Neurobiology of Aging 34 (2013) 2676–2682

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Age-dependent alterations in the number, volume, and localization of islands of Calleja within the olfactory tubercle

Stacey Adjei, Alexandra L. Houck, Katherine Ma, Daniel W. Wesson*

Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, USA

ARTICLE INFO

Article history:
Received 5 September 2012
Received in revised form 8 May 2013
Accepted 16 May 2013
Available online 22 June 2013

Keywords:
Olfaction
Olfactory cortex
Basal forebrain
Odor
Sensory processing

ABSTRACT

The incidence of olfactory perceptual dysfunction increases substantially with aging. Putative mechanisms for olfactory sensory loss are surfacing, including neuroanatomical modifications within brain regions responsible for odor information processing. The islands of Calleja (IC) are dense cell clusters localized within the olfactory tubercle, a cortical structure receiving monosynaptic input from the olfactory bulb. The IC are hypothesized to be important for intra- and extra-olfactory tubercle information processing, and thus olfaction. However, whether the anatomy of the IC are affected throughout normal aging remains unclear. By examining the IC of C57bl/6 mice throughout adulthood and early aging (4–18 months of age), we found that the number of IC decreases significantly with aging. Stereological analysis revealed that the remaining IC in 18-month-old mice were significantly reduced in estimated volume compared with those in 4- month-old mice. We additionally found that whereas young adults (4 months of age) possess greater numbers of IC within the posterior parts of the olfactory tubercle, by 18 months of age, a greater percentage of IC are found within the anterior-most part of the olfactory tubercle, perhaps providing a substrate for the differential access of the IC to odor information throughout aging. These results show that the IC are highly plastic components of the olfactory cortex, changing in volume, localization, and even number throughout normal aging. We predict that modifications among the IC throughout aging and age-related neurodegenerative disorders might be a novel contributor to pathological changes in olfactory cortex function and olfactory perception.

1. Introduction

Aging coincides with numerous levels of changes within the central nervous system, many of which are hypothesized to underlie age-dependent changes in sensory, motor, and cognitive function (Andrews-Hanna et al., 2007; Hof and Morrison, 2004). In particular, olfactory sensory function is strongly affected by aging (Murphy et al., 2002), in a manner even reported to predict mortality (Wilson et al., 2011). Specifically, deficits in odor detection, discrimination, recognition, and learning are commonly reported among aged persons (e.g., Boesveldt et al., 2011; Doty et al., 1984; Murphy et al., 1991; Wilson et al., 2007) and in aged animals (Brushfield et al., 2008; Enwere et al., 2004; Guan and Dluzen, 1994; LaSarge et al., 2007; Nakayasu et al., 2000; Patel and Larson, 2009; Rui et al., 2005; Schoenbaum et al., 2002). Understanding the neural basis of olfactory sensory dysfunction is critical for designing biomarkers to differentiate between age- and disease-associated losses in function and thus enhances diagnostic specificity (Benarroch, 2010).

Age-dependent olfactory loss might stem from insults to multiple stages of odor information processing in the brain. Conductive-based deficits might occur within the nasal epithelium at the level of the olfactory receptor neurons (Kovacs, 2004; Rawson et al., 2012). Alternatively, sensorineural deficits might occur within the olfactory bulb and/or all processing stages following the olfactory bulb. Particularly, following intrabulbar processing stages (Wachowiak and Shipley, 2006), odor information departs the olfactory bulb along mitral and tufted cells into primary sensory cortices (Schwob and Price, 1984; Scott et al., 1980; Shipley and Adamek, 1984; White, 1965). These olfactory cortices, including the piriform cortex, anterior olfactory nucleus, posterolateral cortical amygdala, lateral entorhinal cortex, and olfactory tubercle (OT), represent an especially critical processing level (Cleland and Linster, 2003; Haberly, 2001), hypothesized essential for a range of odor-based behaviors (Barkai and Saar, 2001; Brunjes et al., 2005; Gottfried, 2010; Wesson and Wilson, 2011; Wilson and Sullivan, 2011). Though the olfactory cortex is implicated in olfactory dysfunction observed in age-related neurodegenerative disorders (i.e., Parkinson disease and Alzheimer's disease) (Li et al., 2010;

* Corresponding author at: Department of Neurosciences, Case Western Reserve University School of Medicine, 2109 Adelbert Avenue, Cleveland, OH 44106, USA. Tel.: +1 216 368 6100; fax: +1 216 368 4650.
E-mail address: danielwesson@gmail.com (D.W. Wesson).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.05.014

Ubeda-Bañon et al., 2012; Wattendorf et al., 2009; Wesson et al., 2010, 2011), its direct involvement in olfactory dysfunction accompanying normal aging remains unclear.

We predicted that the islands of Calleja (IC) within the OT are modified during the course of normal aging. The IC are dense cell clusters that extend rostrocaudally throughout most of the OT in mice (Calleja, 1893; Creps, 1974; de Vente et al., 2001; Fallon et al., 1978; Ganser, 1882; Meyer et al., 1989). The IC consists mostly of small, spherical granule cells (Calleja, 1893; Fallon et al., 1978), many of which are GABA-ergic (Hsieh and Puche, 2013; Krieger et al., 1983; Meyer et al., 1989; Millhouse, 1987; Ribak and Fallon, 1982). The granule cells comprising the IC originate from the subventricular zone where they enter the OT off the rostral-migratory stream during late embryogenesis (Creps, 1974; De Marchis et al., 2004; Saaltink et al., 2012). The neurogenesis-dependent nature of the IC and their diverse morphology (de Vente et al., 2001) suggest that the IC are highly plastic components of the olfactory cortex. Supporting this hypothesis, dramatic levels of cell death in the IC are present during early postnatal development (Ahern et al., 2013). Whether this plasticity extends itself through adulthood leading to progressive changes among the IC with aging is unknown.

To address these questions, here we examined the IC of C57bl/6 mice from 4–18 months of age (young to late-middle age). We found that the IC significantly decrease in number with aging and that this is accompanied by a reduced volume of remaining IC—suggesting a progressive pruning in IC with age. Further, we found evidence that the location of the IC within the OT is also altered throughout aging. Together, these results demonstrate a novel anatomical modification in the brain which occurs with aging. We propose that age-related changes in the IC might provide a novel mechanism for olfactory circuit disruption and thus olfactory perceptual dysfunction with aging.

