, y compris notre étude, montrent que le BO est plastique et que divers facteurs peuvent faire évoluer son volume. Cependant, il n'existe toujours pas de consensus sur les mécanismes cellulaires et synaptiques sous-jacents (Huart *et al.*, 2019). Quatre mécanismes ont été proposés pour expliquer la plasticité du BO.

La synaptogenèse est un premier mécanisme possible : l'activité module les connexions entre les neurones, c'est-à-dire dans le cas présent les synapses entre neurones olfactifs et cellules mitrales, et mène à l'augmentation ou la diminution du nombre de synapses, modulant ainsi le volume du BO (Eavri *et al.*, 2013; Zatorre *et al.*, 2012). Ce mécanisme a été proposé pour expliquer la

diminution du volume du BO avec l'âge ou liée à un trouble olfactif : une diminution du nombre de synapses voire de glomérules serait à l'origine de cette diminution (Haehner *et al.*, 2008; Huart *et al.*, 2019).

D'autres mécanismes impliquent l'augmentation du nombre de neurones. La neurogenèse est une propriété remarquable du système olfactif. Ce processus a lieu en continu au niveau de l'épithélium olfactif : les neurones olfactifs se régénèrent en permanence, ce qui est primordial pour maintenir un odorat fonctionnel car les neurones olfactifs sont directement en contact avec l'environnement et peuvent donc être endommagés (Schwob *et al.*, 2010). En stimulant l'épithélium olfactif régulièrement, il peut être suggéré que l'entraînement olfactif stimule la neurogenèse. Les axones des neurones olfactifs nouvellement formés grandissent ensuite et forment des synapses avec les cellules mitrales au sein du bulbe olfactif, ce qui expliquerait pourquoi le volume du BO augmente.

La neurogenèse pourrait également avoir lieu dans la zone supraventriculaire du ventricule latéral et donner des neuroblastes qui migrent vers le BO et se différencient en interneurones, mais ce mécanisme premièrement démontré chez l'animal (Kornack *et al.*, 2001; Lois *et al.*, 1996; Ming *et al.*, 2011) est encore sujet à débat chez l'humain (Huart *et al.*, 2019). En effet, bien que la présence de neuroblastes ait été observée dans le ventricule latéral (Johansson *et al.*, 1999; Sanai *et al.*, 2004), leur capacité à migrer jusqu'au BO n'a pas été démontrée (Curtis *et al.*, 2007; Sanai *et al.*, 2007; Sanai *et al.*, 2011).

Finalement, le quatrième mécanisme concerne une potentielle plasticité intrinsèque du BO. En effet, des cellules progénitrices ont été détectées au sein du BO et il a été suggéré qu'elles pourraient être à l'origine de l'augmentation du nombre de cellules et donc du volume du BO (Pagano *et al.*, 2000). D'après des observations faites chez l'animal, les changements de volume du BO pourraient aussi être dus aux interneurones qui sont chez l'animal continuellement renouvelés et sujets à des modifications structurales dépendantes de l'activité : les épines dendritiques sont relocalisées vers les dendrites des cellules mitrales actives, un processus qui permet un ajustement rapide du réseau puisque la relocalisation des épines dendritiques des interneurones requiert seulement quelques minutes alors que les dendrites des cellules mitrales sont relativement stables au cours du temps (Breton-Provencher *et al.*, 2016; Hardy *et al.*, 2017). Cependant, ce mécanisme et l'existence de neurogenèse dans le BO restent à démontrer chez l'humain (Bergmann *et al.*, 2015).

Divers mécanismes sous-jacents pourraient donc être à l'origine de l'augmentation du volume du BO que nous avons observée chez les étudiants en sommellerie, mais l'état actuel des connaissances ne nous permet pas de savoir précisément quels mécanismes ont été impliqués.

Épaisseur corticale et modèle de surproduction-élagage

Au cours de la formation en sommellerie, l'épaisseur corticale des étudiants en sommellerie a évolué dans cinq régions cérébrales distinctes : nous avons observé une augmentation de l'épaisseur corticale au niveau du cortex entorhinal (CE) droit, ainsi qu'une diminution au niveau du gyrus temporal inférieur (GTI) gauche, de la portion triangulaire du gyrus frontal inférieur (GFIt) droit, du gyrus pariétal supérieur (GPS) gauche et du gyrus frontal supérieur (GFS) gauche.

L'épaisseur corticale, reflet des effets de la plasticité cérébrale

La mesure de l'épaisseur corticale a été utilisée à maintes reprises dans de précédentes études pour rendre compte des effets de la plasticité cérébrale liée à l'entraînement. C'est avec cette mesure qu'il a par exemple été montré que le cortex auditif ainsi que certaines régions cérébrales impliquées dans la cognition de haut niveau sont plus épaisses chez les musiciens (Bermudez *et al.*, 2009). Un autre exemple concerne les effets d'un entraînement des compétences sociales sur une durée de neuf mois : un tel entraînement mène à des changements d'épaisseur corticale au niveau de régions cérébrales connues comme étant impliquées dans des réseaux socioaffectifs et sociocognitifs (Valk *et al.*, 2017).

Des études ont également utilisé la mesure de l'épaisseur corticale pour évaluer les effets de la plasticité cérébrale dans le domaine de l'olfaction. C'est ainsi qu'ont été mises en évidence des différences structurales dues à l'expertise : comparés à des novices, les sommeliers ont un cortex entorhinal plus épais que les novices, et son épaisseur est corrélée au nombre d'années d'expérience (Banks *et al.*, 2016). Sans même parler d'expertise, la mesure de l'épaisseur corticale a également permis de montrer qu'un entraînement olfactif de six semaines menait à un épaississement du cortex dans différentes régions telles que le cortex entorhinal droit, le gyrus frontal inférieur gauche, et le gyrus fusiforme bilatéral (Al Ain *et al.*, 2019).

Des régions cérébrales plus ou moins associées à l'olfaction

Parmi les régions dans lesquelles nous avons observé une évolution de l'épaisseur corticale se trouve le CE. Puisque ce cortex est une des aires olfactives primaires (Patel *et al.*, 2014), il est parfaitement compréhensible qu'une stimulation répétée du système olfactif mène à un épaississement de ce cortex. De plus, notre observation est en accord avec ce qui a été observé chez des novices ayant suivi un entraînement olfactif sur une durée de six semaines, et chez les sommeliers (Al Ain *et al.*, 2019; Banks *et al.*, 2016).

Le GFIt est impliqué dans le traitement de haut niveau de l'information olfactive. Une précédente étude réalisée chez des participants normosmiques a par exemple rapporté que cette région était activée lorsque les participants devaient évaluer la familiarité des odeurs qui leur étaient présentées (Plailly *et al.*, 2005). Cette région a aussi été mise en évidence dans des études sur l'olfaction réalisées chez des patients parkinsoniens : cette région est activée lors d'une stimulation olfactive (Hummel *et al.*, 2010; Welge-Lussen *et al.*, 2009; Westermann *et al.*, 2008) et sa densité est positivement corrélée à la capacité à identifier des odeurs (Pardini *et al.*, 2009). Enfin, cette région fait partie de celles où a été observé un épaississement suite à un entraînement olfactif de six semaines (Al Ain *et al.*, 2019).

Le GTI est une aire faisant partie de la voie ventrale du traitement visuel, qui est impliquée dans la reconnaissance d'objet (Kupers *et al.*, 2011; Kupers *et al.*, 2014; Mishkin *et al.*, 1983; Ungerleider *et al.*, 1994). Cependant, cette région semble également avoir un lien avec l'olfaction : l'entraînement olfactif de six semaines a mené à un épaississement de cette région (Al Ain *et al.*, 2019) tandis que, chez les patients anosmiques, son volume de matière grise est réduit (Peng *et al.*, 2013).

Le GFS est impliqué dans des fonctions cognitives de haut niveau telles que la mémoire de travail (du Boisgueheneuc *et al.*, 2006; Klingberg, 2006). Tout comme dans le GTI, la quantité de matière grise est réduite dans le GFS, ce qui suggère un lien de cette région avec l'odorat (Peng *et al.*, 2013).

La dernière région dans laquelle nous avons observé une évolution de l'épaisseur corticale est le GPS, une aire visuelle faisant partie de la voie dorsale impliquée dans la perception spatiale (Stickel *et al.*, 2019). Il peut sembler surprenant qu'un entraînement olfactif impacte une aire visuelle. Cependant, il a été montré que les aires visuelles étaient activées lors de tâches purement olfactives

(Dade *et al.*, 2002; Zatorre *et al.*, 2000), et que la stimulation du cortex visuel par stimulation magnétique transcrânienne améliorait la capacité à discriminer des odeurs (Jadauji *et al.*, 2012).

Le modèle de surproduction-élagage

Certaines régions où nous avons observé une évolution de l'épaisseur corticale au cours de la formation ont également évolué au cours de l'entraînement olfactif de six semaines ; c'est le cas du CE, du GFIt et du GTI (Al Ain *et al.*, 2019). Cependant, tandis que l'entraînement olfactif de six semaines a mené exclusivement à des épaississements de ces régions, nous avons observé un épaississement du CE mais un amincissement des autres régions.

Un amincissement du cortex ne faisait pas partie de nos hypothèses de départ. En effet, de plus grandes capacités olfactives sont d'habitude associées à des structures cérébrales plus volumineuses et un cortex plus épais (Buschhuter *et al.*, 2008; Frasnelli *et al.*, 2010; Hummel *et al.*, 2003; Seubert *et al.*, 2013) avec en plus, chez les sommeliers, une épaisseur corticale positivement corrélée au nombre d'années d'expérience (Banks *et al.*, 2016). Cependant, dans les autres domaines, le fait qu'un entraînement mène à un amincissement du cortex n'est pas inédit : des amincissements du cortex ont effectivement été observés suite à neuf mois d'entraînement des compétences sociales (Valk *et al.*, 2017) ou encore suite à une semaine d'entraînement visant à améliorer la vitesse de traitement (Takeuchi *et al.*, 2011).

La théorie selon laquelle les changements dus à la plasticité cérébrale liée à l'entraînement ne sont pas linéaires expliquerait les résultats que nous avons obtenus. Cette théorie repose sur les observations faites lors d'une étude durant laquelle des participants droitiers se sont entraînés à écrire et dessiner avec la main gauche pendant sept semaines et ont été testés plusieurs fois au cours de l'entraînement : l'épaisseur corticale de certaines régions a d'abord augmenté pendant les quatre premières semaines, puis a diminué ensuite, malgré une pratique continue et une capacité à écrire et dessiner de la main gauche qui continuait de s'améliorer (Wenger *et al.*, 2017). Ces observations ont donné naissance au modèle de plasticité nommé modèle de surproduction-élagage selon lequel, dans un premier temps, le nombre de synapses augmente fortement et, dans un deuxième temps, seules les connexions pertinentes sont stabilisées tandis que toutes les autres sont éliminées par élagage (Lindenberger *et al.*, 2017). D'après ce modèle, l'épaississement du cortex pourrait être très rapide. C'est ce qui a été observé lors d'un entraînement de jonglage de cinq semaines et durant lequel les participants ont été testés plusieurs fois : la plus forte augmentation de l'épaisseur

corticale a eu lieu au cours de la première semaine, ce qui a mené les auteurs à suggérer que l'apprentissage d'une nouvelle tâche avait plus d'impact sur la structure cérébrale que la pratique continue d'une tâche déjà apprise (Driemeyer *et al.*, 2008).

Ce modèle de surproduction-élagage expliquerait pourquoi nous avons observé, au cours d'une formation d'un an et demi, un amincissement du cortex dans des régions où un entraînement olfactif de six semaines permet d'observer un épaississement : il s'agirait d'une question de timing. Lors de l'étude qui consistait en un entraînement olfactif de six semaines, les participants ont été testés une première fois avant le début de l'entraînement, puis une deuxième fois à la fin. Dans notre étude, nous n'avons été capables de contacter les participants seulement une fois qu'ils avaient débuté leur formation et le temps nécessaire pour trouver des disponibilités pour chaque étudiant a fait que les premiers tests ont eu lieu principalement lors du deuxième mois de leur formation, c'est-à-dire plusieurs semaines après le début de leur entraînement. Les participants ont ensuite été testés à la fin de leur formation, environ un an et demi plus tard. Parce que le timing des deux études était différent, il est possible que l'épaississement du cortex observé suite à l'entraînement olfactif de six semaines corresponde à la première phase de surproduction, tandis que la fin de cette première phase était peut-être déjà proche lorsque nous avons testés nos participants une première, ce qui expliquerait pourquoi nous avons observé un amincissement dans ces mêmes régions dû à la deuxième phase d'élagage durant laquelle plus de synapses seraient éliminées que nouvellement formées (voir Figure 14).

L'existence de différentes phases serait due à l'interaction de différents processus qui interviennent en suivant différentes cinétiques. Des études réalisées chez l'animal soutiennent le modèle de surproduction-élagage et apportent des pistes quand aux mécanismes sous-jacents : l'utilisation de la microscopie à deux photons et de l'optogénétique chez la souris pendant un entraînement moteur a permis de mettre en évidence la formation rapide de nouvelles épines dendritiques suivie d'un élagage des épines plus lent pendant que les épines conservées sont stabilisées. Ce serait sur les connexions nouvellement formées et stabilisées que reposent l'acquisition et l'amélioration de nouvelles capacités (Xu *et al.*, 2009). La surproduction de nouvelles connexions et l'élagage des épines dendritiques sont deux processus qui dépendent probablement de nombreux facteurs tels que la plasticité inhérente à chaque région et la pertinence des connexions ; il serait alors raisonnable de suggérer que, d'une région à l'autre, surproduction et élagage suivent différentes

cinétiques et que les deux phases s'enchaînent donc à différentes vitesses. Il serait également possible d'envisager l'existence d'une troisième phase lors de laquelle le cortex s'épaissirait à nouveau : une fois que toutes les connexions produites en excès et étant finalement impertinentes ont été supprimées et que seules les connexions pertinentes ont été conservées et stabilisées, le processus d'élagage ralentit tandis que le processus de production de nouvelles connexions pourrait se poursuivre, ce qui renverserait de nouveau la balance ; il y aurait plus de connexions nouvellement formées que de connexions éliminées, ce qui mènerait à un épaississement du cortex. Cela expliquerait pourquoi des corrélations positives sont trouvées entre épaisseur corticale du cortex entorhinal et nombre d'années d'expérience en sommellerie (Banks *et al.*, 2016). L'existence de trois phases qui se succèderaient à différentes vitesses en fonction des régions expliquerait pourquoi, alors que nous observons un épaississement du cortex dans une certaine région, nous observons dans le même temps un amincissement du cortex dans d'autres régions où a été observé un épaississement lorsque la durée de l'entraînement était différente (voir Figure 14).

Évaluation de la performance olfactive

Au niveau des tests olfactifs, nous avons rapporté que les étudiants en sommellerie avaient, lors des deux premiers mois de leur formation, de meilleurs scores que le groupe contrôle dans le test d'identification. Nous n'avons obtenu aucun autre résultat concluant lors de notre étude longitudinale, que ce soit pour les tests réalisés avec les Sniffin' Sticks ou pour la tâche olfactive réalisée dans le scanner IRM. Cela ne signifie pas que la performance des sommeliers ne s'est pas améliorée avec la formation ; nous n'avons juste pas réussi à mettre une quelconque amélioration en évidence. Dans notre étude additionnelle visant à évaluer la capacité des sommeliers ainsi que de novices entraînés et non entraînés à identifier des odorants au sein d'un mélange, nous avons observé que les sommeliers étaient meilleurs que les novices pour les mélanges contenant jusqu'à quatre odorants, tandis que les novices entraînés pendant cinq jours étaient devenus aussi performants que les sommeliers pour identifier les odorants seuls, mais pas significativement meilleurs que les novices non entraînés pour les mélanges de deux odorants ou plus.

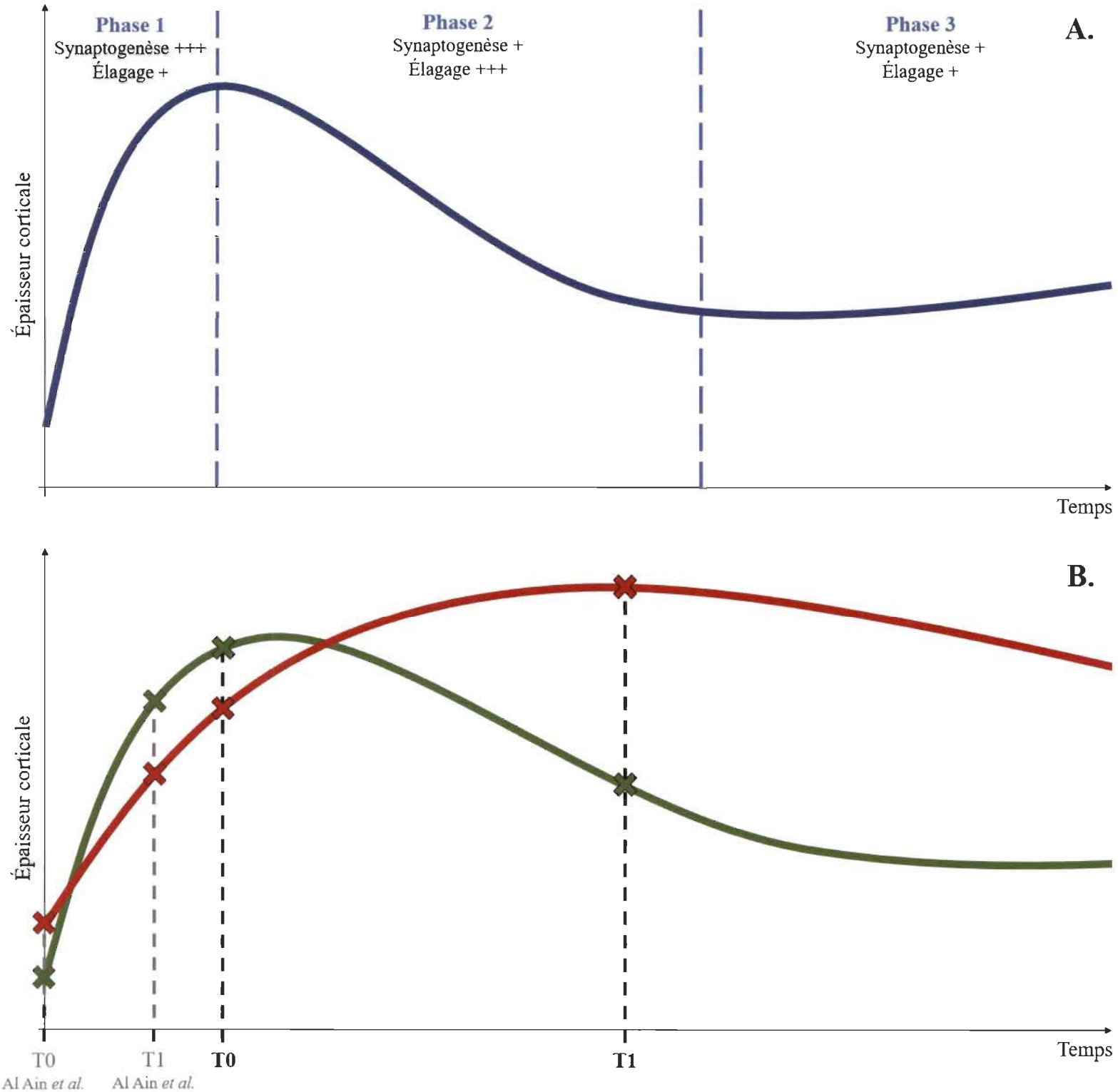

Figure 14. Le modèle de surproduction-élagage.

