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Aging in the olfactory system

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Abstract

With advancing age, the ability of humans to detect and discriminate odors declines. In light of the rapid progress in analyzing molecular and structural correlates of developing and adult olfactory systems, the paucity of information available on the aged olfactory system is startling. A rich literature documents the decline of olfactory acuity in aged humans, but the underlying cellular and molecular mechanisms are largely unknown. Using animal models, preliminary work is beginning to uncover differences between young and aged rodents that may help address the deficits seen in humans, but many questions remain unanswered. Recent studies of odorant receptor (OR) expression, synaptic organization, adult neurogenesis, and the contribution of cortical representation during aging suggest possible underlying mechanisms and new research directions.

Keywords

adult neurogenesis; aging; odorant receptor; olfactory cortex; synaptic organization; rostral migratory stream

Why olfaction and aging?

The decline in the ability to detect and discriminate odors in aged humans leads to a decrease in the quality of life and diminished appetite with accompanying impacts on nutritional status (for a review, see [1]). Decreased olfactory function with aging parallels generalized age-related deficits in sensory functions and cognition that occur in the absence of obvious disease states.

The olfactory system resembles other sensory systems in translating sensory stimuli into distinct perceptions and motivations. However, it is unique in offering an opportunity to use molecular diversity to probe how information extracted from sensory stimuli generates different perceptions. Using this molecular diversity, the field has gained information about the sensory signals generated by different ORs (see Glossary) and begun charting the organization of sensory signals from the nose to the olfactory bulb and hierarchically to the olfactory cortices. These molecular and genetic tools, together with the abundance of information on behavioral responses to odorants, are helpful in dissecting the mechanisms that underlie age-related changes in olfactory function.

The paucity of information available on cellular or molecular changes in the aged olfactory system is startling. A rich and detailed literature documents declines in olfactory acuity, sensitivity, and discrimination in aged human populations, as well as the olfactory deficits that appear with the onset of specific age-related disorders such as Parkinson's disease and Alzheimer's disease (for a review, see [2]). Behavioral studies of olfactory function in aged rodents parallels the findings in humans with decreased sensitivity and ability to discriminate odors (e.g., [3–6]). However, studies of the mechanisms underlying olfaction during nonpathological normal aging are few in number. Rawson and colleagues suggested that isolated human olfactory sensory neurons (OSNs) had a decrease in both sensitivity and selectivity to odorant mixtures, a developmental change that may contribute to age-related declines in olfactory function [7]. However, despite the suggestion that OSN dynamics may change with aging, olfactory bulb glomerular organization of aged humans does not appear to differ from younger subjects [8]. Cellular and molecular mechanisms contributing to age-related changes in olfaction are speculative but may include alterations in neuronal populations, synaptic organization, and/or synaptic function. Elsewhere in the central nervous system such changes are proposed to underlie functional deficits (for reviews, see [9,10]). Preliminary work is also beginning to identify candidate genes that may be up- or down-regulated in the olfactory system of aged or injured rodents; work that is likely to provide important clues regarding the mechanisms or events that underlie aging in the olfactory system (e.g., [11,12]). However, for the moment, there are few substantive insights into the cellular and molecular properties of the aged olfactory system, creating a severe handicap in attempting to understand the mechanisms that underlie decreased olfactory function in the elderly. Several reviews appeared in the past few years summarizing the evidence from human olfaction related to aging, senescence, and disease progression, to which we refer the reader [13,14]. In addition, there is a strong interest in the development of mouse models for studying the molecular and cellular substrates of aging-related neurodegenerative diseases such as Alzheimer's [15]. Here, we focus on normal nonpathological aging in rodents and how it may affect the main olfactory system. The authors apologize to those whose work was not included here due to space limitations.

Overview – olfaction and olfactory circuits

As reviewed in Box 1, odors are detected by sensory neurons located in the olfactory epithelium lining the nasal cavity. Once bound to selective ORs, odors elicit a transduction cascade that culminates at synapses with projection and interneurons in the olfactory bulb. Local circuits within the bulb contribute to processing of odor signals prior to projection neurons extending the signal to higher-order targets.