2. Methods

2.1. Animals

Male C57bl/6 mice (Harlan Laboratories) at 4, 8, 10, 14, and 18 months of age ($n = 5$ per age) were housed with food and water available ad libitum in a room governed by a 12-hour day:light cycle. We specifically selected male mice for use in this study to reduce possible influences of endogenous sex hormone cycles on IC development and maintenance. Mice were injected with urethane (2 mg/kg, intraperitoneal) and transcardially perfused with 0.9% NaCl followed by 10% formalin. The brains were immediately removed and stored in 30% sucrose in 10% formalin for >48 hours at 4 °C. All procedures involving animals were approved by the Case Western Reserve University Institutional Animal Care and Use Committee.

2.2. Histology

Frozen brains were coronally sectioned on a sliding microtome at 20 μm starting immediately posterior to the olfactory bulbs and lasting until the appearance of the anterior commissure. This anterior-posterior span was selected to include the entire distance of the OT (Paxinos and Franklin, 2000) (Fig. 1). Alternately sampled 20-μm sections were placed on gelatin-subbed slides (Southern Biotechnology Associates Inc, Birmingham, AL, USA) in successive

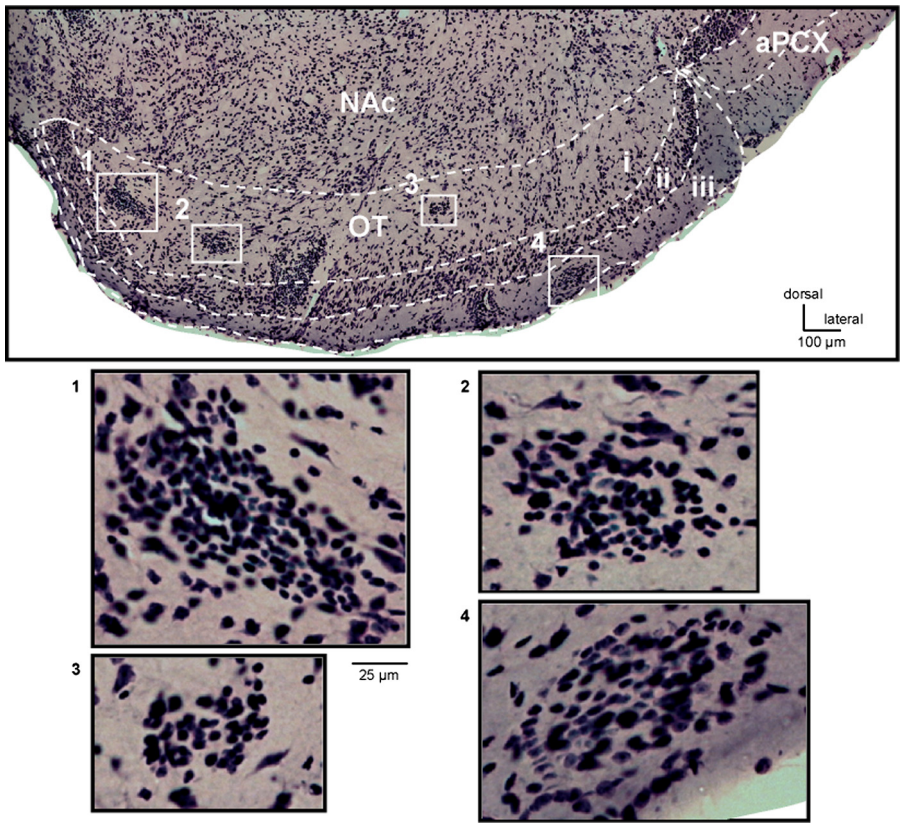

Fig. 1. Localizing the islands of Calleja (IC) within the mouse olfactory tubercle. Photomicrograph of a cresyl violet-stained basal forebrain from a C57bl/6 mouse (20-μm coronal section). Span of the olfactory tubercle (OT) and anterior piriform cortex (aPCX) layers i, ii, and iii are indicated (white dashed lines). Localization of 4 IC are indicated (white squares) and displayed in enhanced magnification in panels 1–4 (lower). Additional IC are identifiable within this section, yet are not indicated nor displayed with enhanced magnification for space purposes. Abbreviation: NAc, nucleus accumbens.

order. Sections were then stained with cresyl violet. First, sections were dehydrated in graded 70% and 50% EtOH (1 minute each). They were then rehydrated in ddH_2O (1 minute) and placed in filtered 0.1% cresyl violet (Sigma Aldrich, St Louis, MO, USA) (7 minutes). The slides were then dipped in ddH_2O (twice) and then in 70% and 95% EtOH for rinsing. Afterward, the slides were twice incubated in Histoclear (National Diagnostic, Atlanta, GA, USA) for 8 and 15 minutes before being coverslipped with Permount (Fisher Scientific). All brains were sectioned and stained in a pseudorandom order by an experimenter blind to the age of each specimen.

2.3. Imaging

Bilateral serial images of the OT and each IC from all mice were captured using a Leica DMM 1000 microscope equipped with a Leica EC3 digital camera. The IC were identified by 2 independent observers (SA and ALH) blind to the age of each specimen based on the following 3 criteria (as exemplified in Fig. 1): (1) clusters composed of small spherical cells within any of the layers of the OT (Meyer et al., 1989); (2) clusters containing ≥10 cells (none less than 20 were found); and (3) the cluster cells had to be smaller and more spherical than the surrounding cells (Millhouse, 1987). Prevention of the corpuscle problem (Mouton, 2002; West, 2012) was accomplished using manual inspection of similarities in localization and morphology of identified IC throughout subsequent sections (an IC identified on 2 successive sections for instance was counted as a single IC). This method was selected to prevent making unfounded assumptions about the size and shape of the IC (e.g., as needed for Abercrombie correction; Abercrombie and Johnson, 1946; Mouton, 2002) and yielded IC numbers in young animals close to those expected based on previous reports (Hsieh and Puche, 2013). After identification, the IC were assigned unique identifier codes and subsequently captured at magnification ×40 for later quantification (see later in text).