A. *Selon ce modèle, les changements de l'épaisseur corticale dus à la plasticité cérébrale liée à l'entraînement ne sont pas linéaires au cours du temps. Synaptogenèse et élagage interviennent simultanément plus ou moins intensément : + symbolise une activité modérée, +++ une activité forte. Durant la première phase, dite phase de surproduction, une forte synaptogenèse mène à un épaississement du cortex. Durant la deuxième phase, dite phase d'élagage, la synaptogenèse ralentit tandis qu'un fort élagage permet d'éliminer un grand nombre de synapses et de ne garder que celles qui sont fonctionnellement pertinentes, ce qui mène à un amincissement du cortex. Lors d'une troisième phase, une pratique continue amènerait synaptogenèse et élagage à s'équilibrer ; un nombre de synapses nouvellement créées légèrement supérieur au nombre de synapses éliminées mènerait à un léger épaississement du cortex au cours du temps, ce qui expliquerait par exemple l'existence de corrélations positives entre épaisseur corticale et nombre d'années d'expertise en sommellerie (Banks et al., 2016).* ***B.*** *Représentation hypothétique de l'épaisseur corticale au cours du temps dans le cortex entorhinal (CE, en rouge) ainsi que dans la portion triangulaire du gyrus frontal inférieur et dans le gyrus temporal inférieur (GFIt et GTI, en vert), suivant le modèle de surproduction-élagage et illustrant le fait que l'épaisseur corticale évolue à différentes vitesses dans différentes régions cérébrales. En fonction du timing, une étude longitudinale peut mener à l'observation d'un épaississement ou d'un amincissement du cortex : T0 et T1, en noir, représentent les points dans le temps correspondant potentiellement à la première visite des étudiants en sommellerie au cours du deuxième mois de leur formation (T0) et à la fin de leur formation (T1), tandis que T0 Al Ain* et al. *et T1 Al Ain* et al.*, en gris, représentent les points dans le temps correspondant aux tests réalisés au tout début et à la fin d'un entraînement olfactif de six semaines (Al Ain et al., 2019). Ces points dans le temps expliquent pourquoi, à cause du timing et de l'évolution de l'épaisseur corticale plus ou moins rapide d'une région cérébrale à l'autre, nous avons observé un épaississement du CE et un amincissement dans le GFIt et le GTI, tandis que l'entraînement olfactif de six semaines d'Al Ain et al. a mené à une augmentation de l'épaisseur corticale dans ces régions.*

Performance olfactive au cours de la formation en sommellerie

Au cours des premières semaines de formation, la performance des étudiants en sommellerie était meilleure que celle des étudiants du groupe contrôle dans les tests d'identification libre et

d'identification avec indices. Les étudiants ont été plus précisément testés entre la troisième et la neuvième semaine de formation ; puisque nous n'avons pas pu contacter les étudiants avant qu'ils débutent leur formation, nous ne pouvons pas déterminer si ces capacités olfactives supérieures dues à un rapide progrès au cours des premières semaines, ou si les étudiants ont intégré la formation en ayant déjà un odorat affiné. Il est possible qu'ils aient progressé rapidement car, au moment des tests, les étudiants en sommellerie pratiquaient dans le cadre de leur formation des exercices olfactifs qui incluaient l'identification d'odeurs.

En les testant de nouveau à la fin de leur formation, nous n'avons noté aucune amélioration significative de leur performance. Nous pensons cependant que leurs capacités olfactives se sont améliorées, mais nous n'avons pas réussi à mettre cette amélioration en évidence avec le test de Sniffin' Sticks. Une première explication reposerait sur le fait que les effets d'un entraînement olfactif peuvent être spécifiques aux tâches réalisées pendant l'entraînement. En effet, c'est ce qui a été observé lors d'entraînements qui consistaient à sentir quotidiennement quatre odeurs : à la fin de l'entraînement, les participants avaient une meilleure sensibilité à ces odeurs-là mais pas à d'autres odeurs (Dalton *et al.*, 2002; Mori *et al.*, 2015). Un effet généralisé a cependant été noté chez les normosmiques lorsque l'entraînement consistait en des tâches plus complexes que seulement sentir passivement (Al Ain *et al.*, 2019), ainsi que chez les patients pour qui sentir quotidiennement quatre odeurs permet de recouvrer l'odorat (Altundag *et al.*, 2015; Damm *et al.*, 2014; Fleiner *et al.*, 2012; Geissler *et al.*, 2014; Haehner *et al.*, 2013; Hummel *et al.*, 2009; Konstantinidis *et al.*, 2013). Une deuxième explication concerne les tests utilisés : les Sniffin' Sticks, initialement mis au point pour distinguer les normosmiques et les patients hyposmiques ou anosmiques, ne sont pas forcément adaptés pour distinguer normosmiques et participants dont la sensibilité olfactive est encore plus élevée. Nous avons remarqué que les étudiants en sommellerie en début de formation n'avaient aucune difficulté avec le test de Sniffin' Sticks tandis qu'à la fin de leur formation, ils semblaient moins à l'aise : leur approche était plus analytique et menait à des hésitations et à des temps de réponse plus longs que ce qu'on observait chez les participants du groupe contrôle. Cela était visible principalement dans le test d'identification. Dans la partie identification libre, le nombre de bonnes réponses était relativement faible. Dans la partie identification avec indices, tandis que, généralement, la présentation des quatre choix de réponses conforte la réponse donnée dans la partie identification libre ou engendre une réaction du type « ah mais oui, c'était ça ! », la présentation des choix de réponses semblait causer chez les étudiants

sommeliers en fin de formation encore plus de confusion, car ils trouvaient que l'odeur ne correspondait soit à aucune des quatre réponses possibles – c'était le cas surtout pour les odeurs fruitées, qui étaient selon eux trop intenses et pas assez naturelles – ou au contraire plusieurs des réponses, comme c'était le cas pour le chocolat qui, selon eux, pouvait également correspondre à l'odeur de vanille et de biscuit. Puisque les résultats obtenus avec les Sniffin' Sticks n'étaient pas significatifs, nous n'avons pu trouver aucune corrélation avec les données de neuroimagerie.

Une dernière tâche faisait partie intégrante de notre étude longitudinale. Il s'agissait de la tâche olfactive réalisée directement dans le scanner IRM, lors de l'IRM fonctionnelle : cette tâche lors de laquelle les participants devaient distinguer vin et jus, ou vin rouge et vin blanc, semblait plus adaptée aux sommeliers car elle était centrée sur le vin. Cependant, nous n'avons obtenu aucun résultat significatif, ce qui est peut-être dû à un nombre d'essais insuffisant, mais il était difficile de prolonger l'IRM fonctionnelle à cause de contraintes au niveau du temps disponible dans le scanner.

Les tests olfactifs réalisés au cours de l'étude longitudinale ne nous ont donc pas permis de mettre en évidence une quelconque amélioration de la performance olfactive.

Reconnaissance d'odorants au sein de mélanges et court entraînement

En plus de notre étude longitudinale, nous avons réalisé une étude transversale dont le but était de tester l'effet d'un entraînement olfactif de cinq jours en comparant la performance de novices en olfaction ayant suivi ce court entraînement à celles de sommeliers et de novices non entraînés. La tâche consistait à identifier des odorants au sein de mélanges. Les novices suivant l'entraînement olfactif étaient entraînés sur cette tâche-là. Les résultats ont montré que les novices entraînés étaient meilleurs que les novices non entraînés pour identifier les odorants seuls, et que leur performance à ce niveau-là n'était pas significativement différente des sommeliers qui, eux, étaient meilleurs que les novices pour identifier des odeurs dans des mélanges allant jusqu'à quatre odorants. Pour les mélanges de cinq odorants, les sommeliers n'étaient pas plus performants, ce qui confirme que l'être humain ne peut identifier que jusqu'à quatre odorants dans un mélange (Jinks *et al.*, 1999; Livermore *et al.*, 1998a) et que cette limite s'applique même aux experts (Livermore *et al.*, 1996). Cette étude montre également qu'un entraînement olfactif de courte durée permet d'améliorer la capacité à identifier des odorants seuls, mais qu'il n'est pas suffisant pour améliorer la performance lorsque la tâche se complexifie avec des mélanges de deux odorants ou plus.

L'avantage principal de cette étude était la nature de la tâche. Premièrement, il s'agissait d'une tâche complexe, ce qui permet de distinguer normosmiques et experts ayant des capacités olfactives supérieures plus facilement que les Sniffin' Sticks qui ont été créés pour distinguer normosmiques et hyposmiques. Deuxièmement, il s'agissait d'une tâche qui ressemble à ce que les sommeliers sont amenés à accomplir quotidiennement au sein de leur profession, puisque le vin est un mélange de nombreux odorants.

Il aurait été intéressant de tester, en plus de nos trois groupes, un quatrième groupe constitué d'étudiants en sommellerie, mais leur emploi du temps était chargé et l'étude longitudinale demandait déjà beaucoup d'investissement de leur part. Il aurait donc été compliqué de les recruter pour une étude supplémentaire.

Des approches de neuroimagerie complémentaires

Dans notre étude longitudinale, nous avons utilisé plusieurs approches d'IRM. Ces approches sont complémentaires car elles nous ont permis d'explorer différentes parties du cerveau : le bulbe olfactif, le cortex, et la matière blanche. En plus de ces approches structurales, l'IRM fonctionnelle nous a permis d'étudier l'activité cérébrale.

Les mesures du bulbe olfactif et de l'épaisseur corticale discutées ci-dessus nous ont permis d'observer des effets de la plasticité cérébrale résultant de la formation en sommellerie. IRM de diffusion et IRMf, cependant, n'ont pas permis de mettre en évidence une quelconque évolution de l'anisotropie fractionnelle ou de l'activité cérébrale au cours de la formation, ni de différences significatives avec les étudiants du groupe contrôle. Ces approches auraient pourtant pu révéler des changements. En effet, elles ont été utilisées auparavant dans d'autres études pour mettre en évidence les effets de la plasticité cérébrale.

En ce qui concerne l'IRM de diffusion, l'augmentation de l'anisotropie fractionnelle est généralement liée à l'apprentissage (Zatorre *et al.*, 2012). Lors d'une étude dans laquelle les participants ont suivi un entraînement de la mémoire de travail, par exemple, une augmentation de l'anisotropie fractionnelle a été observée dans certaines régions du cerveau, et cette augmentation était corrélée au nombre de sessions d'entraînement. Les chercheurs ont suggéré que l'entraînement avait mené à une myélinisation dans ces régions (Takeuchi *et al.*, 2010). Dans le cadre de l'étude

de la plasticité, l'IRM de diffusion peut donc être une approche efficace pour observer des modifications au niveau de la matière blanche.

L'IRMf a été utilisée dans de nombreuses études pour analyser les effets de la plasticité cérébrale. Cette approche a par exemple permis de montrer qu'un entraînement olfactif modifie la connectivité fonctionnelle, que ce soit chez les normosmiques (Royet *et al.*, 2013) ou les anosmiques (Kollndorfer *et al.*, 2014; Kollndorfer *et al.*, 2015). En comparant les données obtenues avant et après un entraînement de piano de six semaines, une autre étude d'IRMf a mis en évidence des circuits neuronaux distincts, un dont l'activation est modifiée par l'entraînement, un autre dont l'activation avant l'entraînement prédit le taux d'apprentissage, apportant ainsi des éléments de réponse à une question centrale de la plasticité, à savoir si les changements obtenus sont le résultat de l'entraînement ou de prédispositions (Herholz *et al.*, 2016). Les experts constituent un autre modèle pour étudier la plasticité liée à l'entraînement, quel que soit le domaine d'expertise : chez les experts en radiologie, des corrélations ont été trouvées entre le nombre d'années d'expertise et l'activation des régions cérébrales impliquées dans le traitement visuel (Harley *et al.*, 2009). De façon similaire, l'activation cérébrale est différente chez les sommeliers lors de la perception du vin (Pazart *et al.*, 2014). Chez l'aveugle, diverses études ont mis en évidence un des processus impliqués dans la plasticité intermodale, consistant en une réorganisation fonctionnelle des aires visuelles qui servent alors à traiter des stimuli non-visuels, ou sont même activées dans des tâches cognitives (Amedi *et al.*, 2003; Burton *et al.*, 2002; Frasnelli *et al.*, 2011; Kupers *et al.*, 2011; Renier *et al.*, 2013; Royet *et al.*, 2013; Theoret *et al.*, 2004). Ces nombreux exemples montrent que l'IRMf constitue également une approche efficace pour l'étude de la plasticité.

Conclusion

Ce projet de thèse consistait à évaluer les effets d'un entraînement olfactif à long terme – la formation en sommellerie – sur l'odorat et le cerveau. En utilisant comme modèle la formation en sommellerie, nous avons étudié les effets d'un entraînement olfactif qui a l'avantage d'être plus écologique qu'un entraînement olfactif réalisé en laboratoire, offrant une motivation autre que simplement prendre part à la recherche scientifique car les étudiants ont choisi cette formation pour faire de la sommellerie leur métier.

Les résultats majeurs de ce projet concernent les observations faites au niveau du bulbe olfactif et de l'épaisseur corticale : au cours de la formation en sommellerie, le volume du bulbe olfactif a

augmenté et des variations locales de l'épaisseur corticale ont été mises en évidence. Le débat quant aux mécanismes cellulaires et moléculaires impliqués est toujours ouvert car, bien que les études réalisées chez l'animal apportent des éléments de réponse, il est difficile de confirmer quels mécanismes sont impliqués chez l'humain. Parmi les différents mécanismes sous-jacents proposés, il est probable que ce soit au niveau des épines dendritiques que la plasticité intervient : que ce soit au niveau du bulbe olfactif ou du cortex, la littérature semble indiquer que formation, relocalisation et élagage des épines dendritiques permettraient de moduler les connexions synaptiques assez rapidement, dépendamment de l'activité. Les méthodes de neuroimagerie que nous avons utilisées ne permettent cependant pas de confirmer l'implication de ce mécanisme dans les changements que nous avons observés, ni de débattre de l'existence de neurogenèse chez l'humain.

À l'échelle plus large, nos observations en rapport avec l'amincissement du cortex mises en relation avec les résultats obtenus dans d'autres études semblent soutenir le modèle de surproduction-élagage selon lequel les effets de la plasticité cérébrale liée à l'entraînement ne sont pas linéaires. Selon ce modèle, dans un premier temps, le cortex s'épaissirait relativement rapidement dû à une surproduction de nouvelles synapses puis, dans un deuxième temps, le cortex s'amincirait à la suite d'un élagage des synapses qui permettrait de garder seulement les synapses fonctionnellement pertinentes. Nous pouvons suggérer que dans un troisième temps, une fois que les synapses produites en excès ont été éliminées, une pratique continue induirait un épaississement du cortex plus lent au cours des années. Ce modèle relativement récent remet en question le principe-même des études longitudinales qui ne testent les participants que deux fois dans le temps, car les changements dus à la plasticité ne seraient pas linéaires et l'idéal pour les observer serait donc de tester les participants plus de deux fois, ce qui peut représenter un défi supplémentaire car cela nécessite un investissement plus important. Comprendre la plasticité, c'est pouvoir apporter des connaissances susceptibles d'aider dans domaines telles que le traitement de maladies neurodégénératives. Il est donc important que des études futures tiennent compte du modèle de surproduction-élagage afin d'approfondir les connaissances sur les mécanismes impliqués dans la plasticité cérébrale.

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Annexe 1 – Smell training improves olfactory function and alters brain structure

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Syrina Al Aïn, Daphnee Poupon, Sebastien Hetu, Noemie Mercier, Jason Steffener, Johannes Frasnelli

Abstract

Training and repeated exposure to odorants leads to enhanced olfactory sensitivity. So far, the efficacy of intensive olfactory training on olfactory function in a healthy population and its underlying neurobiological basis remain poorly known. This study investigated the effects of a 6-week intensive and well-controlled olfactory training on olfactory function and brain structure/neuroplasticity. Thirty-six healthy young individuals were recruited and randomly distributed in three groups: (1) 12 participants underwent daily intensive olfactory training of at least 20 min that included an (a) odor intensity classification task, an (b) odor quality classification task and an (c) target odor detection task, (2) 12 participants underwent an equivalent visual control training, and (3) 12 control individuals did not participate in any training. Before and after the training period, all participants performed a series of olfactory tests and those from groups 1 and 2 underwent structural magnetic resonance (MR) imaging, from which we obtained measures such as cortical thickness and tissue density. Participants improved in the respectively trained tasks throughout the 6-weeks training period. Those who underwent olfactory training improved general olfactory function compared to control participants, especially in odor identification, thus showing intramodal transfer. Further, MR imaging analysis revealed that olfactory training led to increased cortical thickness in the right inferior frontal gyrus, the bilateral fusiform gyrus and the right entorhinal cortex.

