Olfactory bulb volume and cortical thickness evolve during sommelier training

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Abstract
Brain plasticity is essential for experts to acquire the abilities they need. Sommeliers are olfaction experts who display differences in olfactory regions in the brain that correlate with greater olfactory abilities. While most studies on this topic are cross-sectional, we used a longitudinal design and invited 17 sommelier students at the start and end of their training then to compare them to 17 control students to study the effects of training-related brain plasticity. After a year and a half, 5 sommelier students and 4 control students dropped out, leading to 12 sommelier students versus 13 controls. We used magnetic resonance imaging to measure cortical thickness and olfactory bulb volume, as this structure plays a crucial role in olfactory processing. We used the Sniffin' Sticks test to evaluate olfactory performance. During training, olfactory bulb volume increased in sommelier students while there was no significant change in the control group. We also observed that thickness of right entorhinal cortex increased, and cortical thickness decreased in other cerebral regions. Our olfactory tests did not reveal any significant changes in sommelier students. In conclusion, this is the first longitudinal study to report an increase in olfactory bulb volume in olfaction experts in line with the notion of effects of ecological training-related brain plasticity. The mixed results about cortical thickness might be explained by a “overproduction-pruning” model of brain plasticity, according to which the effects of training-related plasticity are non-linear and simultaneously involve different processes.

KEYWORDS
brain, cortical thickness, magnetic resonance imaging, olfaction, olfactory bulb, plasticity, sommelier

1 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 can be observed in hippocampus of London taxi drivers because the hippocampus is involved in spatial memory. With years of experience, taxi drivers memorize the London map better thus need less encoding of new spatial information so they start using their mental map of the city (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), allow radiologists to process, and interpret radiographs more effectively (Harley et al., 2009). These training-related changes in the brain can be structural or functional; they can occur in both cortex and white matter. We can detect this type of changes with neuroimaging techniques such as magnetic resonance imaging (MRI). It is important to know whether these changes can also be detected as a result of ecological training which will give us insight about olfactory plasticity. This has the potential to improve the olfactory training protocols for individuals with the loss of olfactory function.

The olfactory bulb (OB) is an ovoid structure located under the frontal lobe of 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 brain (Gottfried, 2010; H uart, Rombaux, & Hummel, 2013; K. Mori, Nagao, & Yoshihara, 1999).

By using MRI, the volume of this structure can be measured. In healthy people with a normal sense of smell, significant positive correlations between OB volumes and olfactory performance were reported: a bigger OB is associated with better olfactory abilities (Buschhuter et al., 2008; Seubert, Freiherr, Frasnelli, Hummel, & Lundstrom, 2013). Similar correlations were observed in patients with olfactory dysfunction: impairment of olfactory dysfunction is correlated with smaller OB volume (Liu, Hang, Liu, & Han, 2017; Rombaux et al., 2006). Cause of olfactory dysfunction can be congenital in some rare cases, but most of the time the impairment is acquired. Patients with a unilateral complete nasal obstruction are subject to a decrease of ipsilateral OB volume. For example, absence of olfactory input on one side during a few months resulted in a smaller ipsilateral OB, while 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. Similar to olfactory performance, OB volume decreases with age (Hang, Liu, Han, Zhang, & Zhang, 2015).

Olfactory training typically consists of smelling odors every day for a few months, and it is a potential way for patients to recover from olfactory dysfunction/loss. It can improve olfactory performance, and these changes correlate with OB volume increases (Haehner, Rodewald, Gerber, & Hummel, 2008; Rombaux, Duprez, & Hummel, 2009). Olfactory training can have the same effect in people with a normal sense of smell. In one experiment, participants were tested at the beginning and end of a four-month olfactory training but only one of their nostrils was trained. OB on both the trained and contralateral side nostrils became bigger. This confirms the correlation between olfactory function and OB volume. It also indicates that the underlying mechanism is complex and involves top-down processes which allows OB that is not on the stimulated side to grow along with a stimulated OB (Negoias, Pietsch, & Hummel, 2017). Besides receiving input from olfactory epithelium, OB neuronal activity is modulated by centrifugal input from cerebral structures such as primary olfactory cortex, amygdala, hippocampus, locus coeruleus, and raphe nuclei (Lazarini & Lledo, 2011). Changes in OB volume could be due to neurogenesis that happens in adult olfactory system or synaptogenesis between olfactory receptor neurons and mitral cells in OB (Curtis et al., 2007; Eavri & Nedivi, 2013; Lotsch et al., 2014; for a review, see Huart et al., 2013; H uart, Rombaux, & Hummel, 2019).