2.4. Data analysis

Stereological measures of estimated volume and localization were performed using NIH Image J (http://rsbweb.nih.gov/ij). For volume estimates, images at magnification ×40 of serial alternate sections containing a single identified IC were uploaded and analyzed using the Image J Volumest plug-in (Merzin, 2008). The number of successive alternate images possessing each IC, the thickness of the sections, and the appropriate pixel-to-micron equivalence were entered into the Volumest program. Next, the border of each IC was manually traced. The volume estimate (Merzin, 2008) was calculated after correction for the fixed sampling interval (alternate 20-μm sections). Identical methods were used for OT volume estimates to control for the reference trap confound (West, 2012), however, by uploading images (at magnification ×10) possessing the OT and then tracing the OT perimeter within each uploaded section. Next, the location of each IC along the anterior-posterior axis was determined by measuring the distance between the OT's anterior-most aspect (marked consistently throughout all ages by the retraction of the ventral tenia tecta) and before each IC first appeared (20-μm resolution determined by section thickness). Similarly, the location of each IC respective to the medial-lateral axis was determined by measuring the distance from the medial-most aspect of the OT to the medial-most aspect of each IC. Some IC within middle-aged groups (10 and 14 months) had tears through them, perhaps resulting from sectioning or slide mounting, precluding the use of these age groups for accurate volume and localization measures but still allowing highly robust and sensitive quantification of IC number. All measures were collected in a pseudorandom order across ages by an experimenter blind to the age of each specimen.

Data were analyzed using Origin 8.5 (Northampton, MA, USA) using statistics as specified throughout the Results, and Bonferroni correction in cases of repeated analyses of variance to control for the familywise error rate. Values are reported as mean ± standard error of the mean unless otherwise noted.

3. Results

3.1. Progressive decrease in IC number with age

To address whether the number of IC within mice might change with aging, we quantified the total number of IC found across both hemispheres per mouse and across all ages. We observed a significant effect of age on the average number of IC within each hemisphere, with a strong negative correlation between age and IC number ($r(23) = -0.52$; $p < 0.01$, Pearson's correlation) (Fig. 2, black dashed line). Eighteen-month-old mice possessed approximately 21% fewer IC on average than 4-month-old mice per hemisphere (Fig. 2). Significant differences between particular age groups we further observed (4-month vs. 18-month, [$F(1,8) = 6.21$; $p = 0.037$], 10-month vs. 18-month [$F(1,8) = 10.769$; $p = 0.013$]) (Fig. 2), with other groups showing trends toward significance when compared with 18-month-old subjects (8-month [$F(1,8) = 3.70$; $p = 0.095$]; 14-month [$F(1,8) = 3.46$; $p = 0.099$]). Similar levels of significance were observed when analyzing for total IC number (across both

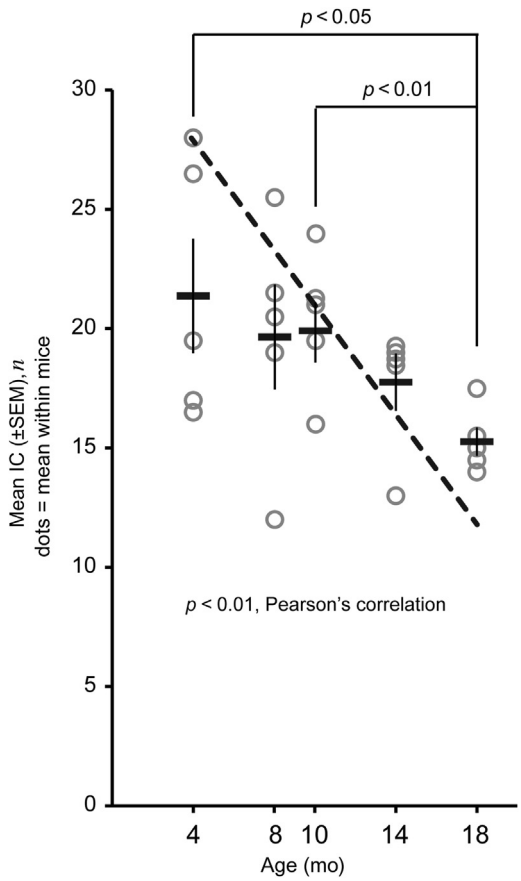

Fig. 2. Progressive decrease in number of islands of Calleja (IC) with aging. Scatter plot represents the mean number of IC across both hemispheres for each mouse (open circles) and the average of this value across all mice (horizontal lines). Black dashed line indicates linear fit. *p* values derived from analysis of variance followed by Fisher's least significant difference test.

hemispheres) instead of average number. Thus, 1 feature of normal aging is a significant, progressive decrease in the number of IC.

3.2. IC in aged mice are reduced in estimated volume

The reduced number of IC observed with aging could be because of: (1) shrinkage or disappearance of the IC (possibly because of reduced neurogenesis with age; Lazarov et al., 2010; apoptosis [Ahern et al., 2013], and/or diffusion of the IC granule cells); or in contrast; (2) convergence of multiple IC into a unitary structure. For an initial test of these possibilities, we used stereological methods (Merzin, 2008) to estimate IC volume across the entire population of IC in 4-month-old and 18-month-old mice (see Methods). Increased IC volume with aging would provide evidence for convergence among the IC with aging, whereas decreased volume would suggest apoptotic and/or diffusive processes. Supporting the latter, we found a significant decrease in estimated volume of the IC with age ($F(1,8) = 16.073$; $p = 0.0039$) (Fig. 3), with IC in 18-month-old mice being on average 59.45 ± 8.9% smaller than IC in 4-month-old mice. Importantly, a similar comparison of total OT volume estimates revealed no change with aging ([$F(1,8) = 0.113$; $p = 0.75$], 4-month [5.51E + 08 ± 2.41E + 07 μm^3] vs. 18-month [5.43E + 08 ± 6.86E + 06 μm^3] mean ± standard error of the mean), suggesting that the observed reduction in IC volume with aging is independent of global changes within the OT (differential tissue shrinkage) (Hatton and von Bartheld, 1999) in the 18-month-old cohort. Thus in aged mice, not only are there fewer IC, but the remaining IC are reduced in volume.