Keywords: odor training, olfactory perception, olfactory system, neuroplasticity, MR imaging

Introduction

Contrary to what was thought for a long time, the adult brain exhibits an impressive degree of neural plasticity. Sensory loss (Merabet and Pascual-Leone, 2010; Reichert and Schopf, 2018), stroke (Borstad et al., 2013; Jones, 2017; Wilkins et al., 2017), brain tumors/irradiation (Brockmann et al., 2011; Duffau, 2008; Fisicaro et al., 2016; Merabet and Pascual-Leone, 2010), but also learning and training (Chang, 2014) lead to neural reorganization. Throughout the lifespan, experience-driven neural plasticity is necessary to cope with and adapt to ongoing environmental modifications. Over the last two decades, numerous studies have highlighted that acquiring and mastering skills, via an intensive training over the life-span (i.e., becoming an expert in a certain domain) or a specific short-term training paradigm (i.e., in the frame of a scientific study) are associated with functional and anatomical modifications in corresponding brain areas. The effects of experience and training have been investigated in various domains, such as music, sport, and games. For example, musicians exhibit changes in brain structure, such as an increased cortical density in auditory and motor cortex (Bermudez et al., 2009; Bermudez and Zatorre, 2005; Draganski et al., 2004; Gaser and Schaulg, 2003a,b; Kleber et al., 2007; Kleber et al., 2010; Maguire et al., 2000; Schlaug, 2015; Sluming et al., 2002; Zarate and Zatorre, 2008). Longitudinal studies juggling training in young (Draganski et al., 2004; Driemeyer et al. 2008) and in older participants (Boyke et al. 2008) suggest similar effects of motor training on brain structure. Moreover, intensive learning of medical students (Draganski et al., 2006), being a taxi driver in London (Maguire et al., 2000s) and an 8-week intensive memory training in elder participants (Engvig et al., 2010), are all associated with structural alterations of memory areas. Taken together, specific long and short-term training induces structural and functional changes in corresponding brain areas, and this neuroplasticity can occur throughout lifespan.

Contrary to other domains, we know much less about the effects of odor learning and odor expertise on the adult human brain. The relatively scarce literature that exists suggests that similar mechanisms apply to the sense of smell: olfactory specialists, e.g., perfumers (Delon-Martin et al., 2013) and sommeliers (Banks et al., 2016), have denser olfactory processing areas compared to untrained individuals. Specifically, professional perfumers exhibited increased gray matter volume in the orbitofrontal cortex which was positively correlated with years of experience (Banks et al., 2016; Delon-Martin et al., 2013). Compared to controls, perfumers who imagine smells (Plailly et al., 2012) and sommeliers who taste wine (Castriota-Scanderbeg et al., 2005; Pazart et al., 2014)

exhibit distinct activation patterns in olfactory processing regions (piriform cortex, orbitofrontal cortex) and hippocampus. Such functional adaptations may lead to the observed structural changes. These studies therefore suggest that sustained olfactory training and experience lead to functional and then structural reorganization of olfactory brain areas of odor experts. In non-expert individuals with a normal sense of smell, a short-term olfactory training improves olfactory performance (Dalton et al., 2002), and repeated exposure to an odorant enhances odor detection (Dalton et al., 2002; Doty et al., 1981; Engen, 1960; Rabin and Cain, 1986). Along the same lines, olfactory training of a few seconds daily is considered as a behavioral therapy in patients with olfactory dysfunction (Abolmaali et al., 2002; Damm et al., 2014; Fleiner et al., 2012; Haehner et al., 2013; Hummel et al., 2009; Konstantinidis et al., 2013; Mueller et al., 2005; Rombaux et al., 2006; Schriever et al., 2014b).

On a microscopic level, sensory experience leads to synapse formation and spine sprouting, and increases cell genesis of glial or neuronal cells (Trachtenberg et al., 2002), which macroscopically may result in an in-crease of gray matter density or thickness. Modern neuroimaging tools enable us to measure structural characteristics of the brain such as cortical thickness (Ad-Dabbagh et al., 2006) or gray matter density (Ashburner and Friston, 2000) on the whole brain. These methods showed that cortical thickness in olfactory processing areas is associated with performance in olfactory tasks (Frasnelli et al., 2010) and that individuals suffering from congenital anosmia or hyposmia due to different etiologies exhibit an altered architecture of these same structures (Abolmaali et al., 2002; Bitter et al., 2010a,b; Collet et al., 2009; Frasnelli et al., 2013; Haehner et al., 2008; Ibarretxe-Bilbao et al., 2010; Mueller et al., 2005; Rombaux et al., 2009a; Rombaux et al., 2009b; Rombaux et al., 2006; Rupp et al., 2005; Wattendorf et al., 2009). Specifically, the volume of core olfactory areas such as the piriform cortex, the orbitofrontal cortex (OFC), and the insular cortex were correlated with olfactory performance (Frasnelli et al., 2010; Seubert et al., 2013). Additionally, healthy individuals possess a bigger olfactory bulb than individuals with a reduced/absent sense of smell (Rombaux et al., 2006). In individuals with a normal sense of smell, olfactory bulb volume is correlated with olfactory performance (Buschhuter et al., 2008). However, most of the studies mentioned above suffer from one or two of the following problems: (1) the lack of precise control over the olfactory training; (2) the lack of a longitudinal component in the study.

The aim of this pilot study was therefore to examine the effects of a well-controlled 6-week olfactory training on olfactory function and on structural measures of the brain, with a particular focus on the olfactory processing areas and olfactory tasks. While participants performed specific tasks during training, we investigated a generalized improvement of olfactory function, extending to olfactory tasks which had not been exercised (intramodal transfer). In order to do so we measured olfactory function on six olfactory tasks in an olfactory training group and two control groups before and after a 6 weeks training period and carried out structural MRI. Since very little data is available on the effects of olfactory training on structural brain measures, we decided to test for cortical thickness and density to determine if one measure is more sensitive to detect training effects than the other.

We hypothesized that a short-term intensive odor training (1) leads to a better performance in the training tasks (training-specific effect), (2) leads to a better performance in non-exercised olfactory tasks (intra-modal transfer), (3) alters cortical density/thickness in olfactory and other brain areas and (4) that the changes in performance are correlated with changes in brain anatomy.

Methods

Participants

Thirty-six healthy participants (21 women and 15 men; mean [range] age = 24 [18–35] years) with normal olfactory function were included into the study. Exclusion criteria were neurological or psychiatric dis-eases, pregnancy, claustrophobia or impaired color vision. Participants were asked to refrain from smoking, eating, or drinking (except water) during the hour prior to training and testing.

All participants gave written consent, as required by the local ethical review board which approved all behavioral procedures and the use of individual MRI scans (CMER RNQ 15-16-10).

Training

Participants were randomly distributed across three groups: (1) Olfactory training group (OT): 12 participants (7 women, 5 men) followed a strict olfactory training paradigm consisting of daily visits to the lab for 6 weeks, (2) Visual training control group (VTC): 12 participants (6 women, 6

men) completed an equivalent visual training paradigm, and (3) Control group (C): 12 participants (8 women, 4 men) did not receive any training.

The training sessions took place in a well-ventilated experimental room in our laboratory at the University du Quebec a Trois-Rivieres (UQTR). Trained participants (OT and VTC groups) were invited to visit the lab for daily training sessions over 6 weeks. Training sessions lasted between 20 and 30 min and consisted in the following 3 tasks: (1) Intensity classification, (2) Quality classification, and (3) Target detection. These tasks were carried out daily (Monday through Friday). For the weekend, we instructed participants to perform a reduced training session with task 1 at home.

Olfactory Training. The goal of the olfactory training was to expose participants to (a) odorants in general and (b) to a specific target odor in order to achieve a controlled, steady and repeated odor exposure. The target odor was either phenyl ethanol (PEA; n = 6) or n-butanol (BUT; n = 6). All of the odorants were contained in opaque glass bottles. For each task, participants were instructed to respond as fast as possible while maintaining accuracy and were allowed to smell as much as they wanted. (1) Odor intensity classification task: We asked participants to order 16 odor samples of the target odor according to its concentration (from the lightest to the strongest concentration of PEA and BUT: 4%; 2%; 1%; 0,5%; 0,25%; 0,125%; 0,0625%; 0,03125%; 0,0156%; 0,0078%; 0,0039%; 0,00195%; 0,000977%; 0,000488%; 0,00024%; 0,00012%; propylene glycol was used as solvent), (2) Odor quality classification task: We asked participants to order 11 odor samples according to the concentration of the target odor (4%) mixed with citrus odor (ratio target odor: citrus, ranging from 0:100 to 100:0), (3) Target odor detection task: We asked participants to identify whether the target odor was present among a set of 14 samples. Seven bottles contained only one non-target odor (e.g., cola, peach, citrus, etc.) whereas seven other bottles contained the target odor mixed with each of these odors (50:50 mixtures of isointense components). If participants terminated the three tasks in less than 20 min, they completed task 1 again. If participants completed task 1 two times, only their performance during the first administration was analyzed.

Visual Training Control. Participants in this group carried out visual control tasks, based on colored paper. Similar to the olfactory training group, stimuli (here, colored papers) were contained in opaque glass bottles and participants could only observe them one at the time but as often as

they wanted. Tasks were designed to be equivalent to the olfactory training tasks and similar instructions were given. (1) Color intensity classification task: We asked participants to order 16 pieces of colored papers of a target color (gray hues) according to its colorimetric intensity (from the lightest to the darkest gray), (2) Color quality classification task: We asked participants to order 11 color samples according to the color gradient of a target color (pink) mixed with the green color (ratio pink: green, from 0:100 to 100:0), (3) Target color detection task: We asked participants whether a target color (purple color with a specific hue) was recognized among 14 purple hues (7/14 were the target purple hue). More specifically, participants had to observe the target color only once, as long as they wanted, and then they had to answer if yes or no it was the same target purple hue. If participants terminated the three tasks in less than 20 min, they completed task 1 once again. If participants completed task 1 two times, only their performance during the first administration was analyzed.

Behavioral tasks

In order to assess the generalized effect of training, we measured a total of six olfactory behavioral tasks (see Table 1 for an overview), based on the Sniffin' Sticks olfactory test kit (Hummel et al., 1997; Kobal et al., 2000) (Burghart, Wedel, Germany) and the UPSIT (Doty et al., 1984). We did this before and after the training in all three groups of participants.

(1) PEA detection threshold and (2) BUT detection threshold: We assessed separate odor detection thresholds for PEA and BUT by using the Sniffin' Sticks and following standardized procedures (Hummel et al., 1997). In short, we assessed detection thresholds for each target odor with a single-staircase, 3-alternative forced-choice procedure. The experimenter sequentially presented three odorized pens for 2s in a randomized order. We asked participants to identify the pen containing the target odor (two pens contained the solvent and the third pen contained the target odor at a specific concentration). The staircase began at the lowest concentration of the target odor (among 16 concentrations). Reversal of the staircase was triggered when the odor was properly detected in two successive trials whereas subsequent reversal of the staircase was performed when the target odor was not correctly perceived. Scores for the odor threshold refer to the mean of the last four of seven staircase reversal points and can range from 1 to 16.

(3) Odor discrimination: Odor discrimination were assessed by using an extended version of the Sniffin' Sticks discrimination task (Frasnelli et al., 2010a). Three pens were sequentially

presented for 2s in a randomized order; two containing the same odor and the third containing the target odor. The target odors were identified in a row of 32 triplets of odors. Scores for odor discrimination task can range from 0 to 32.

We assessed participants' capacity to identify odors using two different tasks:

(4) Free odor identification. For this task we adapted the identification subtest of the Sniffin' Sticks battery. Unlike the standard procedure, where participants choose among four descriptors for each of 16 odors, we asked participants to identify odors without cues (free identification). To assess intramodal transfer, we removed 2 out of 16 odorants (i.e., coca cola and lemon) of the analysis, as olfactory trained subjects were exposed to them throughout the olfactory training (task 3). We counted the number of correct responses (1 point for the correct identification of a given odor, 0.5 points for the correct identification of the category of a given odor); scores for this free odor identification test can range from 0 to 14. We used two different sets of odors for session 1 and 2.

(5) Cued odor identification. For this 4-alternative forced-choice odor identification test we used the UPSIT, a scratch-and-sniff test based on microencapsulated odorants printed on paper sheets, which are released upon scratching. In order to avoid a learning effect between both sessions (before and after training), we selected 20 odors amongst the 40 of the UPSIT, in a pseudorandomized and counterbalanced fashion for each participant. For the session after the training we then tested the remaining 20 odors. Participants identified the odors with the help of four descriptors per odor (Doty et al., 1984). Scores for this adapted version of the UPSIT therefore can range from 0 to 20.

(6) Odor memory: We assessed the ability of the participants in recognizing odors after 24 h. A set of 8 odors from the Sniffin' Sticks test were selected (which participants had smelled in the free identification task) as well as 8 odors from an extra-set of sticks (new odors). The experimenter asked whether the odor has been presented during the odor identification task performed the previous day. We counted the number of hits (0–8) and correct rejections (0–8) and calculated d', in accordance to signal detection theory (Snodgrass and Corwin, 1988).

Brain imaging

We carried out the magnetic resonance imaging sessions at the 3.0 T S Trio scanner of the Unite de Neuroimagerie Fonctionnelle (UNF) at the Institut Universitaire de Geriatrie de Montreal (IUGM) of Universite de Montreal. We acquired a T1-weighted structural volume (voxel size: 1.0 1.0 1.0 mm), using an MPRAGE sequence (repetition time 2530 ms, echo time 1.64 ms, flip angle 7 , 176 contiguous sagittal slices, in-plane field of view 256 mm).

General procedure

Before and after the 6-week training period, all participants took part in two sessions in which we measured olfactory function behaviorally. Therefore, we assessed odor thresholds, odor discrimination, odor identification (on day 1) and odor memory (on day 2) for all 36 participants. In addition to this, both (1) the olfactory training group (n = 12) and (2) the visual control group (n = 12) underwent magnetic resonance (MR) imaging in two separate sessions (before and after training; see Table 2 for an overview).

Data analysis and statistics

Behavioral data

We used SPSS 20.0 (IBM) to analyse behavioral data. Since participants of the olfactory training group had different target odors (either PEA, n = 6 or n-BUT, n = 6) we first compared these two subgroups to each other with separate two-sample t-tests for the six behavioral tasks (i.e, BUT threshold, PEA threshold, odor discrimination, free identification, cued identification, odor memory) at the end of training. We did not observe any difference between both subgroups and therefore merged them together.

Task specific training effect. In order to assess task specific training effects, we carried out the following transformations. First, we averaged performance scores per week. Second, we indexed these scores to the mean of week 1, where the value for week 1 was set a 100. If, for example, the score for week 2 was 50% above that of week 1, we assigned a value of 150, etc. We then computed a repeated measures ANOVA with week (6 levels: weeks 1–6) and training task (3 levels: (1) intensity classification, (2) quality classification, (3) target detection) as within-subject factor (wsf) and group (2 levels: olfactory training group, visual training control group) as between-subject factor (bsf).

General training effect (intramodal transfer). In order to evaluate the effect of training on olfactory function, we calculated the difference be-tween scores before and after training (post-pre) for each of the six olfactory tasks. In order to increase statistical power, we compared the two control groups to each other with two sample t-tests (visual control group and no training control group forming visual þ control group). After verifying that they did not exhibit any significant difference for any variable, we merged both control groups. Next, we z-transformed each of the 6 variables and verified normal distribution using Kolomogorov-Smirnoff test. We then computed a repeated measures ANOVA with task (6 levels: (1) PEA threshold; (2) BUT threshold; (3) odor discrimination; (4) free identification; (5) cued identification; (6) odor memory) as wsf and group (2 levels: olfactory training group; visual þ control group) as bsf. We used t-tests for post hoc analyses, with Bonferroni correction for multiple comparison, unless stated otherwise. The alpha level was set at 0.05.

Imaging data

We analyzed images using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB unless stated otherwise.

Preprocessing

We used the CAT12 toolbox (C. Gaser, Structural Brain Mapping group, Jena University Hospital, Jena, Germany) implemented in SPM12. First, using the longitudinal segmentation tool, structural images from the 1st and 2nd visits were realigned, segmented and normalized to MNI space. Briefly, this tool first realigns the two structural images, calculates a mean structural image and corrects the realigned images for signal inhomogeneities (bias-correction) with regard to the reference image. The mean image is then segmented and spatial normalization parameters are estimated using this segmentation. Normalization parameters are applied using DARTEL spatial registration to the segmented bias-corrected images from the 1st and 2nd visit. Finally, the segmented normalized images from the 1st and 2nd visit are again realigned and underwent quality control (using CAT12 quality control tools).

Cortical thickness

CAT12 uses a projection-based thickness (PBT) approach that uses tissue segmentation to estimated the white matter distance and projects the local maxima (equal to the cortical thickness) to other gray matter voxels by using a neighbour relationship described by the white matter distance

(Dahnke et al., 2012). This results in separate cortical thickness data for the left and right hemispheres. This cortical thickness data was finally resampled and smoothed using a 15 mm FWHM kernel. Each participant's cortical thickness data was entered in a second-level analysis using SPM flexible factorial design with the wsf visit (2 levels: pre-training, post-training) and the bsf group (2 levels: olfactory training group; visual training control group). We also evaluated cortical thick-ness changes between pre- and post-training for the olfactory training group by conducting a second level comparison on the wsf visit (post-- training – pre-training). Results were thresholded at $p < 0.05$ using a FWE correction. We also provide results with a lowered criterion ($p < 0.001$) for predicted regions.

Voxel based morphometry

Cortical density was defined as the relative concentration of gray matter within a voxel. Voxel based morphometry data was resampled and smoothed using a 8 mm FWHM kernel. Each participant's data was entered in a second-level analysis using SPM flexible factorial design with the wsf visit (2 levels: pre-training, post-training) and the bsf group (2 levels: olfactory training group; visual training group). We also evaluated cortical density changes between pre- and post-training for the olfactory training group by conducting a second level comparison on the wsf visit (post-training – pre-training). Results were thresholded at $p < 0.05$ using a FWE correction. We also provide results with a lowered criterion ($p < 0.001$) for predicted regions.

Relation between behavioral and imaging data

In order to assess possible associations between changes in performance and changes in brain morphometry related to the training, we tested the correlation between imaging and behavioral measures within the olfactory training group. First, whole brain second level analyses were first conducted by testing the correlation between changes in morphometry metrics (post-training – pre-training) and changes in performance (post-training – pre-training). Results were thresholded at $p < 0.05$ using a FWE correction. Second, ROI based analyses were conducted. Specifically, ROIs were defined as anatomic regions (from the aparc_2009s atlas available in CAT12, see Destrieux et al., 2010), where the peak of significant clusters from the Visit Group interaction analysis were located. For each participant, morphometry measures were extracted for all voxels within these ROIs and averaged for the post-training and pre-training visits. Then, the differences between post-training and pre-training for morphometry and behavioral measures were entered into a correlation

analysis where we corrected for multiple tests (within ROI) by setting a Bonferroni threshold of .05/6 = 0.008.

Results

Task specific training effect

The repeated measure ANOVA revealed a significant effect of week ($F(5,105) = 6.663$; $p = 0.004$; Greenhouse-Geisser corrected). Post hoc t-tests showed a significant difference between (1) week 1 and all other weeks (all $p < 0.022$; uncorrected); (2) week 2 on the one hand and weeks 4–6 on the other hands (all $p < 0.031$; uncorrected; Fig. 1) indicating a significant improvement of scores over time. We did not observe any effects of group or training task, nor any interaction between any of the variables.