Box 1

Olfactory circuits

Odors are detected by OSNs (1) within the olfactory epithelium lining the nasal cavity (Figure I). Each OSN expresses only one of approximately 1200 different ORs in mice. The odor response profile of a sensory neuron is determined by the receptor expressed. There are 5000–10 000 sensory neurons that express each receptor, leading to a total population of $6\text{--}10 \times 10^6$ sensory neurons. The life cycle of OSNs is short and they are routinely replaced from a population of basal cells within the epithelium.

Sensory neuron axons coalesce to form the olfactory nerve, which after passing through the cribriform plate distributes over the surface of the olfactory bulb, targeting discrete areas using traditional guidance molecules, cell signaling, and activity [84]. Although the sensory neurons expressing any given receptor can be broadly distributed across the olfactory epithelium, their axons converge on glomeruli (2) based on OR expression. The

glomeruli are the site of the first synapse in the olfactory system; the OSN axons form excitatory axodendritic synapses onto secondary projection neurons, mitral (3), and tufted cells (4), and onto a population of interneurons, the periglomerular cells (2). Periglomerular and granule cell (5) interneurons provide feed-forward and feed-back inhibition to the mitral and tufted cells via reciprocal dendrodendritic synapses. Like the OSNs, a process of ongoing adult neurogenesis continuously supplies the olfactory bulb with new interneurons (6) [63].

From the olfactory bulb, the mitral and tufted cell axons target neurons throughout olfactory cortices (7), such as piriform cortex, entorhinal cortex, and the olfactory tubercle, among others. The topographical specificity seen in the coalescence of sensory neuron axons into specific glomeruli is not evident in the cortical projections, which appear broad and diffuse. Beyond these primary olfactory cortices, odor information is passed broadly throughout the neuroaxis to additional cortical and subcortical targets.

Because odors are complex and composed of many potential ligands, the identification of odors is a combinatorial process. Most odors are believed to activate many different ORs based on several factors including concentration, and it is the collective pattern of activation that underlies coding/identification.

Olfactory epithelium – sensory input

Cellular mechanisms of aging in the olfactory epithelium

Alterations in the number of OSNs can affect olfactory function. Ablating the OSNs impairs odor detection/identification until ongoing neurogenesis replaces the lost neurons, a process less efficient in older mice [16].

Age-dependent decreases in OSNs may be accounted for by several mechanisms including: (i) environmental determinants; (ii) global and nonspecific changes in cell cycle such as an increase in the quiescent pool of basal cells in the epithelium with an accompanying decrease in the number of neurons; or (iii) a molecularly defined reduction in subpopulations of OSNs that express specific ORs.

Environmental determinants

Although the turnover of OSNs in animals housed under normal conditions occurs over the course of 6–12 weeks, housing in pathogen-free barrier conditions leads to very long-lived sensory neurons in both young and older rodents [17–19]. These data suggest that some changes in olfactory function may be cumulative over the life of the animal due to repeated harsh environmental exposure, the development of rhinitis, or bacterial infections [19].

Change in cell cycle

In perinatal rodents, neurogenesis in the olfactory epithelium is robust but declines by 90% between 3 and 16 months [16,20]. However, eliminating sensory neurons, either chemically or surgically, upregulates neurogenesis in the olfactory epithelium in both young and older mice [16]. These data suggest that despite an apparent quiescent period of neurogenesis in the aged mouse, sensory neuron replacement can be upregulated in the aged, albeit to a lesser degree than in younger mice [21,22].

The molecular mechanisms underlying the loss of sensory neurons remain poorly defined. Developmentally, the molecular cascades of OSN generation and the role of negative feedback in regulating those processes have been widely studied [23]. How those processes may change during aging has not been addressed. Analyses of the effect of telomere

shortening on proliferation of OSNs suggest two independent mechanisms in regulating olfactory epithelium neurogenesis [24]. Homeostatic maintenance of the epithelium was not affected in telomerase knockout mice but older knockout mice failed to show the injury-induced upregulation in neurogenesis. Knocking out Cdkn1a (cyclin-dependent kinase inhibitor 1a; also known as the cell cycle inhibitor p21), a cell cycle inhibitor, rescued the defect, suggesting that the telomere shortening acted by disrupting p21-dependent cell cycle progression. Therefore, the length of the telomere in aged mice is critical for the regeneration of the olfactory epithelium after lesions, but not for normal replacement of OSNs. In a related context, Getchell and colleagues reported a dysregulation of cell cycle-related genes in the olfactory epithelium of older senescenceprone mice [25]. Collectively, these provide alternative pathways that may contribute to the decrease in the generation of new OSNs during aging.