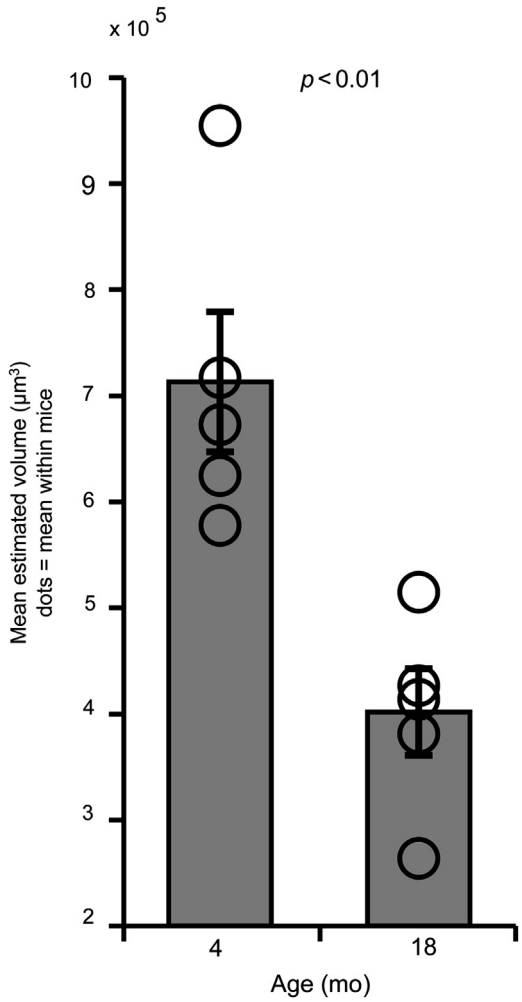

Fig. 3. Islands of Calleja (IC) volume is reduced in aged mice. Scatter plot represents the mean estimated volume of IC across both hemispheres for each mouse (open circles; $n = 5$ mice per group) and the average of this value across all mice (bars) in 4- and 18-month-old mice. Error bars represent standard error of the mean. p = analysis of variance followed by Fisher's least significant difference test.

3.3. Altered spatial distribution of the IC throughout the aging OT

Taken together, the results presented herein (Figs. 2 and 3) suggest that the spatial distribution or regional density of the IC within the OT might be unique in aged versus young adult mice. Therefore, as a final analysis we calculated the localization of all IC in 4- and 18-month-old mice (see Methods). The OT was equally divided into thirds along the anterior-posterior and medial-lateral axes to roughly quantify the spatial distribution of the IC. The total numbers of IC within each hemisphere were normalized to control for effects of age on IC numbers (Fig. 2). This analysis revealed that although both age groups possess a similar percentage of IC within the mesial-most aspect of the OT's anterior-axis ($F(1,12) = 0.27$; $p = 0.61$), the number of IC within the posterior- and anterior-most aspects was inversely affected by age (Fig. 4A). Although 4-month-old mice had a greater percentage of IC in the posterior-most OT compared with 18-month-old mice ($F(1,12) = 5.136$; $p = 0.043$), 18-month-old mice had a greater percentage of IC in the anterior-most OT ($F(1,12)$ 4.93; $p = 0.046$) (Fig. 4A). In contrast, no effect of age on medial-lateral localization of the IC was found in any medial-lateral range ($p > 0.05$; analysis of variance) (Fig. 4B). These results demonstrate that an additional feature of normal aging is an altered spatial distribution of the IC within the OT, suggesting differential access and possibly involvement of the IC in odor information processing in aged versus young adult mice.

4. Discussion

In the present study we explored anatomical changes among the IC throughout normal aging in mice. We predicted that because of the highly plastic nature of the IC (De Marchis et al., 2004; Saaltink et al., 2012), major anatomical alterations in the IC would be observed in aged versus young adult mice. In support of this hypothesis, we observed significant alterations in IC number, volume, and localization throughout early aging (4 to 18 months) which might, as discussed later in text, provide new insights into age-related olfactory sensory dysfunction.

4.1. Functional implications for alterations in IC with age

A major finding of the present work is that the presence of the IC within the OT undergo significant morphological changes in number, volume, and spatial location with aging. The number of IC varies from species to species (Bayer, 1985; Fallon et al., 1978; Meyer et al., 1989; Millhouse and Heimer, 1984; Ribak and Fallon, 1982; Talbot et al., 1988). Although the number of IC is thought to be consistent within species (e.g., Fallon et al., 1978), exceptions, including an increase in the number of IC during early postnatal development exist (Hsieh and Puche, 2013). On the opposite end of the development spectrum from postnatal life, here we found a decreased number and a decreased volume of the IC by 18 months of age. Although herein we only used mice up to 18 months of age (late-middle age), we predict studies in more greatly aged mice (22 months of age and older) will reveal an even more significant decrease in IC number and volume.

Both of these findings might result in possible differential access of information to the IC in aged mice compared with young. The OT receives dense innervation by olfactory bulb mitral and tufted cells

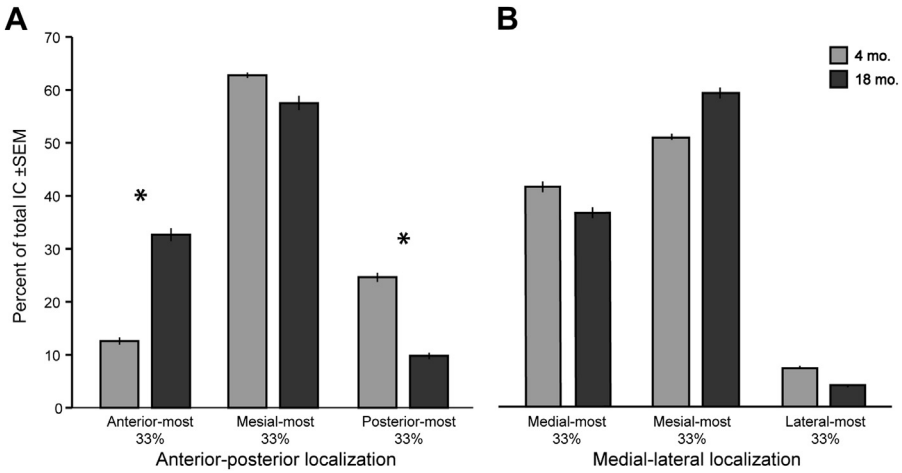

Fig. 4. Altered spatial distribution of the islands of Calleja (IC) within the olfactory tubercle. Histograms of the percentage of total IC found within equally-divided zones along the anterior-posterior (A) and medial-lateral axes (B) of the olfactory tubercle in 4- and 18-month-old mice. $^{*}p < 0.05$, analysis of variance followed by Fisher's least significant difference test. $n = 5$ mice per group. Abbreviation: SEM, standard error of the mean.