Measuring cortical thickness is an efficient way to evaluate changes in gray 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, Lerch, Evans, & Zatorre, 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). Similarly, the ability to perceive and identify odors is also related to brain anatomy as: olfactory performance and cortical thickness in both olfactory and non-olfactory regions are correlated (Frasnelli et al., 2010). Another study targeting sommeliers reported that they 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 6 weeks in different olfactory tasks and testing them before and after the training showed that cortical thickness increased in regions such as right entorhinal cortex, right inferior frontal gyrus, and bilateral fusiform gyrus (Al Ain et al., 2019).

1.1 Objectives and hypotheses

Most studies about brain plasticity in sommeliers and perfumers are cross-sectional, which is an efficient way to compare them with a control group, but it does not allow to see the evolution of brain over time. In olfaction, only in a few studies researchers used a longitudinal design to examine the effects of training-related brain plasticity with MR imaging. As an example, one study tested adults with a normal sense of smell before and after a 6-week-long olfactory training (Al Ain et al., 2019). Though the OB has been the subject of many studies, there has been no report of OB volume in olfactory specialists.

We therefore aimed at examining the effects of sommelier training on the brain and olfactory function in an explorative study. We tested sommelier students at the start and end of their 18 months training and compared them with a group of students whose training did not involve any sense of smell. We used MRI to measure OB volume and cortical thickness. We hypothesized that (a) sommelier training leads to OB volume increases; (b) sommelier training leads to changes of cortical thickness.

2 MATERIALS AND METHODS

2.1 Participants

Initially we recruited 17 sommelier students and 17 control students. Sommelier students were recruited from the Institut de Tourisme et d’Hôtellerie du Québec. Participants did not have any known smell or taste loss/dysfunction. At time point-1 the sommelier group consisted
of 7 women (26.1 ± 4.7 years), and 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. Therefore the control group consisted of 7 women (26.6 ± 4.3 years) and 10 men (25.6 ± 5.7 years). One sommelier student was excluded from the study because of pregnancy, an exclusion criterion for the MRI scan.

At follow up, 5 sommelier students and 4 control participants dropped out of the study resulting in 12 sommelier students and 13 control participants. Therefore at follow up, the sommelier group consisted of five women and seven men (26.6 ± 4.3 years) while the control group consisted of four women and eight men (27.2 ± 4.8 years).

### 2.2 | Sommelier training

The participants in the sommelier group underwent the International Service and Sommelier Training at 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 a professional sommelier. The training consists of 1,200 hr of classes; olfactory training takes place in most of these classes as only 45 hr do not involve any sensory analysis. There is also a minimum of 905 hr work experience obtained during different compulsory internships that include 4 months in an English-speaking establishment outside of 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, such as administration, psychology, life sciences, economics, and humanities, which did not involve any practical olfactory training. We did not monitor whether students were involved in any olfactory-training like activities during the period of training.

### 2.3 | Brain imaging

MRI images were acquired at Prisma Fit 3 Tesla MRI scanner from Siemens of the Unité de Neuroimagerie Fonctionnelle (UNF) at the Institut universitaire de gériatrie de Montréal (IUGM).