General effect of training

The repeated measure ANOVA revealed a significant effect of group ($F(1,34) = 6,56$; $p = 0.014$); with the olfactory training group having significantly better overall results on the six non-trained tasks than the visual þ control group. We next evaluated both groups on all six individual tasks; While being nominally superior on five of the six tasks, the olfactory training group obtained a significantly better score for the free identification task ($p = 0.006$; Fig. 2). We did not observe any effect of task or interactions.

Half of the participants in the olfactory training group had PEA as target odor, whereas the other half had BUT as target odor. We therefore investigated whether detection thresholds for the target odor were more affected than for the non-target odor, by using a paired t-test. We did not observe any significant difference.

Brain imaging

Data from one participant had to be excluded from further analysis due to movement artifacts.

Cortical thickness: when applying FWE correction, the ANOVA revealed a significant interaction between group and visit at two clusters, one located in the right inferior frontal gyrus and one in the left occipital cortex (see Table 3 for detailed information). When we lowered the threshold to $p < 0.001$ uncorrected, we observed significant differences in two additional clusters namely in the

right frontal operculum and in the right superior temporal gyrus stretching into the superior temporal sulcus.

When only comparing between visits for the olfactory training group, we observed again a significant effect in the right inferior frontal gyrus with the conservative criterion. At the more liberal threshold, we additionally observed effects in the left inferior temporal gyrus, the right inferior frontal gyrus, bilateral fusiform gyrus and the right entorhinal cortex (Fig. 3).

Voxel based morphometry: We did not observe any cluster with a significant interaction between group and visit when applying FWE correction ($p < 0.05$). A lower threshold ($p < 0.001$, uncorrected) yielded five small clusters, two of which were located in the occipital cortex bilaterally; three were located in the precentral gyrus bilaterally. Focusing on differences between visits for the olfactory training group, we only observed significant effects using the liberal threshold including in the bilateral inferior frontal gyrus (see Table 4 for detailed information).

Correlation between behavioral and brain measures

Regarding the olfactory group, none of the correlations at the whole brain level reached the statistically significant threshold. ROI correlation analysis between behavioral changes and brain measures yielded no significant correlation that passed Bonferroni correction for either ROIs (see Table 5 where we also provide the correlation results for the visual training group and the whole sample). However, we noted a tendency for a positive correlation between the increase in performance in the olfactory memory task and increases in CT in the left occipital cortex ROI ($r = 0.81$, $p = 0.009$).

Discussion

In this paper, we describe three major results. First, intensive and well-controlled 6-week olfactory and visual trainings both led to improvement in performance specific to the trained tasks. Second, olfactory training, but not visual training led to a generalized improvement of olfactory function, i.e., showed a transfer to olfactory tasks which had not been trained. We observed the largest effect on free odor identification. Third, this olfactory training was associated with a significant increase in cortical thickness and, to a lesser extent, cortical density of several brain regions, including the right inferior frontal gyrus and areas in the temporal lobe.

Behavioral measures

We observed a specific and generalized effect of olfactory training on olfactory function, including a transfer to non-trained tasks.

As expected we observed a specific training effect (i.e., an improvement of performances over weeks in the tasks performed during the training) as olfactory training has proved to have such an effect in multiple studies. For example, participants with specific anosmia (e.g., androstenone, isovaleric acid, lyral) have seen sensitivity to odors they could barely perceive increase after a repeated exposure to them (Croy et al., 2015; Moller€ et al., 1999; Wang et al., 2004). Similar effects have been shown in patients with generalized olfactory dysfunction (Haehner et al., 2013; Hummel et al., 2009), and in non-expert individuals with a normal sense of smell (Dalton et al., 2002; Rabin and Cain, 1986), with repeated exposure to some odorants leading to an increase in the sensitivity to these specific odorants.

Further, such an effect of olfactory training enhancing olfactory function in general has repeatedly been shown for patients with olfactory alteration or loss (Hummel et al., 2009; Damm et al., 2014; Altundag et al., 2015; Konstantinidis et al., 2013; Fleiner et al., 2012; Kollndorfer et al., 2014, 2015; Haehner et al., 2013; Geissler et al., 2014). More precisely, a meta-analysis recently reported positive effect of olfactory training on general olfactory function, with large effects of training on a global olfactory score, odor discrimination and odor identification for patients with olfactory diseases of all types, and small-to-moderate effect on odor sensitivity (Sorokowska et al., 2017). However, its effectiveness on olfactory function in general remained understudied in individuals with a normal sense of smell where results are more heterogeneous. In fact, while one study did not show any generalized effect of olfactory function in individuals with a normal sense of smell (Livermore and Hummel, 2004), a second paper reported even a decrease in sensitivity after olfactory training (Negoias et al., 2017), and other studies reported enhanced odor sensitivity in young and healthy older individuals (Mori et al., 2015; Schriever et al., 2014a). Interestingly, neuroanatomical and electrophysiological studies have demonstrated that repeated odor exposure in humans can increase olfactory bulb volume (Negoias et al., 2017) and increase amplitudes of recordings from the olfactory epithelium (Livermore and Hummel, 2004; Wang et al., 2004).

Our results are therefore contrasting some of these earlier reports, as we observed a significant improvement of olfactory function. The effect in our study was mostly driven by an improvement

in the free identification task. This difference may, at least partly, explained by the fact that our daily 20 min training procedure, including three complex tasks, mobilized higher cognitive abilities than the procedure usually used, consisting in passively smelling 4 odorants, twice a day. Further, we did not observe a target odor specific improvement of the detection threshold, which is congruent with the literature on patients with olfactory dysfunction: training mainly improved their performance in higher order tasks such as odor identification and discrimination rather than odor detection thresholds (Fleiner et al., 2012; Haehner et al., 2013; Sorokowska et al., 2017). Odor detection threshold having less cognitive demand than tasks like identification (Hedner et al., 2010), a possible explanation would be that this short and intensive olfactory training does not impact olfactory sensitivity, but processing of olfactory stimuli at a higher cognitive level. In our study, the positive effect of olfactory training on olfactory function may not only be related to peripheral changes, but seems to also be linked to central changes; i. e., improved cognitive processing of odor stimulation and increased attention to odors. The underlying mechanisms have yet to be discovered. We can note, however, that our results showed an improvement in the free identification task, but not in the cued one; the absence of improvement in this task might be due to a ceiling effect as healthy and young individuals usually already achieve high scores in the UPSIT which is designed to distinguish patients with reduced olfactory function from individuals with normal olfactory function.

Brain imaging

We observed an enhancement of cortical thickness and, to a lesser degree, density due to olfactory training. The link between brain anatomy and olfactory function has been investigated in earlier studies; this literature can be subdivided into four categories of papers. The first set of articles investigated healthy individuals. They show a correlation be-tween olfactory function and neuroanatomical measures such as volume of the olfactory bulb (Buschhuter et al., 2008; Seubert et al., 2013) and the density or thickness of cortical structures, including olfactory pro-cessing areas such as the orbitofrontal, piriform and insular cortex, but also regions which are not classically associated with olfactory processing such as precentral, postcentral and superior temporal gyri (Frasnelli et al., 2013; Segura et al., 2013; Seubert et al., 2013). A second set of papers compared olfactory specialists such as perfumers and sommeliers with healthy controls. Here, specialists, which can be seen as individuals with year-long training, exhibited denser cortex in the orbitofrontal (Delon-Martin et al., 2013), entorhinal and insular cortex (Banks et al., 2016). The

third set of papers examined differences between patients with acquired loss of olfactory function with healthy controls. Here, next to reduced volumes of the olfactory bulb (Rombaux et al., 2006; Rombaux et al., 2008; Rombaux et al., 2010), patients showed thinning of olfactory processing areas such as piriform, insular, orbitofrontal, anterior cingulate cortex and parahippocampal gyrus (Bitter et al., 2010a,b; Gellrich et al., 2017; Peng et al., 2013; Yao et al., 2014). Furthermore, they exhibited thinning in additional brain regions such as subcallosal, superior and middle temporal, middle occipital, fusiform gyri as well as medial prefrontal and cingulate cortex (Bitter et al., 2010a,b; Gellrich et al., 2017; Peng et al., 2013; Yao et al., 2014). Finally, one report investigated the effect of 12 weeks of olfactory training in patients with olfactory dysfunction and found density in hippocampus and thalamus to increase with olfactory function (Gellrich et al., 2017). In summary, these studies indicate a link between olfactory function and neuroanatomical measures, with better olfactory function being related to thicker and denser cortex in olfactory processing areas and other brain regions. We add to this by showing that olfactory training affects brain structures also in healthy participants. We will discuss the most important findings in the following.

We observed the most significant effect of training on cortical thick-ness in the triangular portion of the right inferior frontal gyrus (IFG). In addition, when focusing exclusively on the olfactory training group, we also found increases in cortical density of the bilateral IFG between session 1 and 2, but only at a liberal threshold. While the IFG is commonly reported to be activated after olfactory stimulation, its triangular portion is not typically associated with olfactory processing. Nevertheless, several studies reported involvement of this structure at nearly identical coordinates in olfactory tasks. For example, when judging familiarity of odors, participants exhibited activations of the triangular portion of the IFG (Plailly et al., 2005). The authors interpreted this to reflect the involvement of the region in selection and integration of semantic memory. Three independent studies reported activation to olfactory stimulation to be larger in the triangular portion of the IFG in patients with Parkinson's Disease who exhibit untypically preserved olfactory function compared to controls (Hummel et al., 2010; Welge-Lussen et al., 2009; Westermann et al., 2008). Finally, one study found a positive correlation between density of this structure and the ability to identify odors in patients with corticobasal syndrome (Pardini et al., 2009). While it is difficult to generalize from studies on patients with neurodegeneration this seems to suggest that the triangular portion of the IFG is involved in higher order processing of olfactory function, in line with our finding. However, we did not observe an association be-tween improvement of

olfactory function and changes in cortical thick-ness in the IFG. This suggests that the effect of olfactory training on this particular structure is an all-or-nothing effect. The association between olfactory improvement on the memory test and thickness of the occipital cortex showed a strong correlation although not significant when using a stringent correction. This result is puzzling since this data was obtained in the group of olfactory training. Future studies should investigate these possible links.

Next, we observed an effect of olfactory training in the right superior temporal gyrus (STG). This is in line with earlier reports: in healthy individuals, odor identification and STG thickness are correlated (Frasnelli et al., 2010), whereas in patients with anosmia its density is reduced (Bitter et al., 2010b; Peng et al., 2013). ERP source localization and functional MRI show that STG is involved in early processing of olfactory stimuli (Lascano et al., 2010), especially more complex ones (Pellegrino et al., 2017). Our study suggests that olfactory training affects the STG, possibly caused by the repeated evaluation of complex stimuli.

Further, we observed an effect of training on the bilateral fusiform gyrus. This structure has repeatedly been shown to have a reduced volume in patients with anosmia and hyposmia (Bitter et al., 2010a,b; Peng et al., 2013). In fact, fMRI shows that the fusiform gyrus is involved in odor recognition (Cerf-Ducastel and Murphy, 2006) and correct odor identification (Kjelvik et al., 2012). Again, our results suggest that repeated odor recognition and identification led to an increase of cortical thickness in the fusiform gyrus.

Finally, we also observed a significant increase in thickness of the right entorhinal cortex following olfactory training. The implication of the entorhinal cortex in olfactory processing is well known (Zald and Pardo, 2000), especially with regards to olfactory memory (Wilson et al., 2014). A recent fMRI study showed that odor category learning is asso-ciated with the appearance of specific activation patterns in the ento-rhinal and piriform cortex (Qu et al., 2016). Regarding anatomical measures, its gray matter volume is correlated with the ability to identify odors in patients with different degrees of olfactory dysfunction (Segura et al., 2013).

In summary our results are in line with a notion that olfactory training increases cortical thickness in brain regions involved in olfactory identification, learning, and memory. In addition, there appeared a link be-tween olfactory training and thickness of the occipital cortex.

Voxel based morphometry revealed no effects of training with a conservative threshold. With a more liberal threshold, visual and pre-central areas were found to be changed by olfactory training. Due to the small sample size this result in non-olfactory regions has to be taken with caution, but we have shown that olfactory ability is correlated with thickness of right pericentral areas (Frasnelli et al., 2010). While the exact implication remains unknown, it may have to do with motor con-trol of sniffing. Future studies should show if some people are better sniffers due to a cerebral anatomical predisposition.

There are some limitations to this pilot study. First, the sample size was small, which reduced the power of our statistical analysis. This might have impacted our ability to find significant correlations between olfactory performance and cortical thickness. Next, the duration of the training of 6 weeks was short compared to most other studies, where olfactory training lasted commonly from 2 to 8 months (Fleiner et al., 2012; Hummel et al., 2009; Haehner et al., 2013; Kollndorfer et al., 2014). Moreover, the control group who did not receive training did not undergo MRIs session before and after 6 weeks, limiting any interpretation of their result. However, the biggest strength of our report is the control we had over different aspects of our study. First, we had two control groups. Because the visual training control paradigm was similar to the olfactory training one, we can affirm that any found effect was specifically due to the olfactory training, and not to any unspecific training. Second, since training was carried out in the lab and lasted at least 20 min, we were able to exactly control participants' exposure to odors. This is a great advantage of our study compared to most other ones, where training was typically carried out at home and lasted 20 s on each of 4 odors, twice a day (Fleiner et al., 2012; Hummel et al., 2009; Haehner et al., 2013; Kollndorfer et al., 2014).

In conclusion, our findings confirm that olfactory training can improve olfactory function (Sorokowska et al., 2017), and that changes can occur rather fast as a 6-week training was long enough to observe an improvement. These changes may be related to modifications occurring directly in the brain. Although a recent study investigated longitudinal effects of olfactory training in patients with post-infectious olfactory (Konstantinidis et al., 2016), the question of the effects of the olfactory training duration on behavioral and cerebral changes and the stability of theses changes is still open. Further studies with, ideally, a larger sample size, should in various populations investigate: (1) the effects of a longer olfactory training on olfactory function and on

the brain, (2) the effects of olfactory training on olfactory bulb size, (3) the stability of olfactory improvement beyond the training period, and (4) brain structure connectivity by mapping white matter tractography in the brain using diffusion tensor imaging (DTI). Understanding the underlying mechanisms of neuroplasticity in the olfactory system could be useful to develop efficient ways to use olfactory training as a therapeutic approach for patients with olfactory dysfunction.

Tables and figures

Table 1. Behavioral tasks assessing olfactory function

#	Assessed task	Material *Procedure*
1	Odor threshold 1 for phenyl ethanol	Sniffin' Sticks *Standard*
2	Odor threshold 2 for n-butanol	Sniffin' Sticks *Standard*
3	Odor discrimination	Sniffin' Sticks *Adapted: 32 items instead of 16*
4	Free odor identification 1	Sniffin' Sticks *Adapted: free instead of cued identification*
5	Cued odor identification 2	UPSIT *Adapted: 20 items instead of 40*
6	Olfactory memory	Sniffin' Sticks *Adapted*

Table 2. Overview over procedures

Group	Behavioral testing 1	MRI 1	training	Behavioral testing 2	MRI 2
olfactory training	x	x	x (olfactory)	x	x
visual training control	x	x	x (visual)	x	x
no training control	x			x	

Table 3. Significant effects of olfactory training on cortical thickness. Structures in bold were significant at a $p < 0.05$, FWE level, the remainder at a $p < 0.001$ uncorrected level. Coordinates (x, y, z) are in MNI space. BA: Brodmann area

Contrast	Region	T	Coordinates		
			x	y	z
Interaction: group x time ($Olf_{post\text{-}training}$ - $Olf_{pre\text{-}training}$) - ($Vis_{post\text{-}training}$ - $Vis_{pre\text{-}training}$)	**R inferior frontal gyrus (triangular portion) BA 45**	5.94	54	29	1
	L occipital cortex BA 18	6.53	−8	−102	9
	R inferior frontal gyrus (opercular portion) BA 44	4.20	49	14	4
	R superior temporal gyrus BA 22	3.69	59	−12	−6
Olfactory group: effect of time ($Olf_{post\text{-}training}$ - $Olf_{pre\text{-}training}$)	**R inferior frontal gyrus (triangular portion) BA 45**	6.94	54	30	2
	L inferior temporal gyrus BA 21	4.12	−55	−19	−32
	R inferior frontal gyrus (opercular portion) BA 44	4.09	51	15	6
	R anterior fusiform gyrus BA 20	3.77	33	−12	−35
	L anterior fusiform gyrus BA 20	3.72	−34	−12	−34
	R entorhinal cortex	3.70	22	−6	−31

Table 4. Significant effects of olfactory training on voxel based morphometry. All structures were significant at a $p < 0.001$ uncorrected level. Coordinates (x, y, z) are in MNI space. BA: Brodmann area

Contrast	Region	T	Coordinates		
Interaction: group x time ($Olf_{post\text{-}training}$ - $Olf_{pre\text{-}training}$) - ($Vis_{post\text{-}training}$ - $Vis_{pre\text{-}training}$)	L striate area BA 17	5.46	−9	−99	−2
	L precentral gyrus BA 6	4.74	−39	−2	38
	L precentral gyrus BA 4	4.38	−14	−18	66
	R precentral gyrus BA 4	4.24	39	−16	40
	R occipital area BA 18	3.91	2	−78	6
Olfactory group: effect of time ($Olf_{post\text{-}training}$ - $Olf_{pre\text{-}training}$)	L superior frontal gyrus BA 10	7.35	−21	69	10
	L middle frontal gyrus BA 10	5.04	−36	57	20
	L superior frontal gyrus BA 10	4.85	−28	64	−9
	R middle temporal pole BA 38	4.80	38	22	−39
	R cerebellum	4.65	27	−87	−45
	L inferior orbital frontal BA 47	4.58	−28	32	−3
	R middle frontal gyrus BA 6	4.41	27	0	46
	L middle frontal gyrus BA 6	4.30	−24	3	48
	R caudate BA 48	4.28	10	24	−4
	R occipital pole BA 18	4.18	15	−104	10
	L inferior frontal gyrus BA 46	4.18	−51	45	3
	R inferior frontal gyrus BA 47	4.17	30	30	−4
	R supplemental motor area BA 6	4.10	15	−6	60
	L inferior temporal gyrus BA 20	4.07	−45	−21	−21
	R supplemental motor area BA 6	4.05	16	9	62