(e.g., Haberly and Price, 1978; Imamura et al., 2011; Kang et al., 2011; Millhouse and Heimer, 1984; Nagayama et al., 2010; Schwob and Price, 1984; Scott et al., 1980; Shipley and Adamek, 1984; Sosulski et al., 2011; White, 1965)— positioning it to play a critical role in odor perception. Localized throughout the OT, the IC are hypothesized important for both intra- and extra-OT information processing (Fallon et al., 1978; Meyer et al., 1989; Wahle and Meyer, 1986). Although the IC are positioned strategically to play a role in olfactory processing, their function is likely diverse considering the reciprocal interconnections between IC cells and brain reward and arousal-related structures, including the ventral tegmental area (Fallon et al., 1983), the substantia nigra, and nucleus accumbens (Fallon et al., 1978). Thus, in addition to possibly providing sources for intra-OT synaptic activity, the IC are also integral parts of OT-basal forebrain circuits (Ikemoto, 2010). With this in mind, our finding that the number and volume of IC decreases dramatically with age posits a novel putative mechanism for age-related changes to not only olfactory perception, but also odor hedonics, and possibly even reward behaviors. Particularly, the large presence of GABAergic cells in the IC (Krieger et al., 1983) suggests that a reduced number of IC would render the OT more excitable and/or unable to exert inhibition on the previously listed connected structures. Thus, age-related reductions in IC might entail significant basal forebrain network instability.

Another finding of the present work is a divergent location of the IC in young versus old mice, with aged mice having a greater proportion of IC in the anterior-most aspects of the OT. The particularly dense innervation by mitral and tufted cells into the antero-lateral aspect of the OT suggests that IC in this region might be more integrally involved in odor information processing. It is possible therefore that a shift of the IC into this region (or contrastingly a decrease among IC in the posterior aspect) in aged mice might alter processing of odors in a manner aberrant from that found in young mice. Future recordings from mice across the lifespan to explore whether spatial regions of the OT do indeed change in their processing of odors with age will be important to test this hypothesis.

We used several methods to increase the likelihood that our results are free from stereological bias (Mouton, 2002; Schmitz and Hof, 2005; West, 2012). First, all histological and analytic methods were performed in a pseudorandom order across ages by experimenters blind to the age of each specimen. Second, we controlled for biologically-based reference traps (West, 2012) in our analysis of IC number by verifying that the volume of our reference space (the OT), was indeed not affected by age (see Results, section 3.2). This same analysis also suggests that the reduced IC number found with age is not a product of differential susceptibility to tissue shrinkage in tissue originating from aged mice, an artificially-based reference trap (Schmitz and Hof, 2005). The IC are highly amorphous and vary considerably in their size and distribution throughout the OT (Adjei and Wesson, 2012; de Vente et al., 2001). This considerable biological variation led us to use manual methods to carefully count the total number of IC manually throughout alternately sampled OT sections (vs. progressing with traditional optical dissector methods based on arbitrary selected grid sizes which might have skewed the present results). In our design, the IC were identified by independent observers based on specific objective criteria (see section 2.3) and counted manually again by the same independent observers. Reliability was later assessed by comparing IC numbers and identities between observers. Prevention of the corpuscle problem (West, 2012) was accomplished by manual inspection of similarities in localization and morphology of identified IC throughout subsequent sections (an IC identified on 2 successive sections for instance was counted as a single IC). This method was selected to prevent unnecessary assumption about the size and shape of the IC (e.g., as needed for Abercrombie correction; Abercrombie and Johnson, 1946) and yielded IC numbers close to those expected based on previous reports (Hsieh and Puche, 2013). Thus, through a combination of stereological software platforms (Image J Volumest), operationally defined criteria, and interobserver reliability, the present study surmounted existing difficulties inherent in studying the IC in an unbiased manner.

Based on our methodological approach (cresyl violet staining) we can only speculate mechanisms underlying IC loss (number and volume) with aging. Presently it is unknown whether decreases in neurogenesis with age (Lazarov et al., 2010; Shook et al., 2012), as observed to affect the olfactory epithelium and olfactory bulb (Enwere et al., 2004; Weiler and Farbman, 1997), might alter the formation and number of IC. Irradiation experiments of the mouse subventricular zone demonstrated a powerful control of IC formation by rostral migratory stream neuronal progenitors (De Marchis et al., 2004). Future work exploring whether IC loss as observed herein is result of decreased neurogenesis with age, would be informative in testing this possible mechanism. Alternatively, it is possible that IC loss results from neurogenesis-independent factors, including increased cell death among these unique structures (Ahern et al., 2013) with age. Irrespective of the particular

mechanism, whether particular types of IC neurons (e.g., GABAergic) are more greatly affected by aging remains to be tested and would provide clues to the influence of this morphological loss on OT network activity.

4.2. Effect of aging on the olfactory system

Our results are consistent with the view that age and development severely affects anatomical aspects of the olfactory system and its function. Although particular networks and features of this system appear resilient to mechanisms of normal aging, including the bulk of olfactory bulb organization (Richard et al., 2010) (but see for instance, Cavallin et al., 2010), some aspects of olfactory bulb neurotransmission (Mandairon et al., 2011), and even the volume of the OT itself (see Results), are not as robust. Cell proliferation in the olfactory epithelium decreases >90% in aging rats (postnatal day 1 to 333), resulting in increased surface area of the olfactory epithelium (Weiler and Farbman, 1997) and thus altered access of odorants to odor receptors. Also in aged humans (≥60 years of age), isolated olfactory receptor neurons are more likely to generalize between odors compared with adults (≤45 years of age) which might result in impaired odor discrimination and/or a decline in sensitivity (Rawson et al., 2012). Secondary (cortical) and higher-order olfactory structures also display significant changes (mostly atrophy) with age (Jack et al., 2000; Resnick et al., 2003; Rogalski et al., 2012; Trivedi et al., 2011), along with most of the brain (Fox and Schott, 2004). The results of the present study add the IC, and thus the OT, to the list of structures that are affected during normal aging. Whether anatomical modifications to the IC, similar to those reported here in mice, are observed throughout aging in humans is presently unknown.

Anatomical modifications to central olfactory structures, including gross volume reductions, are reported in Alzheimer's disease and Parkinson disease (Bohnen et al., 2007; De Leon et al., 1997; Kovacs, 2004; Ubeda-Bañon et al., 2012; Wattendorf et al., 2009; Wong et al., 2010). In persons with Alzheimer's disease, congo-negative plaques and dystrophic neurites are found in the IC (van Nes et al., 1993). Exploring the IC in Parkinson disease and animal models of the disease might be especially fruitful considering the close relationship between the OT and the nigrostriatal system (Ikemoto, 2007). This might be strengthened by analysis of particular receptor types, which are uniquely vulnerable to neurodegenerative mechanisms and also highly expressed in the IC (e.g., D_3 receptors; Joyce et al., 2004). Analysis of the IC along these lines might provide new clues to neurodegenerative and age-related mechanisms of olfactory sensory loss.