#### 2.3.1 | OB volume

To measure OB volume, we used a standard protocol resulting in 2-mm-thick T2-weighted images in Turbo Spin Echo mode. Images were obtained in the coronal plane and there was no gap between the 2-mm-thick slices, with voxel size: 0.16 × 0.16 × 2 mm³. This method was described as the most suitable method for OB volumetry (Huart et al., 2013; Seubert et al., 2013). OB volumes were measured for right and left OB by DP without blinding the process. To maintain objectivity, measurements were done twice and made sure that the difference between the two volumes were less than 10%.

#### 2.3.2 | T1-weighted MRI

To obtain whole brain volume and 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 2,300 ms, echo time 2.26 ms, flip angle 8°, in-plane field of view 256 mm). An automated reconstitution of a tridimensional brain image was performed using Freesurfer 6.0 for Linux (http://surfer.nmr.mgh.harvard.edu), which provided us with the whole brain volume.

### 2.4 | Olfactory performance

Olfactory performance was assessed using an extended version of the Sniffin’ Sticks test (Hummel, Kobal, Gudziol, & Mackay-Sim, 2007; Hummel, Sekinger, Wolf, Pauli, & Kobal, 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.

We assessed olfactory performance by using the Sniffin’ Sticks test measure (1) odor threshold, (2) odor discrimination, and (3) odor identification. We further used the same test to carry out an (4) odor memory test, as described earlier (Al Ain et al., 2019).

#### 2.4.1 | Odor detection threshold

We assessed odor thresholds for phenylethyl alcohol 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 containing solvent. Participants had to identify the pen containing the odorant. In this task, we tested 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 (Hummel et al., 1997). Obtained scores ranged between 1 and 16 for each nostril.

#### 2.4.2 | 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, that is, the pen containing the different odorant. The 32-triplets discrimination test is an extended version of the commercially available 16-triplets test (Frasnelli et al., 2010; Haehner et al., 2009). Obtained scores ranged from 0 to 32.
2.4.3 | 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 beginning of training is composed of following odors: orange, leather, cinnamon, peppermint, banana, lemon, licorice, turpentine, garlic, coffee, apple, cloves, pineapple, rose, anise, and fish. The second one that we used at the end of training is composed of 16 other common odors: pear, coke, lilac, grapefruit, grass, raspberry, honey, ginger, coconut, lavender, melon, peach, mushrooms, smoked meat, chocolate, and onion (Haehner 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 were 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 Sniffin’ Sticks test. Therefore, they were same for all participants. In total, we obtained two scores: free and cued identification, each ranging from 0 to 16 and corresponding to number of odors that were correctly identified. In the free identification task, participants scored only if the identification was fully correct, for example, naming lemon or leather for orange would both count as zero.

2.4.4 | Olfactory memory

We assessed olfactory memory by using two sets of 16 pens designed for the identification task. Only eight pens from each set were used for this task; half of the participants were tested with pens labeled with even numbers, the other half with odd numbers. The order of pens 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 Sniffin’ Sticks test. Therefore, they were same for all participants. In total, we obtained two scores: free and cued identification, each ranging from 0 to 16 and corresponding to number of odors that were correctly identified. In the free identification task, participants scored only if the identification was fully correct, for example, naming lemon or leather for orange would both count as zero.

2.5 | Analysis

Alpha was set at .05 and we used Bonferroni–Holm corrections for multiple comparisons.

2.5.1 | OB volume

We used the MIPAV (Medical Image Processing, Analysis, and Visualization) to measure OB volume by manually contouring the OB surface on each coronal slice, from anterior to posterior, with pixel size 0.16 × 0.16 mm². The first slice (most anterior one) we consider is the one on which OB becomes visible. A sudden decrease in the diameter of OB marks the posterior end of the structure and it 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 mm³. This approach is commonly used in studies examining OB volumes, and it was proven to be a reliable and an accurate method (Huart et al., 2013; Seubert et al., 2013; Yousem, Geckle, Bilker, & Doty, 1998).

Extracted OB volumes were analyzed using SPSS 23.0 for Windows. For the analysis, OB volume was our dependent variable. We performed repeated measures of ANOVA with two within-subject factors: time (two levels: beginning of training “T1,” and end of training after 18 months “T2”), and side (two levels: left and right). Group (two levels: sommelier students and control participants) was defined as between-subject factor. We also used whole brain volume as covariate.

