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Stable olfactory sensory neuron *in vivo* physiology during normal aging

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Abstract

Normal aging is associated with a number of smell impairments that are paralleled by age-dependent changes in the peripheral olfactory system, including decreases in olfactory sensory neurons (OSNs) and in the regenerative capacity of the epithelium. Thus, an age-dependent degradation of sensory input to the brain is one proposed mechanism for the loss of olfactory function in older populations. Here, we tested this hypothesis by performing *in vivo* optical neurophysiology in 6, 12, 18, and 24 month-old mice. We visualized odor-evoked neurotransmitter release from populations of OSNs into olfactory bulb glomeruli, and found that these sensory inputs are actually quite stable during normal aging. Specifically, the magnitude and number of odor-evoked glomerular responses were comparable across all ages, and there was no effect of age on the sensitivity of OSN responses to odors or on the neural discriminability of different sensory maps. These results suggest that the brain's olfactory bulbs do not receive deteriorated input during aging and that local bulbar circuitry might adapt to maintain stable nerve input.

Keywords

Aging; Olfactory sensory neuron; Olfactory bulb; Optical neurophysiology; Sensory plasticity; Mouse models

1. Introduction

Despite the widespread prevalence of olfactory impairments in aging populations (Doty et al., 1984; Hummel et al., 2007; Murphy et al., 2002), the underlying neurosensory mechanisms are largely unknown. It has been hypothesized that a loss of peripheral input might underlie some of these olfactory deficits since aging has been associated with a reduced regenerative capacity of the peripheral olfactory system (Brann and Firestein, 2014) and a decreased density of olfactory sensory neurons (OSNs) in the epithelium (Apfelbach et al., 1991; Lee et al., 2009). However, OSN axon projections to the olfactory bulb seem to be

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Disclosure Statement

The authors have no conflicts of interest to disclose.

Appendix A. Supplementary Data

Supplementary data associated with this article can be found in the Supplementary Materials.

preserved during aging (Richard et al., 2010), and studies addressing age-related functional changes in olfactory circuitry have yielded mixed results. For instance, one report identified functional changes in odor tuning of individual OSNs during aging (Rawson et al., 2012), even though odor receptor gene expression remains relatively stable (Khan et al., 2013) and individual OSNs maintain their dynamic range (Lee et al., 2009). An examination of OSN function at the population level is needed to determine if the brain's olfactory bulbs are receiving deteriorated sensory inputs from OSNs during normal aging. While the first synapse in the olfactory system is not easily accessible in humans, in mice, the olfactory bulbs are anterior to the prefrontal cortex and directly below the surface of the skull, rendering them (and their primary sensory afferents) optically accessible *in vivo*. Thus, we utilized a mouse model to assess how normal aging affects primary sensory inputs to the brain's olfactory bulbs, and visualized odor-evoked neurotransmitter release from populations of OSNs into olfactory bulb glomeruli *in vivo* in mice ranging from 6–24 months of age.

2. Materials and Methods

We used 37 mice ($N_{\text{male}} = 19$; $N_{\text{female}} = 18$) on a mixed C57BL/6 and 129SvJ background that expressed synaptophysin (spH) under the control of the olfactory marker protein (OMP) promoter (Bozza et al., 2004). Fluorescence signals in these mice indicate cumulative neurotransmitter release from OSN terminals into olfactory bulb glomeruli. Subjects were either 6 ($N = 7$), 12 ($N = 14$), 18 ($N = 7$), or 24 ($N = 9$) months of age, which roughly corresponds to 20, 40, 60, and 80 years of age in humans (Dutta and Sengupta, 2016). *In vivo* optical neurophysiology was performed as previously described (Czarnecki et al., 2011; Kass et al., 2017; Kass et al., 2013a; Kass et al., 2013b), and as detailed in the Supplementary Materials and Methods. All procedures were approved by the Rutgers University IACUC.