Although the generation of new OSNs in rodents decreases with aging, the evidence is controversial regarding cell death as measured by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, with a reported decrease in cell death in mice and an increase in rats [26,27]. Further studies are required but the mouse data suggests a stable equilibrium during aging whereas the data from rats is consistent with a decrease in OSNs [18,20]. Whether the molecular mechanisms underlying both neurogenic activity in the epithelium and apoptosis are cell autonomous or nonautonomous is unknown.

Reduction of molecularly defined subpopulations

OR gene expression varies significantly across development and aging. Custom microarrays, PCR and *in situ* analyses show individual variability in OR gene expression with peaks occurring between mouse postnatal day 14 and day 60, followed by steep declines for some receptors during aging [12,28,29]. Because the sensitivity of the sensory neurons that continue to express ORs in aged mice appears comparable to younger mice, it seems plausible that receptor copy number for individual cells is not affected during aging [30]. Further, analyses of mouse chromosomal location or subfamily assignment have not revealed any striking patterns related to aging [12]. Recently, an analysis of 531 mouse OR genes, using a NanoString platform, suggested that while some age-related changes were evident, as a whole changes in expression were minimal [31]. Although these recent data remain to be resolved with the earlier analyses, it continues to be interesting to speculate that the changes in OR expression may reflect the role(s) of specific odors at different points in the life cycle as well as environmental influences.

In summary, the number of OSNs in the epithelium declines during aging, most likely reflecting a decrease in the homeostatic maintenance of the sensory neurons by basal cell division. Although lesions inducing a coordinated loss of sensory neurons increase neurogenesis and sensory neuron replacement, it is less robust in the aged than in younger mice. Cell loss from the epithelium does not appear to be uniform. Subpopulations of sensory neurons expressing specific ORs may decrease in number, whereas others can remain stable during aging. The dynamic range and sensitivity of mouse sensory neurons expressing ORs does not diminish with aging, suggesting that the number of receptors/cells may not be affected.

Olfactory bulb – first-level processing, cell populations, and synaptic circuits

Although regions of the neocortex show evidence of atrophy with aging, data on the mouse olfactory bulb remains controversial with both decreases in volume and stability reported during aging [32–34]. Nevertheless, the proportion of the total volume accounted for by each of the olfactory bulb layers appears stable, suggesting that there may not be a disproportionate effect on subsets of cells or circuits.

Cell populations in the aged olfactory bulb

Neuronal populations in the aging mouse olfactory bulb appear stable. No significant changes have been reported for the numbers of NeuN-positive interneurons or Tbx21-positive mitral cells [33]. Molecular diversity among periglomerular cells is well known, but no significant age-related changes were found among those expressing calretinin, calbindin, or tyrosine hydroxylase, suggesting that numbers and proportions of periglomerular cell subpopulations are stable once established [33–35].

Newborn interneurons in the aged olfactory bulb

Fewer new adult-born neuroblasts reach the mouse olfactory bulb and differentiate into interneurons with aging; most likely due to a decrease in cell proliferation in the subventricular zone (SVZ), as discussed further below. There is, however, no significant age-related change in total numbers of interneurons in the mouse olfactory bulb [33]. Consequently, the reduced number of newborn neurons in aged animals must be compensated for by decreased interneuron loss in the olfactory bulb. Potential mechanisms include decreased cell death of existing interneurons and/or an increase in the survival of newborn cells that successfully migrate to the olfactory bulb. Consistent with these mechanisms, apoptotic cell death is suppressed in the aged olfactory bulb. There are fewer TUNEL-positive and cleaved caspase 3-positive cells in the mouse olfactory bulb at 24 months compared to 2 months, as well as a downregulation of procaspase3 [36,37]. Whether the suppression of cell death is caused by the reduction of neurogenesis or occurs independently in the aged olfactory bulb is an interesting question, but remains controversial. Further work is required to understand the dynamic regulation of SVZ proliferation and the stability of olfactory bulb interneuron populations [38–41].