We performed post-hoc repeated measures of ANOVAs in sommeliers and controls separately, with time and side as within-subject factors so that we could investigate if there was a group-specific evolution of OB volume between T1 and T2.

2.5.2 | Cortical thickness

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

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

The automated reconstitution of a tridimensional image of 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 that are defined by X, Y, and Z coordinates. Vertices of white and pial surfaces have the same identity: each vertex of white surface has a corresponding vertex in the pial surface, which allows us to calculate the distance between two surfaces (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 brain as described in the previous paragraph and this is performed independently for each time point. The output is 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 the within-subject template to create, final results for each time point that are more accurate and reliable than the independent cross-sectional runs. Once this preprocessing was done for each participant, we computed the longitudinal data from cortical thickness measures at T1 (first time point, at the beginning of training) and T2 (second time point, at the end of training). These longitudinal data included:

- the average thickness across time: (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 included smoothing, using a five FWHM kernel and resampling onto FreeSurfer average subject FSSaverage.

Finally, a group analysis was performed using a general linear model 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 <.05 when corrected for multiple comparisons, or at p <.0001 for predicted regions. FreeSurfer stores significance as −log10(p-value); a significance of 4 and more corresponds to p <.0001 uncorrected.

See https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/LongitudinalTutorial for more details.

2.5.3 | Olfactory performance

Olfactory performance scores were analyzed using 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, first we analyzed olfactory threshold on each side. We included the other olfactory tasks in a second analysis.

**Olfactory threshold**

Olfactory threshold was our dependent variable. We performed repeated measures ANOVA with two within-subject factors: time (two levels: beginning of training “T1,” and end of training 18 months later “T2”), and side (two levels: left and right). Group (two levels: sommelier students and control participants) was defined as between-subject factor.

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

**Overall olfactory performance**

This analysis included more olfactory scores. Because there were strong correlations between left and right thresholds, we only kept the better threshold for this analysis which reflects the score obtained when both nostrils are tested simultaneously (Frasnelli, Livermore, Soiffer, & Hummel, 2002). The score obtained in the cued identification task is most commonly used in studies with the Sniffin’ Sticks. We performed a repeated measures ANOVA with two within-subject factors: time (two levels: beginning of training “T1,” and end of training 18 months later “T2”), and test (four levels: better threshold, discrimination, cued identification, and olfactory memory). Group (two levels: sommelier students and control participants) was defined as between-subject factor.

2.5.4 | Correlations between brain and olfaction

We calculated evolutions ΔT2 − T1 for each dependent variable and performed Pearson correlations to examine if there was any correlation between left and right olfactory thresholds and left and right OB volumes, between other olfactory tasks and total OB volume (left + right OB volumes), and between olfactory scores and cortical thickness.

3 | RESULTS

3.1 | OB volume

Figure 1 depicts a coronal slice of a brain. OB volumes are represented on Figure 2.

We found a significant interaction time*group ($F_{1,22} = 16.246$, $p = .001$) and a significant effect of whole brain volume ($F_{1,22} = 16.842$, $p <.001$) on OB volumes. There was no significant main effect of time ($F_{1,22} = 2.339$, $p = .140$), group ($F_{1,22} = 1.015$, $p = .325$), side ($F_{1,22} = 0.007$, $p = .934$), or any interaction.

To disentangle the interaction, we compared OB volumes at T1 and T2 in each group separately. In the sommeliers group, we observed a significant main effect of time with OB volume being bigger at T2 than T1 ($F_{1,11} = 12.028$, $p = .005$). In contrast, there was no significant effect of time in controls ($F_{1,12} = 0.474$, $p = .504$); the evolution of OB volume over time was not significant in the control group (Figure 2).