3. Results

To evaluate age-dependent changes in OSN function, we performed *in vivo* optical neurophysiology in 6–24 month old OMP-spH mice (Figure 1A–D). SpH provides a relatively slow signal in response to discrete odor presentations, with the peak response across most glomeruli occurring around the time of odor offset (Figure 1E). While peak response times varied slightly depending on the stimulus being presented (Supplementary Figure S1), they did not differ between age groups (Figure 1F–G, non-significant effect of age, $F_{(3, 33)} = 1.26$, $p = 0.30$, $\eta_p^2 = 0.10$). Further, there were no age-dependent effects on the peak odor-evoked response amplitudes (F_s , Figure 1H, non-significant effect of age, $F_{(3, 33)} = 1.69$, $p = 0.19$, $\eta_p^2 = 0.13$) or the number of glomeruli receiving OSN input during the peak response (Figure 1A–D and 1I, non-significant effect of age, $F_{(3, 32)} = 0.09$, $p = 0.97$, $\eta_p^2 = 0.01$). These negative results were not an artifact of using an absolute change in fluorescence (ΔF) as our response measure because we ensured equal resting fluorescence across subjects (Figure 1J–N, non-significant effect of age, $F_{(3, 33)} = 1.75$, $p = 0.18$, $\eta_p^2 = 0.14$), and did not find any effects of age in control analyses that were performed on odor-evoked $\Delta F/F$ values (Supplementary Figure S2). Further, there were no differences in respiration between age groups (Supplementary Figure S3).

Odor sensitivity can decline in aging populations (Apfelbach et al., 1991; Hummel et al., 2007), so we next assessed OSN response sensitivity across a 4-fold range of concentrations (Figure 1O–T). Increasing the concentration resulted in an increase in both the peak F (Figure 1S, main effect of concentration, $F_{(2, 66)} = 432.03$, $p < 0.001$, $\eta_p^2 = 0.93$) and the number of glomeruli that were recruited into the response maps (Figure 1T, main effect of concentration, $F_{(1.2, 39.2)} = 109.55$, $p < 0.001$, $\eta_p^2 = 0.77$), as expected. However, these concentration-dependent increases were comparable across all 4 age groups (non-significant age $\times$ concentration interactions: Figure 1S, $F_{(6, 66)} = 0.82$, $p = 0.56$, $\eta_p^2 = 0.07$; Figure 1T, $F_{(3.7, 39.2)} = 0.22$, $p = 0.97$, $\eta_p^2 = 0.02$).

The above analyses were performed on static response maps, so we wondered if there were more subtle age-dependent changes in OSN physiology that went undetected. We looked at the time course in which odor-evoked spH signals became decorrelated from baseline fluorescence (Figure 2A–F), as any such differences could be related to age-associated changes in odor detection abilities. Activity that occurred during early response times was weaker in magnitude than activity that occurred later (Figure 2A–D, pre-peak versus peak), but regardless of age, it was still nonetheless discriminable from pre-odor baseline (Figure 2A–D, base versus pre-peak). To confirm this, we calculated the correlation between odor-evoked spH signals and baseline fluorescence on a frame-by-frame basis (Figure 2E), and found no differences between 6–24 month old mice (Figure 2F, non-significant effect of age, $F_{(3, 33)} = 1.14$, $p = 0.35$, $\eta_p^2 = 0.09$).

Finally, we asked if aging could alter the discriminability of OSN synaptic output because deficits in odor discrimination and identification abilities have been observed in older populations (Doty et al., 1984; Murphy et al., 2002). We visualized activity that was stimulated by a pair of ester odors that might be challenging to discriminate (Figure 2G–O; methyl valerate, MV versus ethyl valerate, EV), and found that these odors drove OSN input to highly overlapping, but distinct, populations of olfactory bulb glomeruli (Figure 2G–M). Notably, the proportion of glomeruli that either responded to 1 (MV-responsive or EV-responsive) or both (dual-responsive, examples in Figure 2J–L) of these esters was the same across all age groups (Figure 2M, $\chi^2 = 2.21$, $p = 0.14$). Despite the high degree of spatial overlap, the sensory maps that were evoked by these 2 esters were still somewhat discriminable from each other since the Euclidean distance (ED) between their activity patterns gradually increased over time (Figure 2N, significant effect of time, $F_{(56, 1512)} = 202.28$, $p < 0.001$, $\eta_p^2 = 0.87$). However, there was no difference between age groups in the rate of this increase (Figure 2N, non-significant age $\times$ time interaction, $F_{(112, 1512)} = 0.52$, $p = 1.00$, $\eta_p^2 = 0.04$) or in the overall discriminability of these sensory maps averaged over time (Figure 2O, non-significant effect of age, $F_{(2, 27)} = 0.17$, $p = 0.85$, $\eta_p^2 = 0.01$). We also confirmed and extended these null results by analyzing sensory maps that were evoked by a relatively less-similar pair of ester odors that might be easy to discriminate (Supplementary Figure S4).