4.3. Conclusions

In the present study we found a progressive decrease in IC number, a reduction in IC volume, and an aberrant localization of the IC in c57bl/6 mice during early aging. These results support previous developmental work (Ahern et al., 2013; Hsieh and Puche, 2013) showing that the IC are highly plastic components of the olfactory cortex and suggest that modifications among the IC throughout aging, and possibly development, might be a novel contributor to pathological changes in olfactory cortex function and olfactory perception. Future studies to explore the mechanisms of age-dependent IC loss and the functional outcomes of this loss on OT function and olfactory perception will be critical in understanding the neural basis of olfactory sensory loss in aging and age-related neurodegenerative disorders. Although now the precise role(s) of the IC in olfactory perception remain unclear, studies attempting to modulate IC number or volume, specifically, will be critical in providing evidence that the degeneration of these structures is a necessary component of age-and possibly even disease-dependent olfactory loss.

Disclosure statement

The authors have no actual or potential conflicts of interest to declare.

All procedures involving animals were approved by the Case Western Reserve University Institutional Animal Care and Use Committee.

Acknowledgements

The authors thank the members of the Wesson lab for comments on an earlier version of this report. This work was supported by NSF grant ISO-1121471 and a grant from the University Hospitals Case Medical Center Spitz Brain Health Fund.

References

Abercrombie, M., Johnson, M.L., 1946. Quantitative histology of Wallerian degeneration I. Nuclear population in rabbit sciatic nerve. J. Anat. Lond. 80, 37–50.
Adjei, S., Wesson, D.W., 2012. A Quantitative Analysis of the Islands of Calleja in the Mouse Olfactory Tubercle. Huntington Beach, CA: Association for Chemoreception Sciences Annual Meeting.
Ahern, T.H., Krug, S., Carr, A.V., Murray, E.K., Fitzpatrick, E., Bengston, L., McCutcheon, J., Vries, G.J., Forger, N.G., 2013. Cell death atlas of the postnatal mouse ventral forebrain and hypothalamus: effects of age and sex [e-pub ahead of print]. J. Comp. Neurol. 521, 2551–2569.
Andrews-Hanna, J.R., Snyder, A.Z., Vincent, J.L., Lustig, C., Head, D., Raichle, M.E., Buckner, R.L., 2007. Disruption of large-scale brain systems in advanced aging. Neuron 56, 924–935.
Barkai, E., Saar, D., 2001. Cellular correlates of olfactory learning in the rat piriform cortex. Rev. Neurosci. 12, 111–120.
Bayer, S.A., 1985. Neurogenesis in the olfactory tubercle and islands of Calleja in the rat. Int. J. Dev. Neurosci. 3, 135–147.
Benarroch, E.E., 2010. Olfactory system. Neurology 75, 1104–1109.
Boesveldt, S., Lindau, S., McClintock, M., Hummel, T., Lundstrom, J., 2011. Gustatory and olfactory dysfunction in older adults: a national probability study. Rhinology 49, 324–330.
Bohnen, N., Gedela, S., Kuwabara, H., Constantine, G., Mathis, C., Studenski, S., Moore, R., 2007. Selective hyposmia and nigrostriatal dopaminergic denervation in Parkinson's disease. J. Neurol. 254, 84–90.
Brunjes, P.C., Illig, K.R., Meyer, E.A., 2005. A field guide to the anterior olfactory nucleus (cortex). Brain Res. Brain Res. Rev. 50, 305–335.
Brushfield, A.M., Luu, T.T., Callahan, B.D., Gilbert, P.E., 2008. A comparison of discrimination and reversal learning for olfactory and visual stimuli in aged rats. Behav. Neurosci. 122, 54–62.
Calleja, C., 1893. Olfactory brain region. Nicolas Moya, Madrid.
Cavallin, M.A., Powell, K., Biju, K.C., Fadool, D.A., 2010. State-dependent sculpting of olfactory sensory neurons is attributed to sensory enrichment, odor deprivation, and aging. Neurosci. Lett. 483, 90–95.
Cleland, T.A., Linster, C., 2003. Central olfactory structures. In: Doty, R.L. (Ed.), Handbook of Olfaction and Gustation, second ed. Marcel Dekker, New York, pp. 165–180.
Creps, E.S., 1974. Time of neuron origin in preoptic and septal areas of the mouse: an autoradiographic study. J. Comp. Neurol. 157, 161–243.
De Leon, M.J., George, A.E., Golomb, J., Tarshish, C., Convit, A., Kluger, A., De Santi, S., Mc Rae, T., Ferris, S.H., Reisberg, B., Ince, C., Rusinek, H., Bobinski, M., Quinn, B., Miller, D.C., Wisniewski, H.M., 1997. Frequency of hippocampal formation atrophy in normal aging and Alzheimer's disease. Neurobiol. Aging 18, 1–11.
De Marchis, S., Fasolo, A., Puche, A.C., 2004. Subventricular zone-derived neuronal progenitors migrate into the subcortical forebrain of postnatal mice. J. Comp. Neurol. 476, 290–300.
de Vente, J., Hani, L., Steinbusch, H.E., Steinbusch, H.W., 2001. The three dimensional structure of the islands of Calleja: a single heterogenous cell complex. Neuroreport 12, 565–568.
Doty, R.L., Shaman, P., Applebaum, S.L., Giberson, R., Sikorski, L., Rosenberg, L., 1984. Smell identification ability: changes with age. Science 226, 1441–1443.
Enwere, E., Shingo, T., Gregg, C., Fujikawa, H., Ohta, S., Weiss, S., 2004. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. J. Neurosci. 24, 8354–8365.
Fallon, J.H., Loughlin, S.E., Ribak, C.E., 1983. The islands of Calleja complex of rat basal forebrain. III. Histochemical evidence for a striatopallidal system. J. Comp. Neurol. 218, 91–120.
Fallon, J.H., Riley, J.N., Sipe, J.C., Moore, R.Y., 1978. The islands of Calleja: organization and connections. J. Comp. Neurol. 181, 375–395.