We then investigated the effect of whole brain volume which is correlated with OB volume (left OB at T1: $r = .456$, $p = .016$; right OB at T1: $r = .371$, $p = .033$; left OB at T2: $r = .578$, $p = .006$; right OB at T2: $r = .622$, $p = .004$). There was no effect of group on the whole brain volume ($F_{1,33} = 0.939$, $p = .340$).

3.2 | Cortical thickness

When we applied a correction for multiple comparisons, we found no significant cluster. When we lowered the threshold to $p <.0001,$...
uncorrected, we observed that sommelier training had an effect on cortical thickness in several small clusters: in sommelier students, there was an increase of cortical thickness in right entorhinal cortex, and a decrease of cortical thickness in left inferior temporal gyrus, triangular portion of right inferior frontal gyrus (pars triangularis), left superior parietal, and superior frontal gyri (for more details, see Tables 1 and 2 and Figure 3).

### 3.3 | Olfactory performance

Olfactory scores are depicted in Figure 4.

In our first analysis, we found a significant interaction of time*group ($F_{1,23} = 8.951, p = .007$) and a significant effect of time with thresholds at T2 better than T1 ($F_{1,23} = 7.510, p = .012$). There was no effect of group ($F_{1,23} = 1.435, p = .243$), side ($F_{1,23} = 0.241, p = .628$), or any interaction.

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 ($F_{1,11} = 0.033, p = .860$) or any other variable and interaction. In control group, there was a significant main effect of time with olfactory thresholds being better at T2 than T1 ($F_{1,12} = 29.775, p < .001$; Figure 4).

In our second analysis, which included four olfactory tasks, we found no significant effect of group ($F_{1,23} = 0.176, p = .678$) or time ($F_{1,23} = 0.906, p = .351$), interaction of time*group ($F_{1,23} = 3.591, p = .071$). This means that the olfactory scores did not significantly evolve between T1 and T2. There was no overall significant difference between groups.

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**TABLE 1** Demographic data at baseline and follow-up

|          | Sommeliers | Controls | Statistic | p-value |
|----------|------------|----------|-----------|---------|
| **Baseline** |            |          |           |         |
| Sex (f/m) | 7/10       | 7/10     | $X^2$     | 1       |
| Age       | 26.0       | 26.2     | t-test    | .9      |
| **Follow-up** |          |          |           |         |
| Sex (f/m) | 5/7        | 4/8      | $X^2$     | .67     |
| Age       | 26.6       | 27.3     | t-test    | .7      |

Note: Mean ages were compared with independent samples t-test. p-values of the tests were added.
3.4 | Correlations between OB volume and olfactory performance

There was no correlation between the OB volume and olfactory threshold at T1 and T2 (left: T1: $r = -.241, p = .176$; T2: $r = -.111, p = .598$; right: T1: $r = -.012, p = .947$; T2: $r = .084, p = .690$). We also found no correlation between evolutions of OB volume and olfactory threshold (left: sommeliers: $r = .259, p = .416$; controls: $r = -.234, p = .442$; right: sommeliers: $r = -.315, p = .319$; controls: $r = -.443, p = .129$). There was no significant correlation between OB volumes or cortical thickness and other olfactory tests.

4 | DISCUSSION

We found that sommelier training led to an increase of OB volume and to changes in cortical thickness in five different regions associated with olfactory processing. Also, olfactory tests did not reveal any
improvement of olfactory function; thus, changes in the brain were not correlated with enhanced olfactory performance.

4.1 | OB volume

The increase of OB volume as sommelier students become olfaction experts is a great example of ecological training-related brain plasticity. This result is in line with the literature about OB volume in patients and healthy people with a normal sense of smell. Since greater OB volume is associated with a better sense of smell (Buschhuter et al., 2008; Liu et al., 2017; Rombaux et al., 2006; Seubert et al., 2013), and olfactory training results with the increase of OB volume (Haehner et al., 2008; Negoias et al., 2017; Rombaux et al., 2009), it is plausible that becoming an expert in olfaction 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, that is, how the OB can grow with training. (1) Synaptogenesis is a first possible mechanism: activity modulates the connections between neurons, that is, the synapses between olfactory receptor neurons and mitral cells lead to an increase or decrease in the number of synapses, thus modulating the size of the structure (Eavri & Nedivi, 2013; Zatorre et al., 2012).