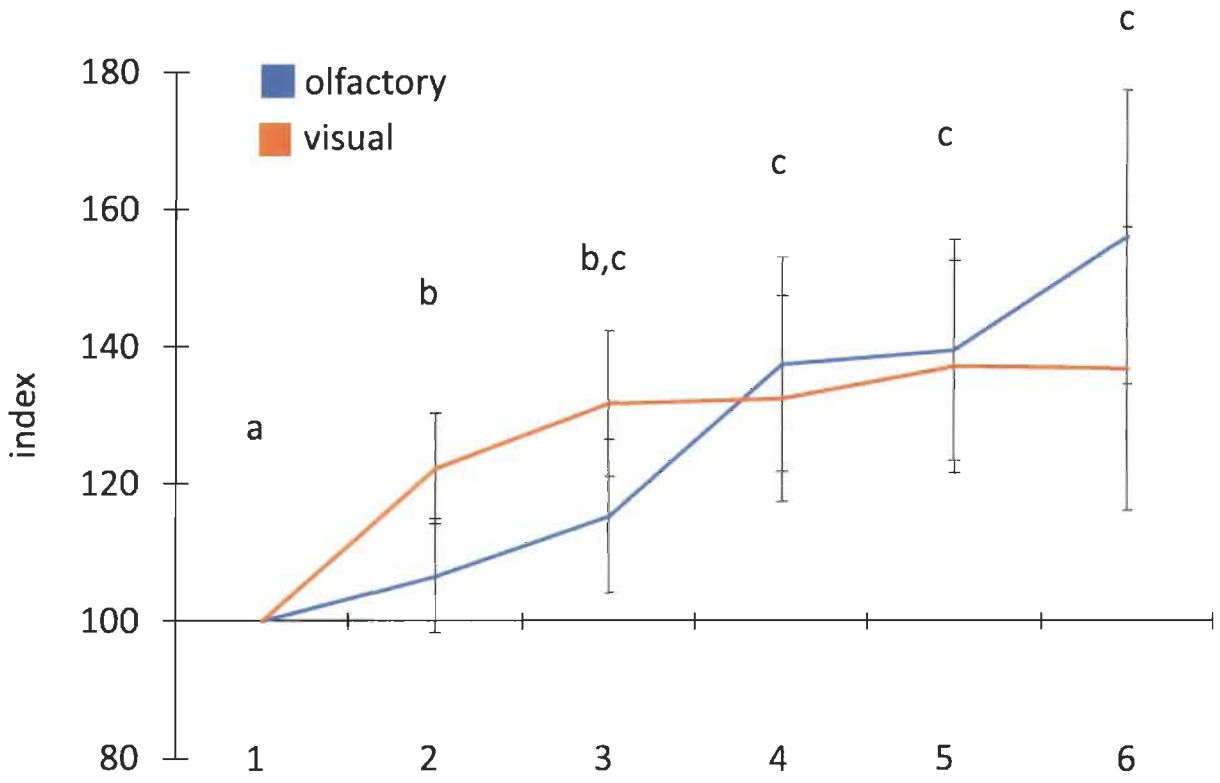

Figure 1. Task specific training effect. Scores were averaged for per week (week 1–6) and task and indexed to week 1 (week 1 = 100) for the olfactory training group (blue) and the visual training group (orange). Error bars indicate standard error of the mean. Letters indicate significant differences between weeks (bars with different letters were significantly different from each other; for example, bars with "a" are significantly different from bars with "b", but both are not significantly different from bars with "a, b").

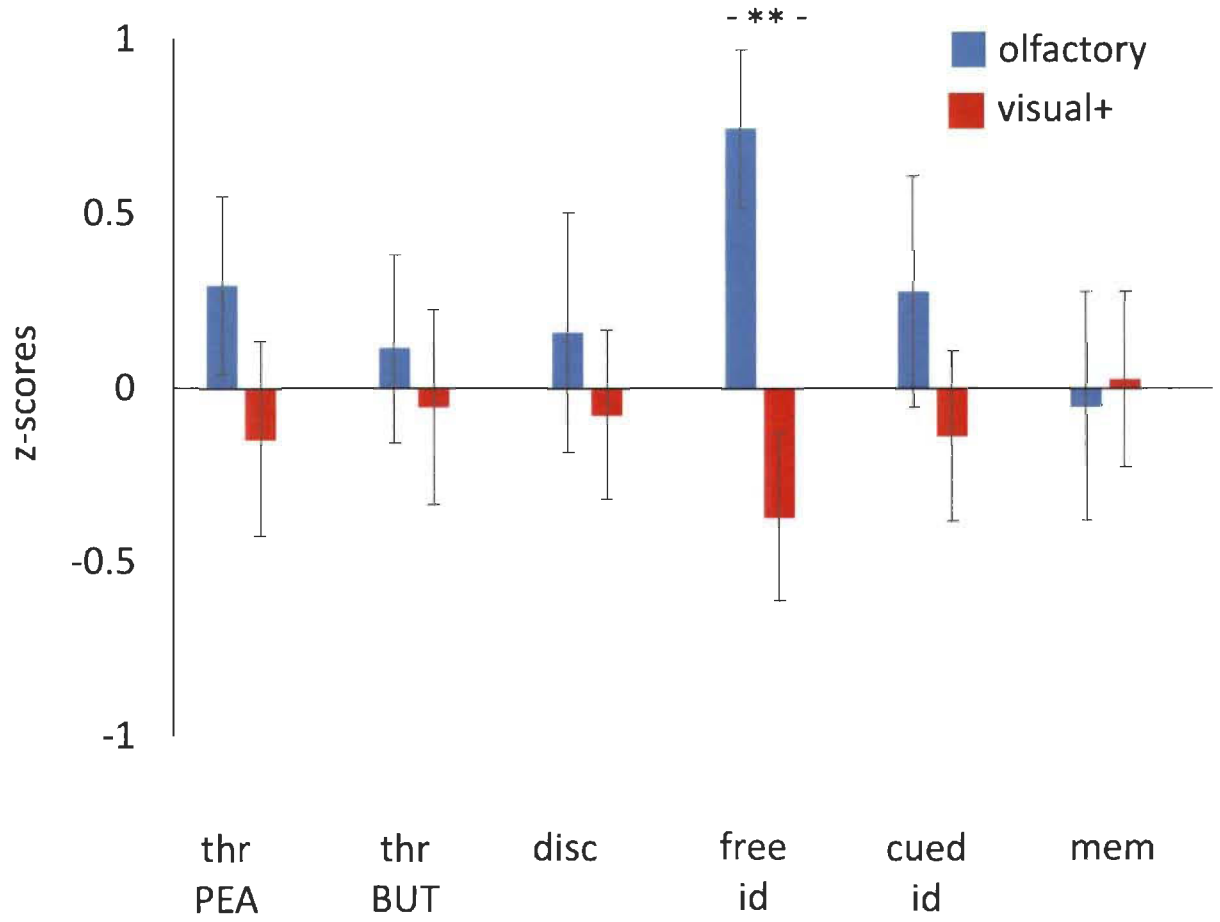

Figure 2. General effect of training. Z-scores for 6 untrained olfactory tasks (thr PEA: detection threshold for phenyl ethanol detection; thr BUT: detection threshold for n-butanol; disc: odor discrimination; free id: uncued odor identification; cued id: cued odor identification; mem: olfactory memory) for the olfactory training group (blue) and the combined control group (visual training control group and untrained control group). Error bars indicate standard error of the mean. Asterisks indicate significant group difference.

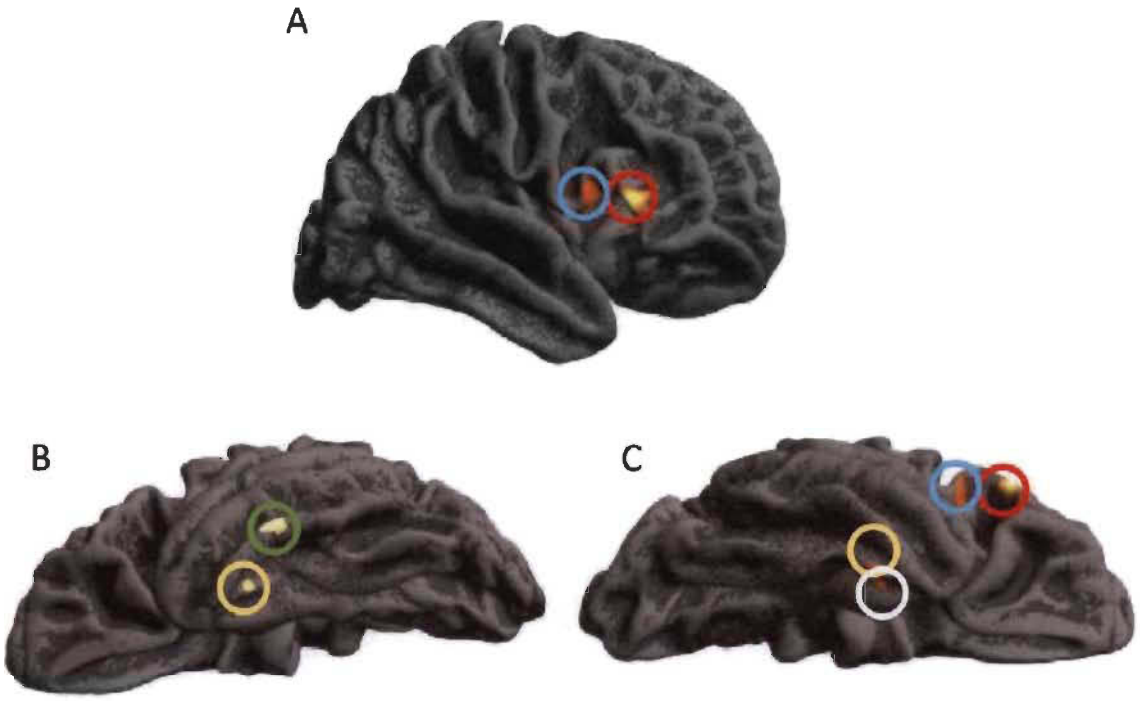

Figure 3. Effect of olfactory training on cortical thickness: Comparison between before and after training in the olfactory training group with increased cortical thickness highlighted. A. frontolateral view of the right hemisphere. B. basal view of the left hemisphere. C. basal view of the right hemisphere. FEW $p < 0.05$: red circle: right inferior frontal gyrus (triangular portion); uncorrected $p < 0.001$: blue circle: right inferior frontal gyrus (opercular portion); green circle: left inferior temporal gyrus; yellow circle: bilateral fusiform gyrus; white circle: right entorhinal cortex.

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Annexe 2 – Early-blind individuals show impaired performance in wine odor categorization

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Simona Manescu, Daphnée Poupon, Jordi Ballester, Hervé Abdi, Dominique Valentin, Franco Lepore, Johannes Frasnelli

Abstract

Blind individuals display superior sensory abilities in other modalities, yet results remain contradictory regarding their performance on olfactory tasks. Using complex ecological olfactory tasks, we evaluated the impact of blindness on olfactory performance. We tested 12 early-blind individuals (M = 49, SD = 13.09) and 12 sighted controls (M = 49, SD = 14.31) who were all blindfolded. Based solely on the wine odors, participants evaluated 24 pairs of wine and determined if both samples belonged to the same category (red wine, white wine, or rosé wine) or not (odor categorization), and if so, whether they were identical or not (odor differentiation). Then, they had to classify 15 different wines (5 red, 5 white and 5 rosé) into red, white, and rosé wines (odor classification). Blind individuals (d': M = 1.3, SD = 1.2) presented lower scores compared to sighted controls (M = 2.2 SD = 0. 8; $p < .05$) in the odor categorization task, but no group difference was observed for the other tasks. For all participants, red wine odors were the easiest to classify (1.8 ± 1.0), followed by white wine odors (0.5 ± 0.6) and finally rosé wine odors (blind and sighted; $F[2; 44] = 11.9$, $p < .001$). In summary, early-blind individuals had a harder time to categorize wine odors. This could be explained by a different construction of internal reference categories for wine in early-blind individuals. Finally, this research is in line with the notion of the absence of higher olfactory sensitivity in blind individuals.

Keywords: Blindness, early-blind, wine odours, olfaction, odour perception, odour categorisation,

Introduction

It has been generally demonstrated that blind individuals compensate for their lack of visual input by displaying supra-normal abilities in their intact modalities (Burton, 2003; Norman and Bartholomew, 2011). These capacities seem to be in part modulated by age of blindness onset because earlier onsets are associated with a better performance (e.g., individuals who lost their sight early in life were better at determining the change in pitch directionality compared to those who lost their vision later in life and sighted controls (Gougoux et al., 2004)). It should be noted that in the literature, the age for defining early-blindness can vary from 1 to 14 years (Lewald, 2002; Wakefield et al., 2004; Cuevas et al., 2010) or not defined at all (Murphy and Cain, 1986), which complicates the understanding of the results within this population. Consequently, it is important to differentiate and consider the age at which sight was lost (i.e., at birth—congenitally blind; within the first few years of their life—early-blind; or during adulthood—late-blind). For the scope of the present paper, we defined early-blind as individuals who lost their sight before the age of 5 (Lewald, 2002). Congenitally and early-blind individuals showed supra-normal abilities in the auditory (Lessard et al., 1998; Simon et al., 2002; Gougoux et al., 2004) and tactile modalities (Alary et al., 2008; Goldreich & Kanics, 2003), whereas results are less systematic within the late-blind population (Voss et al., 2004; Wan et al., 2010). This body of research supports the notion that congenitally and early-blind individuals show supra-normal performance, and more so than late-blind, especially for tasks that are more complex and difficult, where more subtle cues are needed to complete the task (Frasnelli et al., 2011).

Unlike the auditory and tactile modalities, there is less of a consensus regarding the olfactory capacities within the blind population (Majid et al., 2017). Olfactory function is typically investigated by assessing the capacity to detect (threshold), to discriminate and/ or to identify odors. Most studies on odor detection thresholds did not find any difference between the blind and sighted individuals (Rosenbluth et al., 2000; Schwenn et al., 2002; Wakefield et al., 2004; Luers et al., 2014; Kärnekull et al., 2016; Sorokowska, 2016) although a few studies reported that blind people had a lower detection threshold (i.e., had a better sensitivity to odors and needed a lower concentration to be able to detect them; (Cuevas et al., 2010; Beaulieu-Lefebvre et al., 2011; Comoglu et al., 2015)). When it comes to olfactory discrimination, some studies reported no difference between the groups (Schwenn et al., 2002; Beaulieu-Lefebvre et al., 2011; Luers et al., 2014; Sorokowska, 2016), while others suggested that blind individuals outperform the sighted

(Cuevas et al., 2010; Rombaux et al., 2010; Renier et al., 2013; Comoglu et al., 2015). With regards to odor identification it appears that group differences depend on the paradigm that is used. Indeed, in the case of a forced-choice paradigm, the consensus is that blindness does not affect the performance (Smith et al., 1993; Rosenbluth et al., 2000; Schwenn et al., 2002; Cuevas et al., 2010; Beaulieu-Lefebvre et al., 2011; Luers et al., 2014; Comoglu et al., 2015; Gagnon et al., 2015; Sorokowska, 2016). However, in the case of a free naming identification task, several studies suggest that blind individuals outperform the sighted (Murphy and Cain, 1986; Rosenbluth et al., 2000; Wakefield et al., 2004; Cuevas et al., 2010; Rombaux et al., 2010; Renier et al., 2013; Gagnon et al., 2015), while only one team did not find significant results (Sorokowska, 2016; Sorokowska and Karwowski, 2017). Some authors have suggested that the heightened performance of blind individuals in free odor identification may be explainable by a greater ability for blind individuals to generate words (Burton et al., 2002), rather than from genuine increased olfactory ability. Further, shorter response times has been observed in blind individuals, suggesting a faster olfactory processing (Cuevas et al., 2010; Gagnon et al., 2015). Among the studies which reported significant differences between sighted and blind participants, most included only cases with an early blindness onset, but a few compared early-blind and late-blind individuals. While a team reported that blindness onset did not affect performance in olfactory detection, discrimination, or forced-choice identification (Comoglu et al., 2015), another team found that early-blind were better at identifying odors in a free naming paradigm than late-blind (Kärnekull et al., 2016). Altogether, these results seem to suggest that that a supra-normal performance in early-blind individuals is more readily observable if the undergoing tasks present higher levels of difficulty (e.g., free naming identification task); a phenomenon that was previously shown in blind individuals within other modalities (Simon et al., 2002; Alary et al., 2008).

A recent metanalysis (Sorokowska et al., 2018) investigated this body of literature and observed, based on the data of more than thousand observations, an important publication bias, i.e., selective publishing of positive results. In fact, studies that reported significant group differences, typically had small sample sizes. By correcting for the publication bias, the authors provided convincing evidence that blindness does not affect odor identification, odor discrimination and odor thresholds.

While this may lead one to conclude the olfactory function is unchanged in blind individuals, the picture may be more complex. In fact, most studies used tests to evaluate olfactory function that

are designed to differentiate between normal or reduced olfactory function (i.e., Sniffin' Sticks or UPSIT; Doty, Shaman, & Dann, 1984; Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997) and they are fairly easy to complete when no olfactory abnormality is present. Consequently, any differences between two groups would be dampened by a ceiling effect, since these tools are not meant to detect supra-normal performance. Such a ceiling effect, however, could be avoided by using more complex olfactory tasks to discern any potential performance differences between blind and sighted subjects.

An example of a more demanding olfactory task is wine odor assessment, which is challenging even for wine experts (Ballester et al., 2009). In this study, Ballester et al., examined the ability of wine experts, novices, and trained subjects, to classify wine odors—based solely on the wine's olfactory information—as red, white, or rosé wines. All groups could correctly classify red and white wines, but none were able to do so for rosé wines. Similar results were replicated with beer odor categorization (Lelièvre et al., 2009). It is thus possible that, in this kind of task, congenitally and early-blind individuals would outperform sighted controls.

However, in another study, white wines that were colored with an odorless red dye were described as red wines, suggesting that the visual aspects of the wine influenced more its perception than its olfactory aspects (Morrot et al., 2001). Put differently, it seems that the pre-established visual mental categorization of a red wine (i.e., it looks like a red wine, therefore, it should be a red wine) dictates more readily its odor perception than an olfactory mental categorization (i.e., it smells like a red wine, therefore, it should be a red wine). Altogether, these results suggest that wine and beer drinkers rely heavily on visual mental categories rather than olfactory ones to accurately assess these odors. This is further supported by a perceptual olfactory facilitation when odors and visual stimuli are presented congruently (i.e., the smell and the image of orange) versus when they are presented incongruently (i.e., smell of fish and the image of cheese; Gottfried & Dolan, 2003). Additionally, our group has shown that odor perception can be modulated by the labels we give them (i.e., the same odor can be perceived as pleasant and unpleasant if we attribute it a negative or a positive label, respectively). This result suggests that a label activates mental representations which modulate the perception of the odor, and thus provides further support for the influence of internal categories on odor perception (Manescu et al., 2014). Together, these studies highlight the importance of visual mental categorization in olfactory processing. In contrast with what was

previously stated with respect to the blind, the implication of visual mental categorization would support an alternative hypothesis, namely, that congenitally and early-blind individuals would perform worse than controls in a categorization task, because early-blind individuals did not have the opportunity to create visual mental categories. In summary, the literature raises the question of whether blind individuals (i.) present superior performance due to supra-normal olfactory capacities or (ii.) show reduced performance because they do not benefit from visual mental categorization.