4. Discussion

Here, we performed *in vivo* optical neurophysiology on 6–24 month old mice and found no effects of age on several measures of OSN function. There was no difference between age

groups in the latency to onset of peak evoked responses, suggesting that OSN response dynamics are stable during normal aging. Further, there was no effect of age on total neurotransmitter release as the number of odor-responsive glomeruli, and the magnitudes of their peak responses were comparable across ages. The sensitivity of the system to detecting odors also appears to remain constant because there were no differences between age groups in concentration-response functions or in the time that it took for odor-evoked nerve output to become decorrelated from pre-odor baseline activity. Additionally, there were no age-dependent changes in the spatial overlap of glomerular activity that was evoked by chemically-related odors or in the overall neural discriminability of different sensory maps.

Together, these negative findings suggest that the brain's olfactory bulbs may not receive deteriorated sensory input during normal aging. This is perhaps surprising given the age-dependent structural changes that are known to occur in the olfactory epithelium of similarly aged animals (Apfelbach et al., 1991; Brann and Firestein, 2014; Lee et al., 2009). However, the effects of aging on the olfactory system can vary across different strains of mice (Mirich et al., 2002), and some evidence suggests that the anatomy of the olfactory epithelium remains relatively stable in aged rodents that are housed in a laboratory setting, presumably due to minimal exposure to harsh environmental factors and reduced rates of rhinitis (Hinds et al., 1984; Loo et al., 1996). Therefore, it is possible that we did not observe an effect of aging on odor-evoked OSN synaptic output because the structure of the olfactory epithelium may have been relatively stable. An examination of the olfactory epithelium would be necessary to determine whether or not the mice used here exhibit the age-dependent changes in epithelial structure that have been reported in other strains that are housed in similar conditions. While it is possible that the epithelium may have remained relatively intact in these 6–24 month old mice, local circuits in the olfactory bulb can dynamically shape the function of OSNs, so an alternative possibility is that bulbar circuitry adapts to maintain stable sensory inputs from the aging nose. Indeed, the proliferation and death of olfactory bulb interneurons is reduced in aged relative to young animals (Mirich et al., 2002), and aged animals also exhibit a proportional reduction of primary afferent synapses and local circuit synapses in the glomerular layer (Richard et al., 2010). While such age-dependent alterations in bulbar circuitry might ensure sensory fidelity, it is possible that they may also lead to perturbations in downstream signal processing. Thus, age-associated olfactory impairments may be related to changes in higher-order neural circuits that mediate olfactory-driven percepts and behaviors.

While we did not observe any evidence of age-dependent deficits in OSN function, it remains unclear if OSN function would be similarly preserved during normal aging in humans, or how these negative results would relate to the compromised olfactory abilities that can manifest in older adults. First, there may be a disconnect between human and mouse aging effects on olfaction since aging-related diseases such as Alzheimer's disease (AD) are associated with a number of olfactory impairments in humans, whereas mouse models do not consistently identify AD-related olfactory deficits (Phillips et al., 2011; Xu et al., 2014). Second, the olfactory system has some notable organizational differences between mice and humans, including differences in odor receptor gene expression, the number of olfactory bulb glomeruli that receive input from OSNs, and the absolute and relative sizes of the epithelium and olfactory bulb (McGann, 2017), and these organizational differences may

interact with aging effects in different ways. Finally, it is possible that sensory impairments during aging in humans are confounded by the inclusion of people who suffer from a pre-clinical disorder as “normal” subjects. If this were true, then there may actually be less instances of olfactory dysfunction in normal, aging adults, in which case one might expect OSN function to remain relatively stable during normal aging, as we observed in the present experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Odor-evoked synaptic output from OSNs into olfactory bulb glomeruli is relatively stable during normal aging.