Other mechanisms consist of an increase in the number of neurons. Neurogenesis is a remarkable ability of the olfactory system. (2) Continuous neurogenesis occurring at the level of the olfactory epithelium is a second proposed mechanism. The olfactory epithelium contains stem cells that can differentiate into new olfactory receptor neurons (Schwob & Costanzo, 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. This could explain the increase in OB volume.

A third possible mechanism relies on (3) neurogenesis in the supra-ventricular 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 the OB growth. However, this mechanism, which was first demonstrated in adult rodents and monkeys (Kornack & Rakic, 2001; Lois, Garcia-Verdugo, & Alvarez-Buylla, 1996; Ming & Song, 2011) is still debated in adult humans. If neural stem cells have been observed along the lateral ventricle in humans (Johansson, Svensson, Wallstedt, Janson, & Frisen, 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., 2011; Sanai, Berger, García-Verdugo, & Alvarez-Buylla, 2007).

Finally, (4) 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 that they could be
4.2 Cortical thickness

We observed an increase of cortical thickness in sommeliers’ right entorhinal cortex, and a decrease in the inferior temporal gyrus, the superior parietal and the superior frontal gyrus (all left) as well as the triangular portion of right inferior frontal gyrus. It is important to point out that the group difference in cortical thickness were only significant with a liberal threshold of \( p < 0.001 \), uncorrected, while a Bonferroni correction yielded no significant difference. Our approach is in line with earlier research on the association between structural measures in olfactory specialists/training (Al Ain et al., 2019; Banks et al., 2016; Delon-Martin, Plailly, Fonlupt, Veyrac, & Royet, 2013), as using a conservative threshold such as Bonferroni correction comes with the risk of false negative results. We therefore primarily discuss predicted olfactory regions, that is, regions that have shown an effect in earlier studies. This is only true for the right entorhinal cortex which increased in sommelier students. A similar increase to the one we observed was reported following a 6 week long olfactory training (Al Ain et al., 2019). In fact, the entorhinal cortex—one of the primary olfactory regions (Patel & Pinto, 2014)—has a larger volume in sommeliers, and its cortical thickness is positively correlated with years of sommelier experience (Banks et al., 2016).

Other regions with significant differences were not predicted, and conclusions are therefore speculative. We observed a decrease in cortical thickness in left inferior temporal gyrus (ITG), triangular portion of right inferior frontal gyrus (rIFG), left superior parietal gyrus, and left superior frontal gyrus in sommelier students. These regions are not typically found to be involved in olfactory processing. 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 before (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 the two brain regions where we observed a decrease: left ITG and right rIFG.

However, in other fields than olfaction, there were reports of learning-dependent decreases of cortical thickness. For example, following a 9 months training of social skills (Valk et al., 2017) or after a week-long training aiming at improving processing speed (Takeuchi et al., 2011). This suggests 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 7 weeks long training during which right-handed participants practiced writing and drawing with left hand, cortical thickness increased in the first 4 weeks but then decreased despite continued practice and increasing task proficiency (Wenger et al., 2017). This led to the “overproduction—pruning” model of plasticity according to which, number of synapses increases greatly at the beginning—resulting in increased cortical thickness—and then, behaviorally-relevant connections are stabilized while connections that prove to be functionally irrelevant are eliminated by pruning (Lindenberger, Wenger, & Lovden, 2017)—associated with reduced cortical thickness.

This model is supported by the evidence of 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 were stabilized and probably function as the physiological substrate for skill acquisition and improvement (Xu et al., 2009). It 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 5 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, Boyke, Gaser, Buchel, & May, 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 6 weeks and participants were tested before it started; in our study, training lasted 18 months and we tested sommelier students when their training had already started, mostly during the second month. Because the timing was different, it is possible that Al Ain et al. observed the increase of cortical thickness that happens at the beginning 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 in second phase during which more synapses would be eliminated by pruning than the ones that were 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.