To answer this question, we set out to examine olfactory performance in an early-blind population on more difficult and ecological tasks, which include a strong visual mental categorization such as classification of wine odors into red, white, and rosé wines and different control tasks. More specifically, we evaluated performance of blindfolded participants (blind and sighted) on (a.) a wine odor categorization task ("do these two wines belong to the same category?"), (b.) a wine differentiation task ("are these two wines the same wine or different wines?"), (c.) a wine odor classification task ("is this a red, white or rosé wine?"), (d.) a general odor identification task; always exclusively based on the odors with no visual input. For all assessments, we put a particular emphasis on meticulously matching groups in terms of age and gender.

Experimental procedures

Participants

We tested 12 early-blind (age M = 49, SD = 13, range 24 to 65 years, 3 women) and 12 controls (age M = 49, SD=14, range 25 to 71 years, 3 women). All participants were congenitally blind, apart from one who was completely blind since the age of 3 years and a half. Since all blind participants lost their vision within the first few years of life, we will refer to them as the early-blind group. Causes of blindness include retinopathy of prematurity (5/12), congenital cataracts (2/12), microphthalmia (1/12), retinoblastoma (1/12), retinal detachment (1/12), congenital eye defect (1/12) and unknown (1/12). A bit less than half of the early-blind participants had some residual vision in at least one eye (5/12). Early-blind and sighted participants were matched in terms of age, gender, and smoking habits (1/12 in both groups). Participants were also asked about their consumption of red, white and rosé wine, and were matched in terms of how many glasses of wine they drank on average per week (Early-blind: M = 1.6, SD =1 .2; Sighted: M = 1.4, SD = 1.2).

All participants declared that they did not suffer from any medical condition that could affect their sense of smell at the time of the testing and did not have any history of alcohol abuse. Participants were instructed not to eat or drink anything besides water one hour prior to the experiment. Before taking part in the study, subjects gave their written informed consent. After completion, they received a $60 monetary compensation for their participation as well as reimbursement of their travel expenses. The Center for Interdisciplinary Research in Rehabilitation of Greater Montreal (CRIR) approved this study.

Stimuli

Fifteen different wines, five red, five white, and five rosé, were bought locally at the Société des Alcools du Québec (SAQ). For the differentiation task (see below), 2 additional bottles per category were purchased from these initial 15 wines selection. Wine information can be found in Table 1. To preserve the wine for a longer period, each 750mL bottle was transferred into ten 60mL amber glass bottles. Once the wine was transferred, it was refrigerated for a maximum of 12 days after which it was discarded if it was not used in the experiment. When testing occurred, the wine was transferred into wine glasses (Palma; INAO Tasting glass 200mL) which enabled participants to smell the wine within a relatively ecological setting. Each of the 15 wines had their own coded bottles and glasses to avoid any cross-contamination between the wines.

Tasks and Procedures

Two hours prior to each testing, all the wines used for testing were taken out of the refrigerator to reach room temperature (23°C). Before the participant's arrival, the wines were transferred in their corresponding glasses. All participants, including the early-blind participants, were blindfolded for the rest of the two hours of the experiment to maintain uniformity amongst the participants and to avoid any biased effect associated with wearing a blindfold (e.g., pressure on the nose, blindfold odors, etc.). Additionally, tissue paper was inserted between the blindfold and the participant's eyes to avoid any sighting of the wine. Then, we administered the different olfactory tasks, which are further detailed in the following sections. Each of these tasks required the participants to respond based solely on the odors of each wine; there was no wine tasting. For all the tasks, wines were served in wine glasses which were placed in front of the blindfolded participants and guided towards their hands, allowing the participants to know the location of the glass. Once they grasped the glass, they could either bring it to their nose and smell it, or, bring their nose to the glass while

the glass remained on the table. All wines in all tasks were presented randomly. After the experiment, the used wine was discarded, and all the glassware was washed and air dried. After every wash and before every experiment, the experimenter verified that there was no lingering odor in the glasses.

Wine odor categorization and differentiation

From the total of 15 wines, two red, two white, and two rosé wines were used for the wine odor categorization and differentiation tasks. Recall that participants were blindfolded and had to respond based solely on the odor of the wine. The participants were presented simultaneously with two wine glasses and they had to answer by "yes" or "no" the two following questions: (1) "do these wines belong to the same category?" knowing that categories referred to red, white, or rosé wine (categorization; task a) and (2) "do these two glasses contain the same wine or two different wines?" (differentiation; task b). Categorization and differentiation tasks were embedded, the second task depending on the outcome of the first one: after smelling one after the other the two glasses of wine placed in front of them, participants first had to determine whether the wines belonged to the same category (by answering "yes" or "no") without attempting to name the category (task a). Then, if they said that the wines were from the same category, they were asked to determine whether both wine glasses contained the same exact wine, or two different wines (task b). If the participant said that both glasses were not from the same category, it automatically meant that the participant determined that both wine glasses contained different wines. 24 pairs of wines were presented only once in a random order. More specifically, we presented 6 pairs of identical wines (same wine and same category), 6 pairs of wines from the same category (different wines from the same category) and 12 pairs of wines from different categories (different wines from different categories). Participants could take as much time as needed to give their response, but a 40-second wait period was taken between each presentation to avoid olfactory fatigue, which is in line with the literature in the domain (Hummel and Kobal, 1999).

Wine odor classification

Participants had to correctly classify each of the 15 different wines (5 red, 5 white, and 5 rosé) by labelling each of them as "red wine," "white wine," or "rosé wine" (task c). Wine order presentation was randomized for every participant before the experiment. Participants were presented with one glass of wine at a time. They could either smell the wine by taking the glass and bringing it to their

nose or by bringing their nose to the glass while it remained on the table. Similarly, to the previous task, participants could smell the wines for as long as needed. Response times were measured with a stopwatch, from the moment when they put their nose over the glass, about to take their first sniff, to the moment when their response was given.

After they classified the wine, we additionally asked participants to provide three different descriptors for each of the 15 wines. They were free to give any descriptor they wanted, without restriction as to the type of descriptors they could use (e.g., citrus, grass, leather), but were told that they should refrain from comparing the wines between them (e.g., "this wine smells more like grapes compared to the one before"). If they gave less than three descriptors, they were encouraged to generate more descriptors to sum up to three. When they gave more than three descriptors, we asked them to choose which of the given descriptors described the wine best. Thus, they provided a total number of 45 descriptors (three for each of the 15 wines). Similarly, to the last task, a 40-second delay was incorporated between each wine presentation to avoid olfactory fatigue.

Odor identification

In order to assess the participants' ability for odor identification (task d), we administered the identification subtest of the "Sniffin' Sticks" (Hummel et al., 1997). This subtest consists of the presentation of 16 common odors (e.g., apple, rose, leather) in felt-markers which the participants had to correctly identify. The test was administered under two conditions. First, we presented each of the 16 odors to the participants and they had to identify the odors without any cue (free condition). Answers were scored as correct when the participants gave the exact correct label of the odor. If they gave a category (e.g., "fruity" for the banana odor), they were asked to be more specific. Then, we presented the same odors a second time, but this time they had to choose between four alternative choices (forced-choice condition) which were presented verbally to them. For both conditions, we calculated the total number of right responses out of a possible score of 16. Therefore, every participant had a score for the free and the forced-choice conditions. The same researcher scored all responses to assure reliability within the scoring.

Statistical analyses

For the analysis of wine odor categorization, differentiation and classification, we computed sensitivity index d' and bias criterion C (Signal Detection Theory: Snodgrass & Corwin, 1988). Here, d' indicates the sensitivity to accurately detect a stimulus by comparing the correct hits (e.g.,

correctly categorizing a wine as a red wine) to the false alarms (e.g., incorrectly categorizing a wine as a red wine). C, in turn, represents the participant's criterion when responding; a positive value represents a conservative approach (e.g., a bias towards not responding that the wine is red) and a negative value represents a liberal approach (e.g., a bias towards responding that the wine is red). Both variables (d', and C) were taken as dependent variables and analyzed in separate ANOVAs.

All analyses were conducted with SPSS Statistics 24 (IBM, Corp, Armonk, NY). For each measure, we examined whether there were any outliers beyond three standard deviations; none were found. Specifically, for all our dependent variables, we carried out the following analyses: first, z-transformed data; the first on the whole sample of 24 participants and the second on the 12 participants of the early-blind group. We then verified whether any of the blind subjects had a z score larger than 3. We did not find any outlier, indicating that the sample of blind participants was indeed homogenous.

Secondly, we then analysed homogeneity of variances for both groups by using the Levene's test. This yielded a significant difference for one variable, namely d' for the wine odor differentiation ($p=0.002$). Therefore, for only this measure we additionally conducted a non-parametric Mann-Whitney Test to compare both groups. For all analyses, age, gender, wine consumption, and educational level were used as covariates but were removed if they did not impact the results. For all analyses, Bonferroni corrections were applied to correct for multiple comparisons. The alpha level was set at $p = .05$.

For both the wine odor categorization task (task a) and the wine odor differentiation task (task b) we used blindness (2 levels: early-blind and sighted controls) as a between-subject factor on the dependent variables d' and C.

For the wine odor classification task (task c) we used category (3 levels: 1. Red 2. White 3. Rosé) as a within-subject factor and blindness as a between-subject factor. Furthermore, we computed a third repeated measures ANOVA for the dependent variable response times with the same within-subject and between-subject factors.

For the general odor identification task (task d), we conducted a repeated measure analysis of variance (ANOVA) by using odor identification (2 levels: free and forced-choice) as a within-subject factor and blindness as a between-subject factor.

For the exploratory analysis of the use of wine descriptors, we counted how many different descriptors the participants gave: they provided a total number of 45 descriptors but, because they could use the same words to describe different wines, the number of different descriptors was less than 45 and varied across participants. We calculated this number of different descriptors separately for each of the three categories (red, white, and rosé). Then, we performed a repeated-measures analysis of variance (ANOVA) for the dependent variable number of descriptors, with category and blindness as within-subject and between-subject factors, respectively. We analysed the descriptors in two ways: first, we performed the analysis based on the number of descriptors given to the actually presented wine (e.g., descriptors provided for a white wine were considered as white wine descriptors). Because three descriptors were provided for each wine and there were five wines from each category, there can be up to 15 different descriptors for each category. Second, because participants classified each wine before giving descriptors, we knew whether they perceived it as a red, white, or rosé wine, which allowed us to perform another analysis, this time depending on the perceived wine (e.g., descriptors provided for a red wine classified as a white wine were considered as white wine descriptors).

Results

No significant group difference in terms of age, gender and wine consumption frequency was found. However, the sighted controls had a higher level of education (M = 17.8 years; SD = 3.0) compared to the early-blind individuals (M = 12.9; SD = 3.1; $t[22] = 3.2$; $p < .005$). We also verified if the performance of the one non-congenitally blind participant modulated our results. Since this was not the case, this participant was included in all analyses.

Wine odor categorization

For the (a) categorization task, ANOVA with the sensitivity index d' as dependent variable revealed a main effect of group ($F[1,22] = 4.7$; $p < 0.05$; $\eta p2 = 0.18$) with sighted controls (2.2±0.8) being able to categorize wine odors better than early-blind individuals (1.3±1.2). See Figure 1.

With the criterion C analysis as dependent variable, we do not observe an effect of group ($F[1,22] = 1.4$; $p > .05$; $\eta p2 = .06$).

Wine odor differentiation

For the (b) differentiation task, the ANOVA yielded no significant group effects, neither for d' ($F[1,22] = 2.1$ $p > .05$; $\eta p2 = .09$) nor criterion C ($F[1,22] = 2.8$; $p > .05$; $\eta p2 = .12$) as dependent variables. See Figure 2. Sighted controls (2.4 ± 0.5) and early-blind individuals (1.9 ± 1.2) had comparable results for d'. Mann-Whitney Test for d' which was also non-significant ($z = -.96$; $p > .05$).

Wine odor classification

For the (c) classification task, the ANOVA with d' as dependent variable revealed a main effect of category ($F[2,44] = 31.6$; $p < .001$; $\eta p2 = .60$). Post-hoc t-tests (see Figure 3) revealed that for all participants, red wine odors were the easiest to classify (1.8 ± 1.0), followed by white wine odors (0.5 ± 0.6), and finally by rosé wine odors (-0.2 ± 0.8; $p < .001$ and $p < .01$ respectively; corrected comparisons). Importantly, there was no main effect of blindness ($F[1,22] = .15$; $p > .05$; $\eta p2 = .01$) nor an interaction between the two factors ($F[2,44] = .16$; $p > .05$; $\eta p2 = .01$). The ANOVA with C as dependent variable revealed a main effect of category ($F[2,44] = 11.9$; $p < .001$; $\eta p2 = .35$), yet there was no main effect of blindness ($F[1,22] = .04$; $p > .05$; $\eta p2 = .00$) nor an interaction between the two factors ($F[2,44] = .89$; $p > .05$; $\eta p2 = .04$). Post-hoc t-tests revealed that participants were more conservative when classifying a rosé wine compared to when classifying a white and red wine [for all participants, rosé (0.5 ± 0.2) > white (0.3 ± 0.3; $p < .05$, corrected) and rosé > red (0.2 ± 0.1; $p < .001$, corrected), See Figure 3].

Although response times were slightly longer in blind individuals than in sighted participants, the ANOVA with response times as dependent variable yielded no significant main effect of category ($F[2,44] = 1.24$; $p > .05$; $\eta p2 = .06$), blindness ($F[1,22] = 2.80$; $p > .05$; $\eta p2 = .12$) nor an interaction between the two factors ($F[2,44] = .02$; $p > .05$; $\eta p2 = .00$).

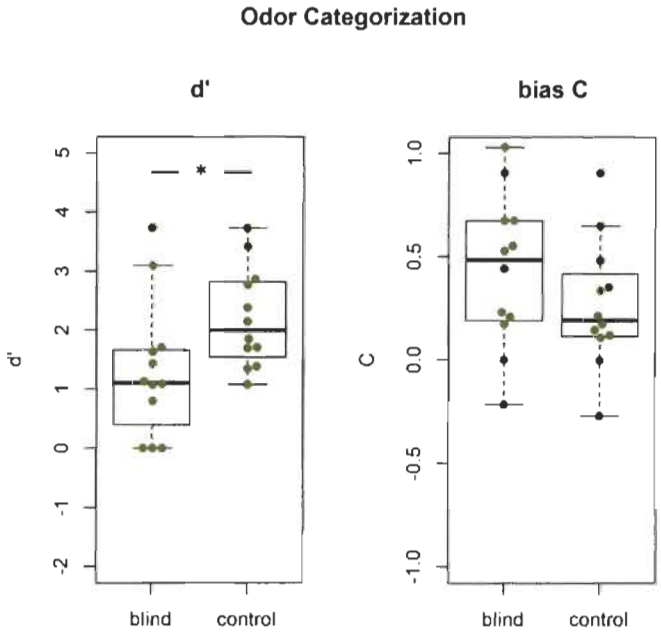

*Figure 1. Signal detection theory scores (d' and criterion C) for wine odor categorization task. Each dot represents one participant. Black line: median, Box: upper and lower quartiles; Whiskers: extreme values; Outliers: more than 1.5 times the interquartile range from the box. *p <0.05.*

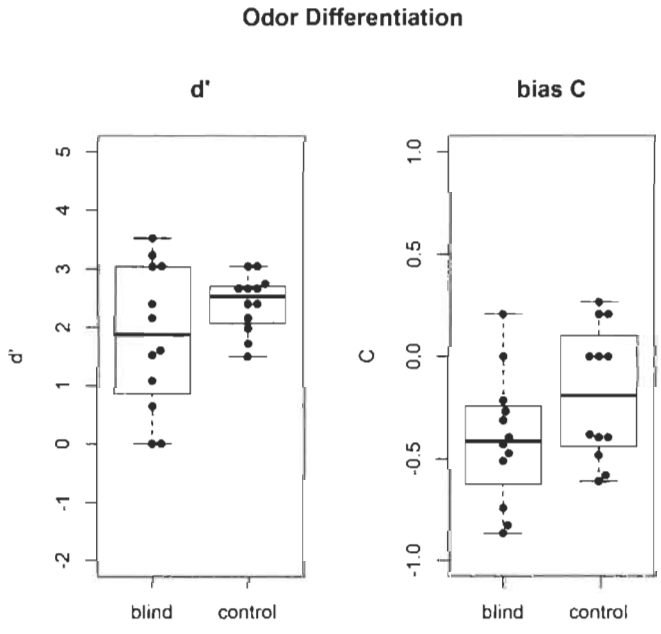

Figure 2. Signal detection theory scores (d' and criterion C) for wine odor differentiation. Each dot represents one participant. Black line: median, Box: upper and lower quartiles; Whiskers: extreme values.