Changing neuron morphology

Age-related changes in neuronal morphology are region and neuron specific throughout the brain (reviewed in [42]). In mouse adult-born periglomerular cells, there is an increase in total dendritic length and number of branching points between 0.5 and 1.5 months post-lentiviral injection (mpi) in SVZ at 2 months of age [43]. Dendritic length decreased during 3–11 mpi (animal age of 5–13 months), whereas the number of branching points remained stable. Interestingly, the dendritic structure of 1.5-mpi cells in 13-month old mice (newborn neurons in an old mouse) was significantly different from that of 1.5 mpi in 4-month old mice (newborn neurons in a young mouse), whereas it was more comparable to 11 mpi in 13-month old mice (old neuron in an old mouse). It appears that dendritic length of adult-born periglomerular cells decreases with age and is determined by the age of the animal more so than neuronal age. Because glomerular diameter is stable during aging, there may be compensatory mechanisms that modulate the effects of shortening periglomerular cell dendrites [33]. New insights into the age-related morphological changes in all olfactory bulb neurons, as well as OSN axons, are necessary.

Synaptic changes

Although the number of neurons in the mouse olfactory bulb is not profoundly affected by aging, there are significant layer-specific decreases in synapses. The number and diameter of glomeruli are stable across aging but the glomerular synapse density at 24 months is decreased to ~50% of that at 2 months [33]. In the glomerular neuropil, the apical dendrites of mitral/tufted and periglomerular cells receive axodendritic synapses from OSN axons and establish reciprocal dendrodendritic synapses among themselves. Both axodendritic and dendrodendritic synapse densities decreased with aging [33]. Because declines in synapse number are smaller than the decline in the number of sensory neurons in rats, compensatory remodeling of glomerular synapses has been proposed [18]. Although provocative, this

remains speculative and without direct measures of axonal arborization or glomerular synaptogenesis. By contrast, in the external plexiform layer the density of dendrodendritic synapses between mitral/tufted cells and granule cells was stable [33].

The effects of aging on glomerular synaptic circuits may reflect the decrease in OSNs innervating the glomeruli as well as the age-dependent decrease in the dendritic arbors of the periglomerular cells. The impact of a 50% reduction in glomerular synapses on odor processing is not clear. Following methyl bromide lesions of the nasal cavity, rodents with only 5% of the olfactory epithelium intact still had a 45% correct odorant identification (chance was equal to 20%) [44]. It seems that the most immediate effect of aging on processing of olfactory information occurs at the first synaptic relay in the glomeruli, with a preservation of downstream circuitry in the olfactory bulb. It will be important to determine if these effects reflect aging among specific subpopulations of sensory neurons that express the same OR, and are therefore specific to individual glomeruli.

Central adult neurogenesis

The aging SVZ

Throughout life new olfactory bulb interneurons are generated from stem cells in the SVZ [45]. The SVZ lines the dorsolateral walls of the lateral ventricles and consists of four cell types: Type A neuroblasts, Type B astrocytes, Type C transit-amplifying neural progenitor cells, and Type E ependymal cells. Type B cells give rise to Type C neural progenitor cells, which in turn produce Type A neuroblasts. Neuroblasts produced in the SVZ migrate in chains through the rostral migratory stream (RMS) to the olfactory bulb where they differentiate into either granule cells (95%) or periglomerular cells (5%) [46]. Interest in endogenous neural stem cell populations in the adult brain is high because they offer the hope of innovative therapeutic intervention and may be implicated in responses to traumatic brain injury [47,48].