4.3 Olfactory performance

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 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, Doolittle, & Breslin, 2002; E. Mori, Petters, Schriever, Valder, & Hummel, 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 (E. 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, Lau, & Goktas, 2012; Geissler, Reimann, Gudziol, Bitter, & Guntinas-Lichius, 2014; Haehner et al., 2013; Hummel et al., 2009; Konstantinidis, Tsakiropoulou, Bekiaridou, Kazantzidou, & Constantinidis, 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 of 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, Heatherbell, & White, 2002; Poupon, Fernandez, Archambault Boisvert, Migneault-Bouchard, & Frasnelli, 2018; Poupon, Fernandez, & Frasnelli, 2019). One key argument may explain the potentially conflicting results for the OB (sommelier students but not controls exhibit an increase in volume) and the behavioral test (controls but not sommelier students exhibit improved scores): the Sniffin’ Sticks test was designed to detect olfactory dysfunction and to distinguish between individuals with olfactory dysfunction from those with no such problem. One may therefore argue that there is a ceiling effect for olfactory specialists, and this test might not be suited for accurately discriminating between people with a normal sense of smell and olfactory experts. We have shown that other tasks may be better suited to distinguish between individuals with normal olfactory function and olfactory specialists, for example, the identification of components within a mixture of odors (Poupon et al., 2018). Further, anomalously, we noticed that sommelier students have a more analytical 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. This was most prominent for the chocolate odor, for which the list of descriptors also included vanilla and biscuit. In other case, mostly 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. In this context, it is important that the odorants used in the Sniffin’ Sticks are artificial odors rather than the actual odor source. An alternative explanation could be put forward to explain the lack of association between OB volume changes and changes in olfactory scores, namely that OB volume is not specifically associated with olfactory function. This explanation is however unlikely: first, the association between OB volume and olfactory function is well established (Buschhuter et al., 2008; Haehner et al., 2008; Liu et al., 2017; Negoias et al., 2017; Rombaux et al., 2006, 2009; Seubert et al., 2013). Secondly, the sommelier students do get better at sensory and thus olfactory evaluations over the course of the 18 months of training, as novices would not be able to pass the final exams. There are other behavioral tests which would have been a better choice such as the identification of components in a mixture. We have shown that sommeliers outperform controls in this task (Poupon et al., 2018). Unfortunately, this test was not included in the present study.

Further studies should consider these points.

5 | LIMITATIONS

We did not observe any significant changes in the olfactory performance of sommeliers. This could be due to the fact that the Sniffin’ Sticks test is a clinical test which may not be suitable for professionals’ performance evaluation. In fact, the sommeliers reported that the odors smelled unnatural potentially interfering with their tests. Further, it is unclear why olfactory thresholds improved in the control group but not for sommeliers; it may be that training-induced threshold improvement had already taken place at T1 for the sommeliers. Future studies should take these questions into account.

It is also worth to note that we have relatively small sample for an imaging study. While the sample at baseline consisted of 34 participants, nine participants dropped out over the observation period of 18 months yielding a final sample size of 25. Our results have therefore to be interpreted cautiously.

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. Future longitudinal studies should also be designed while keeping in mind the idea that the effects of training-related plasticity are not linear to further test the overproduction-pruning model: having more than two time points would be ideal to fully observe the effects of training-related brain plasticity.
6 CONCLUSION

In conclusion, this study aimed at exploring the effects of training-related brain plasticity in brain. 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; we also observed local increases or decreases of cortical thickness that support the overproduction-pruning model of plasticity according to which changes in the brain are nonlinear. It is worth to note that the positive changes in entorhinal cortex and the negative changes in other regions might be a question of timing. It could be that not every region evolves at the same rate.

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CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

ETHICS 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.

DATA AVAILABILITY STATEMENT
Because of a confidentiality clause in agreement with the Ethics Committees, research data are not available.

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