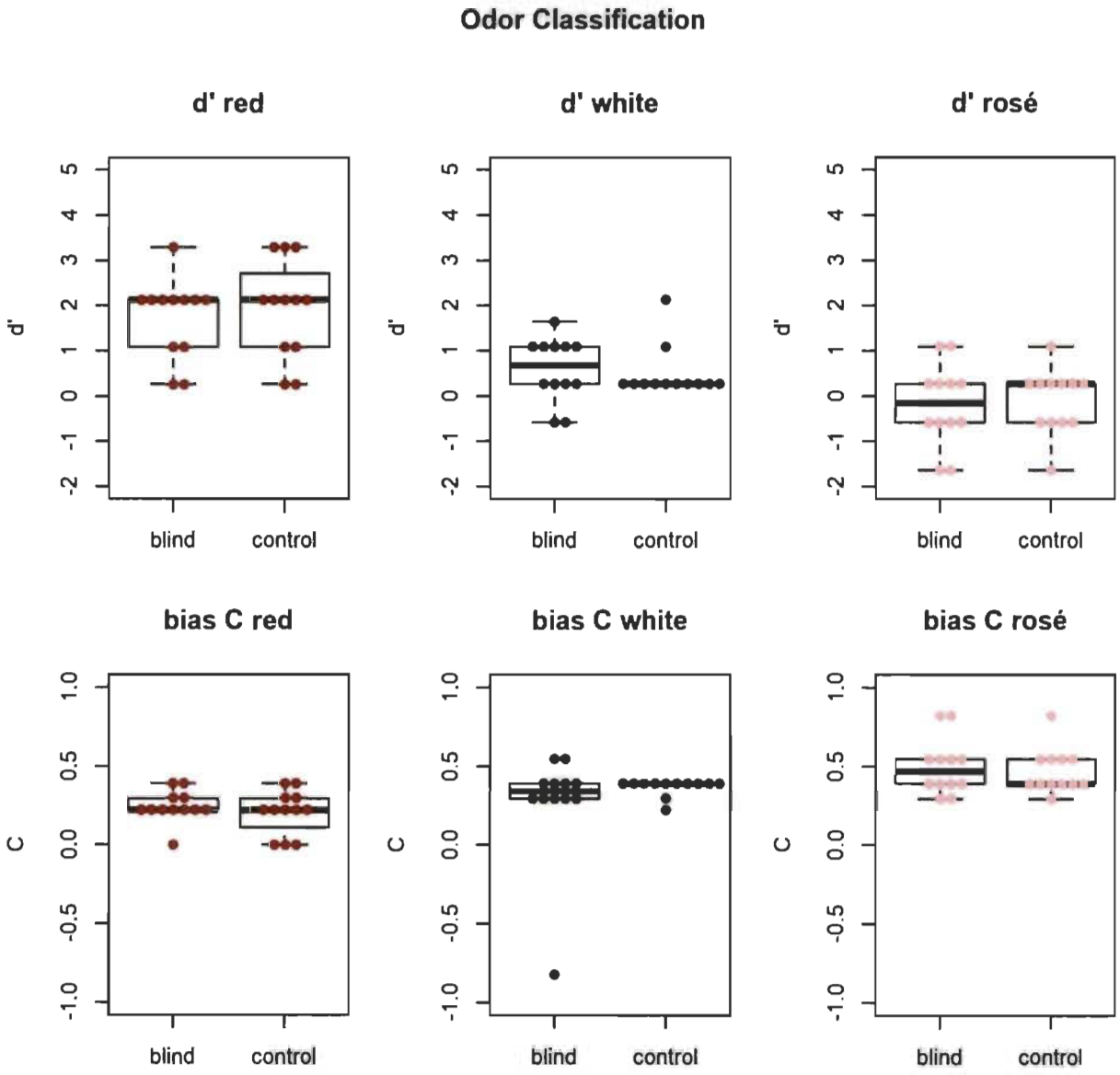

Figure 3. Signal detection theory scores for wine odor classification. d' and criterion C scores for each type of wine are shown. Each dot represents one participant. Black line: median, Box: upper and lower quartiles; Whiskers: extreme values; Outliers: more than 1.5 times the interquartile range from the box.

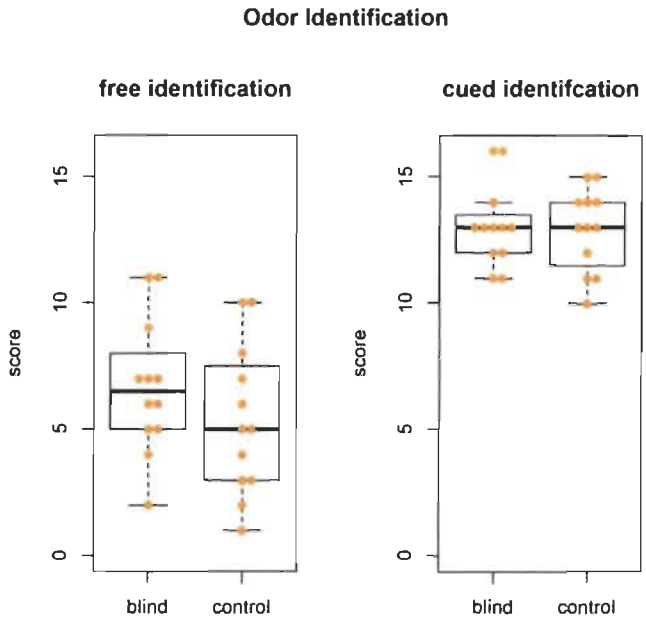

Figure 4. Scores for odor identification under the free and cued conditions. Each dot represents one participant. Black line: median, Box: upper and lower quartiles; Whiskers: extreme values; Outliers: more than 1.5 times the interquartile range from the box.

Odor identification

For the (d) general odor identification task, the ANOVA revealed a main effect of odor identification ($F[1,21] = 147.40$; $p < .001$; $\eta p2 = .88$) with higher scores in the forced-choice condition (13.0 ± 1.6; blind: 13.1± 1.6; sighted 12.9 ± 1.6) compared to the free condition (6.0 ± 2.9; blind 6.7 ± 2.7; sighted: 5.4 ± 2.8), a result suggesting that it was easier to identify odors when a multiple choice was presented to the participants (See Figure 4). There was no main effect of blindness ($F[1,21] = .7$; $p > .05$; $\eta p2 = .04$) nor an interaction between blindness and identification task ($F[1,21] = 1.1$; $p > .05$; $\eta p2 = .05$).

Wine odor description

Our exploratory analysis of wine odor description yielded no significant differences between sighted and early-blind for neither of the variables (all analyses: $F<2.0$; $p > .05$; $\eta p2<.08$).

Discussion

The goal of the present study was to evaluate whether early-blind individuals present different olfactory abilities while undergoing a more complex and ecological task such as a wine odor

assessment. We observed that early-blind individuals had a harder time to determine whether two simultaneously presented wine odors belonged to the same category or not (red, white, or rosé; odor categorization). However, early-blind participants were as good as sighted individuals to (1.) differentiate between wine odors, (2.) to classify wine as red, white, or rosé, and (3.) to identify odors. Therefore, we did not find any olfactory superiority in early-blind subjects, but rather lower performance in one specific task, for which participants have an important comparison of pure sensory input to make.

The main finding of the present study is that, compared to controls, early-blind individuals were less able to determine if two wines odors belonged to the same category or not (both red, white, or rosé wines), but other olfactory measures appear to be unaffected. This finding provides some support for the hypothesis that the lack of visual input in blind individuals penalised them in learning and constructing internal categories such as red, white, and rosé wines and their respective odors. One could have expected that early-blind participants would perform better either due to potentially heightened olfactory abilities (e.g., (Renier et al., 2013)) and/or due to increased verbal memory (e.g., (Amedi et al., 2003)). However, our current results seem to provide support for a stronger association between visual-olfactory processing compared to verbal-olfactory processing, at least for wine odors assessment. Additionally, increased mental imagery abilities could be positively linked with olfactory task performance (i.e., increased accuracy in odor detection when visualizing the tested odor; Djordjevic et al., 2004). Similarly, we can expect that the capacity to imagine the odor of a glass of red wine will aid its accurate odor categorization. Although few studies have supported the notion that early-blind individuals also exhibit mental imagery in other modalities (e.g., mental imagery of shapes, De Volder et al., 2001) and visual-spatial imagery (Vanlierde et al., 2003), it remains unknown whether early-blind individuals can exhibit olfactory-related mental imagery, i.e., creating a visual mental representation when smelling an odor. Consequently, it is possible that a lack of olfactory mental imagery in early-blind individuals explains our current results. A similar mechanism should be at play in the classification task; however, we did not observe a corresponding effect of blindness on the performance in this particular task. One may argue that the classification task was more challenging than the categorization task and this would diminish any differences. Nevertheless, blind individuals took more time to classify wine odors in red, white, and rosé, a pattern which could indicate that they struggled more with the task than did the sighted group. This result was not significant, but it is

possible that with a larger sample size, blind individuals would have shown worse performance in the classification task as well. Therefore, to further our understanding of the mechanisms at play, it will be interesting to compare early-blind and late-blind individuals on similar tasks in future studies and evaluate whether the previous experience with mental imagery will increase the performance in the latter group.

Although early-blind individuals had a harder time to determine if two wine odors belonged to the same category, their ability to discriminate wine odors (i.e., to evaluate whether two wine odors stem from the same or from different wines) was no different from sighted controls. These results are in line with previous research in which blind individuals did not outperform sighted controls on odor discrimination tasks (Cuevas et al., 2010; Beaulieu-Lefebvre et al., 2011; Sorokowska et al., 2018), even when the tasks are more complex, as in the present study.

We also did not find any significant group differences with regards to free odor identification, despite a small advantage for the early-blind individuals, in line with Sorokowska (2018). We also examined whether early-blind individuals were better at generating odor descriptors (Burton et al., 2002), which may explain better free odor identification performance reported in some studies. However, we did not observe any group difference in terms of number of descriptors, whether the analyses concerned the actually presented wine category (i.e., presentation of a red wine) or the perceived wine odor category (i.e., perceived a white wine when in fact it was red). Therefore, we can speculate that the heightened free odor identification found in the literature in the early-blind population could be due to heightened attention (Collignon et al., 2006) or verbal memory (Roder et al., 2001; Amedi et al., 2003).

Finally, our results also show that all participants (blind and sighted) were more sensitive to correctly classifying the odors of red wines, followed by the odors of white wine and finally those of rosé wine. This is in line with previous work (Ballester et al., 2009), and that white wine odors were easier to categorize compared to rosé wine odors. The use of different wines could explain this difference (i.e., our rosé and white wine categories could have been more distinguishable odors compared to the white and rosé wines used in Ballester et al. (2009).

As previously mentioned, one of the possible limitations of the present study is its small sample size. Although this could have dampened our results, we prioritized highly controlled inclusion criteria. Namely, not only we recruited solely early-blind individuals who are very rare, but they

also had to drink wine frequently enough without having a history of alcohol abuse or any olfactory abnormalities. Additionally, we took great precaution to closely match our groups regarding age, gender, and wine consumption. Another limitation may be that two of the tasks were not completely independent: the differentiation task depended on the outcome of the categorization task, since a participant saying two wines are not from the same category will not need to say whether these wines are the same or not, because not being from the same category automatically implies they are different. This might have influenced the results of the second task as, for example, a false negative in the first task (i.e., saying the wines are not from the same categories when they are) will lead to a false negative in the second task if the wines are actually the same. A closer look at the data shows that there is indeed a correlation between the numbers of false negatives in the first and second tasks (Spearman's, $p=0.006$). However, there is no correlation between the global sensitivity scores in both tasks, suggesting that the bias is slim and did not affect the overall results.

To sum, the goal of the present study was to evaluate whether early-blind individuals present different olfactory abilities compared to sighted matched controls while undergoing various complex and ecological tasks by means of wine odor discrimination. We found that early-blind individuals had a harder time to determine whether two simultaneously presented wine odors belonged to the same category or not (wine odor categorization in red, white, or rosé). The reason for this, however, does not appear to be due to differences in olfactory abilities between sighted and blind, but rather in different construction of internal reference categories. The present study is one of its first to explore olfactory discrimination in early-blind individuals using complex and ecological tasks.

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Annexe 3 – Imagerie chez le sommelier

Daphnée Poupon, Johannes Frasnelli

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La plasticité cérébrale

L'apprentissage fait partie de notre vie dès notre plus jeune âge. Il permet d'acquérir de nouvelles capacités et de les améliorer. L'acquisition et l'amélioration de ces capacités sont généralement liées à des modifications qui ont lieu directement dans le cerveau et qui peuvent se produire à différentes échelles, du microscopique au macroscopique, et ceci même chez l'adulte ; pendant de nombreuses années, il a été pensé que le cerveau adulte n'évoluait plus, mais nous savons désormais que, même chez l'adulte, des changements peuvent avoir lieu. Le cerveau est donc modulable : c'est ce qu'on appelle la plasticité cérébrale.

Ce phénomène est essentiel pendant l'enfance, mais il existe toujours à l'âge adulte, même si le cerveau est alors moins malléable. Le cerveau s'adapte et se modifie pour nous permettre d'acquérir et améliorer nos capacités. Cette plasticité rend ainsi notre cerveau unique, reflétant nos aptitudes dans divers domaines.

Les modifications se produisent dans des régions du cerveau spécifiques à la nature de l'apprentissage ou de l'entraînement. En effet, le cerveau est constitué de nombreuses régions qui sont impliquées dans différentes fonctions telles que la perception, la motricité, le langage, la mémoire, les émotions, etc. En s'entraînant à effectuer une tâche encore et encore, certaines régions spécifiques sont mobilisées de manière répétée. C'est à ce moment-là que la plasticité intervient : le cerveau se modifie de manière à ce que la mobilisation de ces régions soit plus efficace, pour ainsi faciliter l'exécution de la tâche en question.

Les modifications du cerveau peuvent être structurales ou fonctionnelles. Les modifications structurales sont caractérisées entre autres par des différences de taille ou de densité de certaines régions cérébrales. Une augmentation de la taille ou de densité d'une certaine région pourrait être dû à une augmentation du nombre de neurones, mais ce phénomène est rare chez l'humain adulte. Si une augmentation de taille ou de densité est observée, il est plus probable que ce soit dû à une augmentation du nombre de connexions – les synapses – que les neurones établissent entre eux. Les modifications fonctionnelles, quant à elles, modulent par exemple l'activation de certaines régions cérébrales lors de l'exécution d'une tâche.

La neuroimagerie

La neuroimagerie permet d'observer et étudier les effets de la plasticité cérébrale. Une des méthodes les plus répandues est l'imagerie par résonance magnétique, ou IRM. Cette méthode permet d'obtenir une image tridimensionnelle du cerveau. À partir de cette image, les tailles de diverses structures et régions cérébrales peuvent être mesurées. L'IRM permet également d'observer quelles régions cérébrales sont activées lors de l'exécution d'une tâche.

C'est ainsi que diverses études scientifiques ont montré, chez des personnes qualifiées d'expertes dans leur domaine, que leur cerveau se modifiait de manière à leur fournir exactement ce dont elles avaient besoin. La plasticité peut par exemple être observée chez des musiciens ou sportifs de haut niveau, ou encore chez des personnes exerçant une certaine profession si cette profession requiert des aptitudes particulières. Une étude pionnière a été réalisée chez des chauffeurs de taxis londoniens et a révélé qu'ils présentaient des spécificités au niveau de leur cerveau, et plus précisément au niveau de l'hippocampe. L'hippocampe est une structure cérébrale dont un des rôles est de faciliter la mémoire spatiale lors de déplacements. Cette structure est subdivisée en deux régions : l'hippocampe antérieur, responsable de mémoriser les informations spatiales de nouveaux environnements, et l'hippocampe postérieur, mobilisé lorsque des informations spatiales déjà mémorisées sont utilisées. Chez les chauffeurs de taxis, l'hippocampe postérieur est plus volumineux tandis que l'hippocampe antérieur est plus petit, et ces différences s'accentuent avec le nombre d'années d'expérience : plus un chauffeur de taxi exerce sa profession longtemps, plus son hippocampe postérieur est volumineux et son hippocampe antérieur est petit. En effet, avec les années d'expérience, le chauffeur connaît de mieux en mieux Londres : les nouvelles informations spatiales sont donc de moins en moins nombreuses et l'hippocampe antérieur est de moins en moins

mobilisé, tandis que la carte de la ville qu'il a enregistrée dans sa mémoire est de plus en plus détaillée et requiert donc de plus en plus de place, d'où un hippocampe postérieur de plus en plus volumineux (Maguire et al., 2000). Similairement, chez les joueurs de badminton professionnels, l'adaptation du cerveau favorise un mouvement plus coordonné, tandis que chez les musiciens, elle facilite la synchronisation des deux mains (Amunts et al., 1997; Di et al., 2012). Le cerveau des radiologistes présente également des spécificités ; ces différences sont liées à un sens de l'observation plus aiguisé qui leur est nécessaire pour remarquer tout détail important sur une radiographie (Harley et al., 2009).

Plasticité et olfaction

Dans le cadre de l'olfaction, les études de neuroimagerie ont également apporté de nombreuses connaissances concernant la plasticité cérébrale et les liens qui existent entre odorat et structure du cerveau.

Liens entre odorat et cerveau

Lorsqu'une odeur est détectée, le signal contenant toute l'information sur l'odeur est envoyé à une structure appelée le bulbe olfactif, qui est accolé à la face inférieure du cerveau, puis transmis au cortex olfactif primaire, qui se constitue de différentes structures nommées le tubercule olfactif, le cortex piriforme, l'amygdale et le cortex entorhinal, au niveau de la jonction entre lobes frontal et temporal. De nombreuses connexions lient le cortex primaire à des structures telles que le cortex orbitofrontal, l'hippocampe, l'hypothalamus, le thalamus, le cortex périrhinal ou encore l'insula, qui composent le cortex olfactif secondaire. C'est principalement dans ces régions cérébrales que les effets de la plasticité sont visibles.

Il existe un lien entre capacités olfactives et structure du cerveau. Ce lien est évalué grâce à deux outils : tandis que la structure du cerveau est analysée grâce à la neuroimagerie, les capacités olfactives sont mesurées grâce à des tests standardisés. Les plus importants exemples de tests commercialisés sont les Sniffin' Sticks et le UPSIT. Le Sniffin' Sticks consiste en des feutres remplis d'odeurs avec lesquels plusieurs tests peuvent être réalisés, ce qui permet d'évaluer plusieurs aspects de la performance olfactive (Hummel et al., 1997). Le premier test permet de mesurer le seuil de détection d'une odeur, c'est-à-dire la concentration à partir de laquelle le participant est capable de détecter une odeur. Dans le deuxième test, appelé test de discrimination,

trois feutres sont présentés à chaque fois au participant. Parmi ces trois feutres, deux contiennent la même odeur, par exemple l'odeur d'orange, et le troisième contient une odeur différente, par exemple l'odeur de citron. Le participant doit déterminer quel feutre contient une odeur différente. Le troisième test est un test d'identification : seize odeurs sont présentées au participant qui doit les nommer en choisissant, pour chacune, une réponse parmi une liste de quatre réponses proposées. Le participant obtient un score pour chacun de ces trois tests, et les trois scores peuvent être additionnés pour obtenir un score global appelé score SDI (Seuil – Discrimination – Identification). Le UPSIT (University of Pennsylvania Smell Identification Test) est un autre test communément utilisé qui permet d'évaluer la capacité du participant à identifier des odeurs. Celui-ci se présente sous la forme de quarante bandelettes que le participant peut gratter pour ainsi libérer les odorants contenus dans des microcapsules. De même que dans le test d'identification du Sniffin' Sticks, le test se présente sous forme de questionnaire à choix multiples et le participant identifie l'odeur en choisissant sa réponse parmi un choix de quatre réponses (Doty et al., 1984).