Neurogenesis decreases in the mouse SVZ during aging, as shown by bromodeoxyuridine (BrdU) labeling [3,49]. BrdU incorporation decreases significantly (~50%) in Type B cells during aging, reflecting an increase in the Type B cell quiescent pool [50–52]. Atrophy occurs within the SVZ niche, including stenosis of the ventral lateral ventricle wall restricting neurogenesis to the dorsolateral region of the lateral ventricle [53,54]. The remaining ventricular cavity becomes enlarged and the ependymal monolayer becomes populated with cuboidal-shaped astrocytes that were once mitotically active [53]. Growth factors including epidermal growth factor, transforming growth factor- α , and peripheral growth hormone can rescue age-dependent decreases in proliferation in mice [3,55,56]. Additional mechanisms, including a downregulation of mammalian target of rapamycin (mTOR), cannabinoid signaling, or changing the systemic milieu, may also influence proliferation in the aged SVZ [57–60]. These studies suggest that the SVZ retains the potential for proliferation and that quiescent periods may be reversible. However, given the structural changes to the ventricle and the decrease in the size of the neurogenic niche, it is not clear how long rescued proliferation could be sustained. A correlation of molecular, neurogenic, and structural changes to the SVZ during normal aging will be important for understanding its potential neurogenic ability.

The aging RMS

The rodent RMS can be divided into three distinct regions: (i) the vertical arm descends from the SVZ and underlies the corpus callosum; (ii) the elbow curves rostrally towards the olfactory bulb at the base of the vertical arm; and (iii) the horizontal arm is the final rostral extension into the sub-ependymal zone of the olfactory bulb. The migratory path is defined by astrocytes that surround and interdigitate with the neuroblasts forming a tube-like

structure. The vasculature within the RMS is pervasive and neuroblasts use both the vasculature and astrocytes as a migratory scaffold [46,61–63]. Neuroblast migration occurs in longitudinally oriented chains requiring 10–14 days from the SVZ through the RMS to the olfactory bulb, a distance of ~5 mm in mice [46]. The importance of the RMS cytoarchitecture to neuroblast migration highlights the importance of further investigation; however, no age-related effects on the RMS cytoarchitecture have been reported [35].

During RMS migration, neuroblasts retain the ability to divide, although there is a decrease during aging that parallels that occurring in the SVZ [35,64]. Age-dependent decreases in cell division in the vertical arm suggest that the vertical arm may be more similar to the SVZ than the elbow, where proliferation decreases are not significant, or the horizontal arm, where uniform proliferation occurs across ages. Overall, the decreases in cell division in both the SVZ and RMS result in fewer migrating neuroblasts occupying a smaller area of the RMS. Despite a change in the density of migrating neuroblasts within the mouse RMS, migration efficacy does not appear to be affected because clearance of labeled neuroblasts from the RMS was not age dependent [35]. The downstream effect of decreased proliferation in both the SVZ and RMS is fewer adult-born interneurons in the olfactory bulb [35]. Because new adult-born neurons are strongly implicated in the acquisition of odor-cued associative tasks, decreases in this subpopulation of interneurons may contribute to age-related deficits in olfactory driven behaviors [4,6,65].

Olfactory cortex – second-level processing, cell populations, and synaptic circuits

The mitral and tufted projection neurons in the olfactory bulb make diffuse projections to nine cortical regions: the anterior olfactory nucleus, olfactory tubercle, anterior and posterior piriform cortex, lateral entorhinal cortex, the anterior and posterolateral cortices of the amygdaloid nuclei, the nucleus of the lateral olfactory tract, and tenia tecta [66]. The rule(s) governing the innervation of these regions by the olfactory bulb projection neurons is of widespread interest but thus far terminal fields appear diffuse and broadly spread without clear evidence of an anatomically or functionally defined topography [66,67].

Data is especially sparse on normal nonpathological effects of aging on olfactory cortices. In the rat anterior olfactory nucleus, neuropeptide Y neurons decrease significantly with age [68]. Studies of entorhinal cortex in rodents have focused on its role in learning and memory without any special attention on olfaction. However, it is notable that the neurofibrillary tangles associated with Alzheimer's disease are found very early in the entorhinal cortex of mouse models [69–71]. The literature using mouse models of Alzheimer's and other neurodegenerative diseases to assess olfactory function has shown a strong correlation of amyloid-beta burden and tau aggregates with olfactory dysfunction [15,72]. Similarly, decreases in Reelin expression, a glycoprotein implicated in synaptic plasticity, and in the number of Reelin-positive neurons in rat entorhinal cortex correlates with cognitive declines [71]. By contrast, aggregates of Reelin are found in rodent models of Alzheimer's disease suggesting that Reelin expression can have profound effects on cognitive measures, including olfaction, due to over- or under-expression [70].