Fox, N.C., Schott, J.M., 2004. Imaging cerebral atrophy: normal ageing to Alzheimer's disease. Lancet 363, 392–394.
Ganser, S., 1882. Comparative anatomical studies on the brain of the mole. Morphol. Jahrb. 7, 591–725.
Gottfried, J.A., 2010. Central mechanisms of odour object perception. Nat. Rev. Neurosci. 11, 628–641.
Guan, X., Dluzen, D.E., 1994. Age related changes of social memory/recognition in male fischer 344 rats. Behav. Brain Res. 61, 87–90.
Haberly, L.B., 2001. Parallel-distributed processing in olfactory cortex: new insights from morphological and physiological analysis of neuronal circuitry. Chem. Senses 26, 551–576.
Haberly, L.B., Price, J.L., 1978. Association and commissural fiber systems of the olfactory cortex of the rat. II. Systems originating in the olfactory peduncle. J. Comp. Neurol. 181, 781–807.
Hatton, W.J., von Bartheld, C.S., 1999. Analysis of cell death in the trochelar nucleus of chick embryos: calibration of the optical disector couting technique reveals systematic bias. J. Comp. Neurol. 409, 169–186.
Hof, P.R., Morrison, J.H., 2004. The aging brain: morphomolecular senescence of cortical circuits. Trends Neurosci. 27, 607–613.
Hsieh, Y.C., Puche, A.C., 2013. Development of the Islands of Calleja. Brain Res. 1490, 52–60.
Ikemoto, S., 2007. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res. Rev. 56, 27–78.
Ikemoto, S., 2010. Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. Neurosci. Biobehav. Rev. 35, 129–150.
Imamura, F., Ayoub, A.E., Rakic, P., Greer, C.A., 2011. Timing of neurogenesis is a determinant of olfactory circuitry. Nat. Neurosci. 14, 331–337.
Jack Jr., C.R., Petersen, R.C., Xu, Y., O'Brien, P.C., Smith, G.E., Ivnik, R.J., Boeve, B.F., Tangalos, E.G., Kokmen, E., 2000. Rates of hippocampal atrophy correlate with change in clinical status in aging and AD. Neurology 55, 484–489.
Joyce, J.N., Der, T.C., Renish, L., Osredkar, T., Hagner, D., Reploge, M., Sakakibara, S., Ueda, S., 2004. Loss of D3 receptors in the zitter mutant rat is not reversed by L-dopa treatment. Exp. Neurol. 187, 178–189.
Kang, N., Baum, M.J., Cherry, J.A., 2011. Different profiles of main and accessory olfactory bulb mitral/tufted cell projections revealed in mice using an anterograde tracer and a whole-mount, flattened cortex preparation. Chem. Senses 36, 251–260.
Kovacs, T., 2004. Mechanisms of olfactory dysfunction in aging and neurodegenerative disorders. Ageing Res. Rev. 3, 215–232.
Krieger, N.R., Megill, J.R., Sterling, P., 1983. Granule cells in the rat olfactory tubercle accumulate 3H-gamma-aminobutyric acid. J. Comp. Neurol. 215, 465–471.
LaSarge, C., Montgomery, K., Tucker, C., Slaton, G.S., Griffith, W., Setlow, B., Bizon, J., 2007. Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. Neurobiol. Aging 28, 928–936.
Lazarov, O., Mattson, M.P., Peterson, D.A., Pimplikar, S.W., van Praag, H., 2010. When neurogenesis encounters aging and disease. Trends Neurosci. 33, 569–579.
Li, W., Howard, J.D., Gottfried, J.A., 2010. Disruption of odour quality coding in piriform cortex mediates olfactory deficits in Alzheimer's disease. Brain 133, 2714–2726.
Mandairon, N., Peace, S.T., Boudadi, K., Boxhorn, C.E., Narla, V.A., Suffis, S.D., Cleland, T.A., 2011. Compensatory responses to age-related decline in odor quality acuity: cholinergic neuromodulation and olfactory enrichment. Neurobiol. Aging 32, 2254–2265.
Merzin, M., 2008. Applying Stereological Method in Radiology. Volume Measurement. Tartu: University of Tartu.
Meyer, G., Gonzalez Hernandez, T., Carrillo Padilla, F., Ferres Torres, R., 1989. Aggregations of granule cells in the basal forebrain (islands of Calleja): Golgi and cytoarchitectonic study in different mammals, including man. J. Comp. Neurol. 284, 405–428.
Millhouse, O.E., 1987. Granule cells of the olfactory tubercle and the question of the islands of Calleja. J. Comp. Neurol. 265, 1–24.
Millhouse, O.E., Heimer, L., 1984. Cell configurations in the olfactory tubercle of the rat. J. Comp. Neurol. 228, 571–597.
Mouton, P.R., 2002. Principles and Practices of Unbiased Stereology. An Introduction for Bioscientists. The Johns Hopkins University Press, Baltimore.
Murphy, C., Cain, W.S., Gilmore, M.M., Skinner, R.B., 1991. Sensory and semantic factors in recognition memory for odors and graphic stimuli: elderly versus young persons. Am. J. Psychol. 104, 161–192.
Murphy, C., Schubert, C.R., Cruickshanks, K.J., Klein, B.K., Klein, R., Nondahl, D.M., 2002. Prevalence of olfactory impairment in older adults. JAMA 288, 2307–2312.
Nagayama, S., Enerva, A., Fletcher, M.L., Masurkar, A.V., Igarashi, K.M., Mori, K., Chen, W.R., 2010. Differential axonal projection of mitral and tufted cells in the mouse main olfactory system. Front. Neural Circuits. 4.
Nakayasu, C., Kanemura, F., Hirano, Y., Shimizu, Y., Tonosaki, K., 2000. Sensitivity of the olfactory sense declines with the aging in senescence-accelerated mouse (SAM-P1). Physiol. Behav. 70, 135–139.
Patel, R.C., Larson, J., 2009. Impaired olfactory discrimination learning and decreased olfactory sensitivity in aged C57Bl/6 mice. Neurobiol. Aging 30, 829–837.
Paxinos, G., Franklin, K., 2000. The Mouse Brain in Stereotaxic Coordinates, second ed. Academic Press, San Diego.
Rawson, N.E., Gomez, G., Cowart, B.J., Kriete, A., Pribitkin, E., Restrepo, D., 2012. Age-associated loss of selectivity in human olfactory sensory neurons. Neurobiol. Aging 33, 1913–1919.