Ce sont à partir de ces scores que des liens entre odorat et structure du cerveau ont été observés. Il n'y a même pas besoin d'être un expert de l'olfaction pour que les deux soient liés : même chez des novices en la matière, des corrélations sont visibles. En effet, nous avons tous un odorat plus ou moins fin et des régions cérébrales responsables de l'odorat plus ou moins développées, et il se trouve que ceux qui ont des structures cérébrales plus volumineuses sont ceux qui ont de meilleures capacités olfactives.

La première structure concernée est le bulbe olfactif : deux équipes de chercheurs ont observé que son volume était positivement corrélé au score SDI ; plus le bulbe olfactif est volumineux, plus le score SDI est élevé. Une de ces équipes a rapporté que le volume du bulbe olfactif était aussi corrélé aux scores obtenus aux tests d'identification et de seuil de détection. L'autre équipe a également observé une corrélation avec le test d'identification, mais pas avec le test de seuil de détection.

Ces observations ne se limitent pas au bulbe olfactif : des corrélations semblables ont été trouvées au niveau de régions cérébrales olfactives telles que le cortex piriforme, le cortex entorhinal, le cortex orbitofrontal et l'insula ; plus ces régions sont épaisses, meilleures sont les capacités olfactives. Des régions qui ne sont généralement pas citées comme ayant un rôle dans l'olfaction sont également liées à la performance olfactive. C'est le cas pour le cortex occipital, principalement impliqué dans la vision, et le cortex somato-moteur, responsable de la motricité et des mouvements.

Les corrélations sont spécifiques à différents tests olfactifs : le volume du cortex orbitofrontal prédit la performance au test de seuil de détection ainsi que le score SDI ; cortex piriforme, cortex entorhinal et cortex occipital sont liés aux scores obtenus au test d'identification ; le résultat au test de discrimination dépend de la taille du cortex orbitofrontal, de l'insula et du cortex somato-moteur (Buschhuter et al., 2008; Frasnelli et al., 2010; Seubert et al., 2013).

Les effets d'un entraînement olfactif

Les liens entre odorat et structure du cerveau sont visibles dans la population en général, mais ni l'un ni l'autre ne sont fixes. Effectivement, plusieurs facteurs peuvent faire varier performance olfactive et structure du cerveau. Avec l'âge, par exemple, les capacités olfactives diminuent (Hummel et al., 2007). Les structures cérébrales ont également tendance à rétrécir avec les années (Buschhuter et al., 2008). Un entraînement olfactif, qui consiste simplement à sentir des odeurs régulièrement, peut avoir l'effet inverse.

À de nombreuses reprises, l'entraînement olfactif s'est révélé être un moyen efficace d'améliorer l'odorat ; si efficace qu'il est considéré comme piste thérapeutique pour les patients dits hyposmiques, c'est-à-dire des patients qui sentent peu les odeurs. En effet, plusieurs études ont rapporté les effets d'un entraînement olfactif durant lequel les patients sentaient quatre odeurs chaque jour pendant trois à huit mois. Tester les patients en début et en fin d'entraînement olfactif a révélé que leur performance olfactive s'améliorait, principalement dans les tests de discrimination et d'identification (Altundag et al., 2015; Damm et al., 2014; Fleiner et al., 2012; Geissler et al., 2014; Haehner et al., 2013; Hummel et al., 2009; Konstantinidis et al., 2013; Sorokowska et al., 2017).

Il n'est pas nécessaire d'avoir des troubles olfactifs pour bénéficier des effets d'un entraînement olfactif : sentir des odeurs de manière répétée peut améliorer l'odorat même si celui-ci se situe déjà dans les normes. L'entraînement peut par exemple permettre d'augmenter la sensibilité à une certaine odeur (Croy et al., 2015; Dalton et al., 2002; Moller et al., 1999; Mori et al., 2015; Rabin & Cain, 1986; Wang et al., 2004).

L'amélioration des capacités olfactives s'accompagne de changements dans le cerveau. En effet, l'entraînement olfactif peut mener à des modifications structurales. Sentir quotidiennement quatre odeurs pendant quatre mois mène par exemple à une augmentation du volume du bulbe olfactif

(Negoias et al., 2017). Dans une autre étude, des participants ont suivi un entraînement olfactif de cinq semaines réalisé directement au laboratoire et constitué de différentes tâches olfactives. À la fin de cet entraînement, les participants avaient de meilleures capacités olfactives, surtout pour l'identification d'odeurs, et le cortex de plusieurs régions s'était épaissi. C'est le cas du gyrus frontal inférieur, généralement rapporté comme étant une zone active après une stimulation olfactive (Al Ain et al., 2019).

L'entraînement mène éventuellement à l'expertise. C'est à ce niveau que se situent les sommeliers.

Le cerveau des sommeliers

En sentant et dégustant de nombreux vins, les sommeliers mobilisent constamment leur odorat. Leur nez est expert des odeurs et cela s'accompagne, tout comme chez les parfumeurs (Delon-Martin et al., 2013; Plailly et al., 2012), de modifications dans le cerveau. En effet, des études de neuroimagerie ont été menées chez les sommeliers, comparant ces experts de l'olfaction à des novices en la matière, et des différences ont été observées.

Au niveau structural, certaines régions olfactives sont plus volumineuses chez les sommeliers. C'est le cas pour le cortex piriforme, le cortex entorhinal, l'insula, ainsi que la région qui entoure le sillon olfactif. L'épaisseur du cortex augmente avec le nombre d'années d'expertise, alors que chez les novices, le cortex s'amincit avec l'âge (Banks et al., 2016; Royet et al., 2013).

Au niveau fonctionnel, les sommeliers réagissent plus vite aux odeurs de vin : une activation plus précoce du cortex olfactif primaire permet une analyse plus rapide de la familiarité et de l'agréabilité de l'odeur (Pazart et al., 2014). De plus, les connexions entre les différentes régions olfactives sont renforcées chez les sommeliers, rendant ainsi le traitement de l'information olfactive plus efficace, ce qui leur permet par exemple d'identifier une odeur plus rapidement. L'activation est plus rapide et les connexions sont renforcées, mais l'activation de ces régions est cependant moins intense car, avec l'expérience, l'analyse de l'odeur requiert moins d'efforts (Royet et al., 2013). En plus de ces différences visibles au sein du réseau olfactif, d'autres régions telles que le précunéus, le noyau caudé et le putamen sont considérablement mobilisées chez les sommeliers. Ces régions sont connues pour être impliquées dans des processus cognitifs de haut niveau comme l'attention, la mémoire ou l'imagerie mentale (Sreenivasan et al., 2017). La

mobilisation de fonctions de plus haut niveau permet d'approfondir l'analyse de l'odeur, fournissant ainsi aux sommeliers plus d'informations sur l'odeur perçue.

De nouvelles frontières

Toutes les études de neuroimagerie réalisées chez les sommeliers comparent experts et novices à un temps donné. Notre équipe à l'Université du Québec à Trois-Rivières a mis en place une nouvelle étude de neuroimagerie dans laquelle les sommeliers ne sont pas testés une seule fois à un moment donné, mais deux fois. Plus précisément, nous avons travaillé avec des étudiants en sommellerie de l'Institut du Tourisme et d'Hôtellerie du Québec (ITHQ) à Montréal. Nous les avons testés une première fois au début de leur formation de sommellerie, puis une deuxième fois un an et demi plus tard, à la fin de leur formation. Nous avons comparé ces étudiants en sommellerie à d'autres étudiants suivant une formation qui n'implique pas l'odorat, que nous avons également testés deux fois. Ce design expérimental nous permettra, une fois l'étude terminée, de voir si des différences étaient déjà visibles en début de formation, ce qui pourrait par exemple indiquer une prédisposition, et d'observer l'évolution de leur cerveau au cours de leur formation.

Le test consistait en une session d'IRM nous permettant d'observer différents aspects structuraux et fonctionnels du cerveau. En ce qui concerne les caractéristiques structurales du cerveau, nous serons capables de mesurer l'épaisseur du cortex ainsi que le volume de structures cérébrales telles que le bulbe olfactif. Les précédentes études montrant que le volume du bulbe olfactif est corrélé aux capacités olfactives ont été réalisées chez des novices en olfaction (Buschhuter et al., 2008), mais rien n'a été rapporté à ce sujet par rapport aux sommeliers. Les premiers résultats préliminaires semblent confirmer notre hypothèse et suggèrent que le volume du bulbe olfactif des sommeliers a augmenté au cours de leur formation tandis que la taille du bulbe olfactif des étudiants novices en olfaction n'a pas évolué. Il nous reste à analyser les caractéristiques structurales du reste du cerveau pour voir si par exemple certaines régions sont devenues plus épaisses, ainsi que les caractéristiques fonctionnelles : grâce à un dispositif permettant d'envoyer des odeurs directement dans le scanner IRM, nous pourrons voir quelles régions du cerveau sont activées lorsque les étudiants en sommellerie sentent du vin, et si cette activation évolue au cours de la formation.

Les résultats ne sont pas encore disponibles, mais cette étude apportera des connaissances sur la plasticité cérébrale et ce qu'il se passe dans le cerveau lors d'un entraînement, car nombreux sont les secrets du cerveau qu'il reste à percer. De futures études pourraient s'intéresser à l'étendue des

effets d'un entraînement olfactif : cet effet est-il limité aux régions olfactives, ou est-ce que d'autres régions du cerveau qui sont associées à d'autres fonctions sont également modifiées ? Est-ce que, en plus d'améliorer l'odorat, un entraînement olfactif peut améliorer d'autres capacités ? Les sommeliers ont-ils une mémoire supérieure à des spécialistes d'autres sens tels que des musiciens ? Puisqu'il existe des liens entre olfaction et cerveau, quelles informations sur la structure et la fonction du cerveau pourrions-nous obtenir à partir de simples tests olfactifs ?

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Annexe 4 – Functional MRI: inconclusive results from our longitudinal study in sommelier students

Introduction

Expertise in a specific field leads to changes in brain structure and function. While different studies reported that sommeliers, who are experts in olfaction, displayed structural differences (Banks *et al.*, 2016; Royet *et al.*, 2013), differences have also been observed in brain function. Indeed, during chemosensory stimulation, while naïve individuals activate primary gustatory and olfactory brain areas (Castriota-Scanderbeg *et al.*, 2005; Sreenivasan *et al.*, 2017), activation and functional connectivity in high-level cognition areas, e.g. areas involved in sensory memory, episodic memory, working memory, semantic memory, multisensory integration and selection of behavioral strategies, are greater in sommeliers (Castriota-Scanderbeg *et al.*, 2005; Pazart *et al.*, 2014; Sreenivasan *et al.*, 2017). Activation of primary areas is also faster (Pazart *et al.*, 2014). On the contrary, while performing certain tasks such as odor mental imagery, activation in certain olfactory brain areas correlates negatively with years of expertise: with experience, these tasks require less effort which explains why a greater expertise comes with a lesser activation (Royet *et al.*, 2013).

All the studies mentioned in the previous paragraph were cross-sectional. Our study uses a longitudinal approach which is interesting to observe the effects of training-related brain plasticity. More precisely, we tested sommelier students at the start and at the end of their training to see if we could observe any difference in brain function. To do this, we used BOLD fMRI to see brain activation during an olfactory task. Using an olfactometer, odors were sent to the participant who had to determine, in the first part of the task, if the odor was wine or juice and, in the second part, if it was red wine or white wine. We compared the performance and brain activation of sommelier students to that of control participants. Our hypotheses were that we would observe effects of group and time on performance in the olfactory task and on brain activation patterns.

Materials and methods

Participants

17 sommelier students enrolled at the Institut de Tourisme et d'Hôtellerie du Québec in Montreal took part in this study. The group was composed of 7 women aged 26.1 ± 4.7 years at the time of the first visit, and of 10 men aged 25.7 ± 5.0 years. This first visit took place between 3 and 9 weeks after the start of their training. The control group consisted of 17 students from the University of Montreal or the University of Quebec in Montreal. These participants were chosen to match sommelier students in age and gender and was therefore composed of 7 women aged 26.6 ± 4.3 years, and of 10 men aged 25.6 ± 5.7 years. One sommelier student was excluded from the study because of pregnancy, defined by the Ethics Committee as a contraindication for the MRI scan.

A year and a half later, at the end of sommelier students' professional training, 12 sommelier students and 13 control participants returned for the second part of the study.

Brain imaging

MRI images were acquired at the Unité de Neuroimagerie Fonctionnelle (UNF) at the IUGM. The UNF provides access to a Prisma Fit 3 Tesla MRI scanner from Siemens.

We used blood-oxygen level-dependent (BOLD) fMRI with the following parameters: repetition time = 785 ms, echo time = 30 ms, flip angle = 54°, in-plane field of view = 192 mm, voxel size = 3x3x3 mm^3, 42 slices.

We also acquired a T1-weighted structural volume using an MPRAGE sequence. This sequence provided 176 contiguous sagittal slices with an isotropic spatial resolution of 1 mm3 (repetition time = 2300 ms, echo time = 2.26 ms, flip angle = 8°, in-plane field of view = 256 mm).

Olfactory task

Odorants

We used four odorants: red wine (J.P. Chenet), white wine (Cellier des Dauphins), 100% grape juice (Welch's), 100% apple juice (Oasis). To make sure participants relied on the olfactory aspect of wine and not the trigeminal perception of alcohol, we added ethanol to the juice so that the percentage of alcohol in wine and in juice was the same (12.5%): we added 1 mL of ethanol to 7 mL juice. In each glass bottle, 6 mL of wine or juice + ethanol were absorbed on cotton pads.

Olfactometer

We used an olfactometer from Osmic Enterprises (http://www.osmicenterprises.com/about.html), a device which allowed to send odors to the participant directly in the MRI scanner. A constant air flow of 1500 mL/min originating from an air pump went through the apparatus and was delivered to the participant via a nasal cannula. During most of the experiment, air was only composed of odorless vector air. During olfactory stimulation, vector air only had a flow of 1000 mL/min while some air went through one of the glass bottles containing the odors at a flow of 500 mL/min. The olfactometer was controlled by computer: the air flow was regulated by a program designed on LabView.

Design

The experiment consisted in two runs of 366 seconds with eight 12-second olfactory stimuli and 30-second inter-interval stimuli. During the first run, each of the four odors was sent twice. During the second run, we only used red wine and white wine, which were both sent four times (see Figure 1). Participants had a joystick that they used every time they perceived an odor to say if they smelled 1. wine or juice in the first run, 2. red wine or white wine in the second run.

For each run, score ranged from 0 to 8 and corresponded to the number of odors correctly identified. In addition, we calculated a sensitivity index d':

$$d' = z(hit\ rate) - z(false\ alarm\ rate)$$

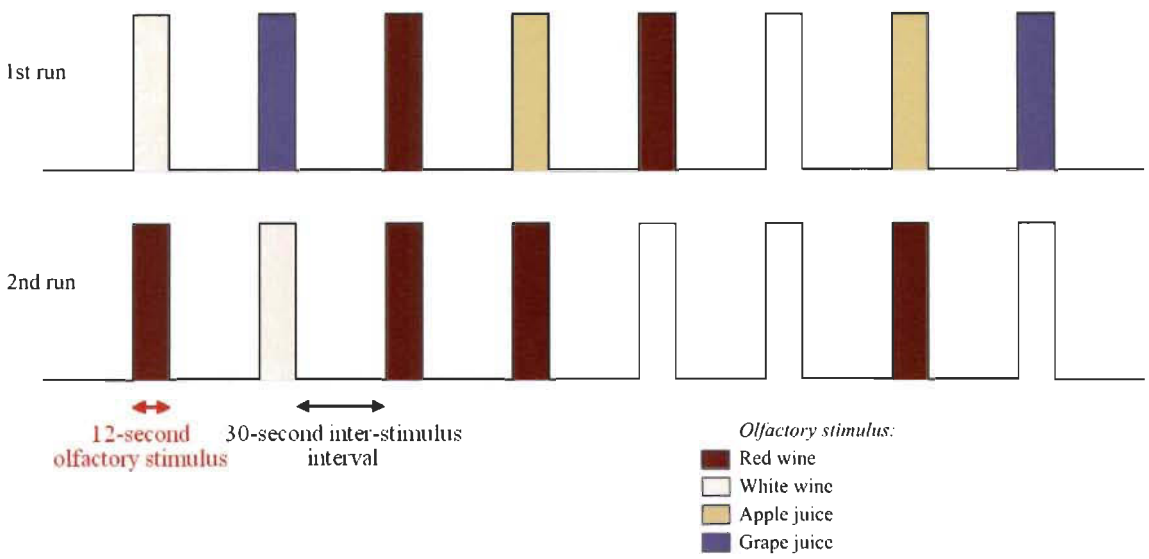

Figure 1. Experimental design of the olfactory task.

Analysis

Brain images were analyzed using SPM12. Preprocessing the images was the first step: realignment, coregistration, segmentation, normalization and smoothing allowed to align anatomical and functional scans and to reconstitute a tridimensional image of the brain in the MNI space. Then, we performed a first-level analysis for each participant to observe brain activation during olfactory stimulation: we defined contrasts that allowed to compare brain activation during the different conditions *wine*, *juice* and *vector air*. Finally, a second-level analysis allowed us to test the effect of *group* by comparing sommeliers and controls at T1 and at T2 separately, and the effect of *time* by comparing T1 and T2 in both groups separately.

To analyze scores obtained in the olfactory task, we used SPSS 23 to perform repeated-measures ANOVAs with *scores* and *sensitivity indexes* as our independent variables, *time* as within-subject factor (2 levels: T1 and T2) and *group* as between-subject factor (2 levels: sommeliers and controls) to see if there was an effect of sommelier training on the performance.

Results

fMRI analysis did not reveal any significant effect of *time* or *group* on brain activation at a FWE $p < 0.05$ level or at an uncorrected $p < 0.0001$ level.

As for the olfactory task, the ANOVA on *scores* revealed no effect of *group* ($F(1;21) = 0.386$, $p = 0.541$) or interaction of *group* with *time* ($F(1;21) < 0.001$, $p = 0.985$). The ANOVA on sensitivity indexes did not show any effect of *group* ($F(1;21) = 1.384$, $p = 0.253$) or interaction of *group* with *time* ($F(1;21) = 0.168$, $p = 0.686$) either. This means that this olfactory task did not show any effect of sommelier training.

Discussion

We did not observe any effect of group or time on performance in the olfactory task or on brain activation patterns.

A greater number of stimulations could have increased the probability to observe significant results, but because each olfactory stimulus lasted 12 seconds, i.e. long enough for the participant to breathe in odorized air, and a 30-second inter-stimulus interval is required to reset the nose, and because we had other scans and the time in the MRI scanner was limited for each participant, we could not afford to make the experiment last longer.

The absence of significant results in this study does not mean sommelier training does not affect brain function. There are probably effects that our study did not show and that future studies could reveal.

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