Beyond its role in generating new olfactory bulb inter-neurons, adult neurogenesis has also received attention in relation to olfactory cortices during aging. For example, in aged rhesus monkeys, a significant decrease was found in doublecortin-positive cells (a marker of migrating neurons) in the amygdala and entorhinal cortex [73]. In mice a small number of adult-born neurons originating in the SVZ migrate to the olfactory tubercle and give rise to granule neurons in the Islands of Calleja, dense clusters of mostly GABAergic cells that extend rostrocaudally throughout most of the tubercle [74]. From 4 to 18 months the number

of neurons in the tubercle decrease significantly with a related increase in autophagosomes that regulate cellular homeostasis by degrading defective and long-lived proteins/organelles [75,76]. These data suggest the possibility of disequilibrium in aged neurons and ensuing cell death and are an important example of aged-related dysfunction in higher-order olfactory centers.

There has been a recent focus on the piriform cortex in olfactory processing in young and adult rodents [77–81]. Although the anterior and posterior piriform cortices are poised as the next frontier of aging olfactory research, data remains sparse for the present [82]. One recent study reports a loss of synaptic AMPA receptors in aging anterior piriform cortex suggesting there may be subtle changes in glutamatergic synaptic function [83]. These data help to underscore the dearth of knowledge about aging in olfactory cortices and provide a springboard for future studies. To date, the tenia tecta and nucleus of the lateral olfactory tract have not been included in any study of normal aging, suggesting additional opportunities for future research.

Concluding remarks and future directions

It is apparent that the mechanisms underlying aging-related deficits in olfactory acuity, discrimination, or sensitivity remain to be determined (Box 2). Although incremental age-associated changes are recognized in neurogenesis in both the epithelium and SVZ, neither these nor the changes in synapse number seem sufficient to fully account for behavioral changes occurring during normal aging.

Box 2

Olfactory cognition

Aging-related changes occurring in the olfactory epithelium and olfactory bulb do not seem sufficient to account for the reports of decreased cognitive olfactory function and odor perception in the elderly. Conflicting reports from human studies argue that the number and odor selectivity of OSNs may decline during aging [7,85] whereas others suggest that odor detection thresholds are less affected by age [86,87]. There is, however, broad agreement that in humans, odor identification and memory decreases significantly, particularly in the seventh and eight decades and beyond. This raises the possibility that age-related declines in olfactory function may predominate in higher centers, including those involved in multisensory integration [88,89]. Support for this notion comes from diverse findings. For example, work from the Wilson lab has demonstrated the multisensory integration of both auditory and olfactory information in the olfactory tubercle, a region recently reported to show age-related losses of GABAergic neurons [75,90]. The Wilson lab also recently reported on the importance of ‘top-down’ processing in the piriform cortex during odor processing [91]. The lateral entorhinal cortex, a region strongly implicated in cellular changes during aging, regulated regional processing of activity in piriform cortex [71]. In a complementary study, the Sobel lab demonstrated changes in the cognitive identification of odors with aging whereas odor detection remained unchanged [92]. These data implied that peripheral function, that is initial odor detection, was not impaired whereas the higher order integration of odor coding and discrimination was subject to the influences of aging. This is consistent with the work of Lee *et al.* (2009) showing that the responses of individual OSNs from mouse did not change during aging [30]. Similarly, Murphy *et al.* (2000) failed to find a significant change in odor thresholds during aging whereas both the N1/2 and the P3 component of the electroencephalogram (EEG) both showed deficits in amplitude and latency suggesting changes in cognitive processing [93].

These data contrast with the auditory system where age-related declines in function are associated with a decrease in detection due to loss of cochlear hair cells (for a review, see [94]). Similarly, the somatosensory system shows deficits in sensitivity related to a loss of sensory receptors in the skin (for a review, see [95]).