Resnick, S.M., Pham, D.L., Kraut, M.A., Zonderman, A.B., Davatzikos, C., 2003. Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. J. Neurosci. 23, 3295–3301.
Ribak, C.E., Fallon, J.H., 1982. The island of Calleja complex of rat basal forebrain. I. Light and electron microscopic observations. J. Comp. Neurol. 205, 207–218.
Richard, M., Taylor, S., Greer, C., 2010. Age-induced disruption of selective olfactory bulb synaptic circuits. Proc. Natl. Acad. Sci. U.S.A 107, 15613–15618.
Rogalski, E., Stebbins, G.T., Barnes, C.A., Murphy, C.M., Stoub, T.R., George, S., Ferrari, C., Shah, R.C., deToledo-Morrell, L., 2012. Age-related changes in parahippocampal white matter integrity: a diffusion tensor imaging study. Neuropsychologia 50, 1759–1765.
Rui, D.S., Luciano, C.B., Reinaldo, N.T., 2005. Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats: involvement of adenosine A1 and A2A receptors. Neurobiol. Aging 26, 957–964.
Saaltink, D.J., Håvik, B., Verissimo, C.S., Lucassen, P., Vreugdenhil, E., 2012. Doublecortin and doublecortin-like are expressed in overlapping and non-overlapping neuronal cell population: implications for neurogenesis. J. Comp. Neurol. 520, 2805–2823.
Schmitz, C., Hof, P.R., 2005. Design-based stereology in neuroscience. Neuroscience 130, 813–831.
Schoenbaum, G., Nugent, S., Saddoris, M.P., Gallagher, M., 2002. Teaching old rats new tricks: age-related impairments in olfactory reversal learning. Neurobiol. Aging 23, 555–564.
Schwob, J.E., Price, J.L., 1984. The development of axonal connections in the central olfactory system of rats. J. Comp. Neurol. 223, 177–202.
Scott, J.W., McBride, R.L., Schneider, S.P., 1980. The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat. J. Comp. Neurol. 194, 519–534.
Shipley, M.T., Adamek, G.D., 1984. The connections of the mouse olfactory bulb: a study using orthograde and retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase. Brain Res. Bull. 12, 669–688.
Shook, B.A., Manz, D.H., Peters, J.J., Kang, S., Conover, J.C., 2012. Spatiotemporal changes to the subventricular zone stem cell pool through aging. J. Neurosci. 32, 6947–6956.
Sosulski, D.L., Lissitsyna Bloom, M., Cutforth, T., Axel, R., Datta, S.R., 2011. Distinct representations of olfactory information in different cortical centres. Nature 472, 213–216.
Talbot, K., Woolf, N.J., Butcher, L.L., 1988. Feline islands of Calleja complex: I. Cytoarchitectural organization and comparative anatomy. J. Comp. Neurol. 275, 553–579.
Trivedi, M., Stoub, T., Murphy, C., George, S., deToledo-Morrell, L., Shah, R., Whitfield-Gabrieli, S., Gabrieli, J., Stebbins, G., 2011. Entorhinal cortex volume is associated with episodic memory related brain activation in normal aging and amnesic mild cognitive impairment. Brain Imag. Behav. 5, 126–136.
Ubeda-Bañon, I., Saiz-Sanchez, D., de la Rosa-Prieto, C., Martinez-Marcos, A., 2012. α-Synuclein in the olfactory system of a mouse model of Parkinson's disease: correlation with olfactory projections. Brain Struct. Funct. 217, 447–458.
van Nes, J.A., Kamphorst, W., Ravid, R., Swaab, D.F., 1993. The distribution of Alz-50 immunoreactivity in the hypothalamus and adjoining areas of Alzheimer's disease patients. Brain 116, 103–115.
Wachowiak, M., Shipley, M.T., 2006. Coding and synaptic processing of sensory information in the glomerular layer of the olfactory bulb. Semin. Cell Dev. Biol. 17, 411–423.
Wahle, P., Meyer, G., 1986. The olfactory tubercle of the cat. II. Immunohistochemical compartmentation. Exp. Brain Res. 62, 528–540.
Wattendorf, E., Welge-Lussen, A., Fiedler, K., Bilecen, D., Wolfensberger, M., Fuhr, P., Hummel, T., Westermann, B., 2009. Olfactory impairment predicts brain atrophy in Parkinson's disease. J. Neurosci. 29, 15410–15413.
Weiler, E., Farbman, A.I., 1997. Proliferation in the rat olfactory epithelium: age-dependent changes. J. Neurosci. 17, 3610–3622.
Wesson, D.W., Borkowski, A.H., Landreth, G.E., Nixon, R.A., Levy, E., Wilson, D.A., 2011. Sensory network dysfunction, behavioral impairments, and their reversibility in an Alzheimer's beta-amyloidosis mouse model. J. Neurosci. 31, 15962–15971.
Wesson, D.W., Levy, E., Nixon, R.A., Wilson, D.A., 2010. Olfactory dysfunction correlates with β-amyloid plaque burden in an Alzheimer's disease mouse model. J. Neurosci. 30, 505–514.
Wesson, D.W., Wilson, D.A., 2011. Sniffing out the contributions of the olfactory tubercle to the sense of smell: hedonics, sensory integration, and more? Neurosci. Biobehav. Rev. 35, 655–668.
West, M.J., 2012. Introduction to Stereology. Cold Spring Harb. Protoc. 2012. pdb.top070623.
White, L.E., 1965. Olfactory bulb projections of the rat. Anat. Rec. 152, 465–479.
Wilson, D.A., Sullivan, R.M., 2011. Cortical processing of odor objects. Neuron 72, 506–519.
Wilson, R.S., Schneider, J.A., Arnold, S.E., Tang, Y., Boyle, P.A., Bennett, D.A., 2007. Olfactory identification and incidence of mild cognitive impairment in older age. Arch. Gen. Psychiatry 64, 802–808.
Wilson, R.S., Yu, L., Bennett, D.A., 2011. Odor identification and mortality in old age. Chem. Senses 36, 63–67.
Wong, K.K., Muller, M.L., Kuwabara, H., Studenski, S.A., Bohnen, N.I., 2010. Olfactory loss and nigrostriatal dopaminergic denervation in the elderly. Neurosci. Lett. 484, 163–167.