Much work remains to be done on the development of a fundamental understanding of odor representation in primary olfactory cortices and beyond, during development, adulthood, and aging. However, the data thus far suggest that hypotheses related to changes in cognitive olfactory function due to normal aging may be addressed most productively beyond the initial processing centers in the olfactory epithelium and bulb.

Many questions remain unanswered (Box 3). For example, although the number of synapses decrease in the glomeruli, it is not clear if this is due to a loss of sensory neurons or decreased axonal branching. Moreover, the strength and plasticity of the glomerular synapses has not yet been studied. Perhaps the most promising horizon is found in the olfactory cortices. Because olfaction depends upon a combinatorial scheme for odor coding, it may be that the more complex integrations occurring in the primary olfactory cortices and beyond will provide new clues about mechanisms operative during olfactory system aging. Given the potential contribution of adult neurogenic mechanisms in the olfactory system, and that the size of the aged population is rapidly increasing, identifying and understanding these mechanisms is imperative.

Box 3

Outstanding questions

- What mechanisms regulate changes in the cell cycle of olfactory epithelium basal cells or SVZ stem cells?
- Are there changes in the number of ORs inserted into the membrane of aged versus young OSNs?
- How do epigenetic mechanisms and the environment affect the nasal cavity and trafficking of odorants to the epithelium?
- How is the discrepancy in measures of OR gene expression resolved?
- Does aging differentially affect subpopulations of OSNs expressing specific ORs?
- Do odor maps in the olfactory bulb change during aging?
- What accounts for the decrease in glomerular layer synapses in the aged mouse?
- Do compensatory changes in olfactory circuitry compensate for declines in adult neurogenesis or synapses?
- Can the contribution of adult-born neurons from the SVZ be regulated during aging based on experience?
- Does the preferential effect of aging on complex odor tasks suggest that deficiencies are occurring at higher order centers and not during initial detection and processing?
- What changes, if any, occur in the olfactory cortices during aging?

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Glossary

Dendrodendritic synapses	synapses occurring between two dendrites. Held in contrast to axodendritic synapses that are polarized from an axonal bouton onto a dendrite
Glomerulus	spherical neuropil containing the terminal fields of OSNs and the apical dendrites of mitral, tufted, and periglomerular cells. The glomerular neuropil is compartmentalized into regions containing either the axodendritic synapses of OSNs or the local circuit dendrodendritic synapses between mitral/tufted cell dendrites and the periglomerular cell dendrites. Each glomerulus is molecularly specific in that it receives afferent input from OSNs expressing the same OR
Interneurons	periglomerular and granule cells, the inhibitory neurons of the olfactory bulb both function to inhibit mitral cells via feed-forward and feedback reciprocal dendrodendritic synapses
Mitral and tufted cells	the primary projection neurons found in the olfactory bulb and receiving synapses from OSNs
Odorant receptor (OR)	a 7-transmembrane G-protein coupled receptor that binds molecular features of odorants with moderate selectivity. Binding of an odorant initiates a signaling cascade that opens cyclic nucleotide-gated channels and an action potential. There are ~1200 different ORs identified thus far in the main olfactory epithelium of mice
Olfactory cortex	a general term that can include any regions receiving input from the primary projection neurons in the olfactory bulb
Olfactory sensory neuron (OSN)	a bipolar neuron whose apical dendrite arborizes into multiple cilia at the surface of the olfactory epithelium within the lumen of the nasal cavity. The cilia are surrounded by mucous. A thin 0.2 μ m unmyelinated axon extends from the basal pole of the OSN and travels as part of the olfactory nerve to the olfactory bulb, without branching until it arborizes within 1 of the ~3700 glomeruli in the olfactory bulb
Subventricular zone (SVZ)	a developmental remnant of the specialized proliferative epithelia surrounding the lateral ventricles. Located on the lateral wall, it is composed of stem cells, intermediate progenitor cells, neuroblasts, and glia. In contrast to most ventricular regions, the SVZ continues to generate new interneurons, periglomerular and granule cells, in the adult. These migrate to the olfactory bulb via an extremely well delineated pathway, the rostral migratory stream

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Figure I. Olfactory circuits. Abbreviations: OE, olfactory epithelium; GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; GRL, granule cell layer; RMS, rostral migratory stream; OC, olfactory cortex.