(**A-D**) Resting light intensity (RLI) images (top) and pseudocolored difference maps (bottom) from a representative subject in each age group. (**E**) Mean±SEM fluorescence records corresponding to the glomerular callouts in **A-D**. The boxed region notes the approximate frames that were used for peak response maps (**A-D**) and analyses (**H-I**). Difference maps (**A-D**) and response amplitudes (ΔF ; **E**) are averaged across 4 trials of 15 au 2M2B. (**F-G**) The latency (in sec) to onset of peak odor-evoked responses is shown for distributions of latency values pooled across individual ΔF s (**F**) and for group means±SEMs calculated across individual subjects (**G**). (**H-I**) The mean±SEM odor-evoked ΔF (**H**) and number of glomerular responses (**I**) is shown for each age group. Data are pooled across 3

concentrations of each of 4 odors in **F-I**. (**J-M**) LED levels were adjusted prior to each imaging session to ensure equal RLIs across subjects. These images show RLIs from representative subjects in each age group. All 4 images are scaled relative to the overall maximum RLI (in mV) across subjects, with individual maximums indicated in the top left corner of each image. (**N**) There was no difference in the mean $\pm$ SEM resting fluorescence through the cranial window across age groups. (**O-R**) Difference maps (top) show patterns of activity that were evoked by the highest concentration (in arbitrary units, au) of 2HEX in each representative subject. The corresponding sets of traces (bottom) show the F_s that were evoked in each sample glomerulus across a 4-fold range of concentrations of 2HEX. Boxed regions note the approximate frames that were used to generate response maps (top) and calculate concentration analyses (**S-T**). Maps (top) and traces (bottom: solid lines $\pm$ shading, mean $\pm$ SEM) are averaged across 4 repeated trials. (**S-T**) Mean $\pm$ SEM concentration response functions showing the odor-evoked F (**S**) and the number of evoked responses (**T**) pooled across 4 odors for each age group. In **G-I** and **N** individual subjects are represented by dots. The example subjects shown in **A-D**, **J-M**, and **O-R** are represented by red, green, and yellow dots, respectively.

Figure 2. No effects of aging on the sensory detection of discretely presented odors or on the discrimination of highly similar sensory maps.

(A–D) Pseudocolor maps show the average (4 trials, 15 au *n*-BA) of 1 sec of pre-odor baseline activity (left, base), 1 sec of activity about half-way through the odor presentation (middle, pre-peak), and 1 sec of activity centered on the approximate peak of the odor-evoked response (right, peak) from representative subjects in each age group. Maps are scaled relative to the overall maximum across time bins per subject. (E) The correlation between *n*-BA-evoked spH signals and baseline fluorescence is averaged across 4 trials (solid lines $\pm$ shaded areas, means $\pm$ SEMs) and shown relative to the time of odor presentation for each of the example subjects in A–D. Boxed regions note the frames that were used to generate the maps in A–D. (F) Mean $\pm$ SEM summary plot that is pooled across frames and odors to demonstrate that odor-evoked OSN activity was equally decorrelated from background noise in all groups. (G–L) Difference maps (G–I) and corresponding fluorescence traces (J–L) that were evoked by a pair of similar ester odors (MV and EV) in representative subjects ranging from 6–24 months of age. Chemical structures are shown in the top right corner of maps. (M) Proportion of glomeruli per age group that received input from OSNs that were stimulated by just one ester from the pair (EV- or MV-selective) or that were stimulated by both esters (dual-responsive). (N) Frame-by-frame ED between MV- and EV-evoked spH signals from the representative subjects in G–I. Inset shows the mean $\pm$ SEM

ED between ester odors collapsed across frames per subject. (**O**) Mean $\pm$ SEM summary plot that is pooled across frames to demonstrate that the overall discriminability between MV- and EV-evoked sensory maps was equivalent across age groups. In **F** and **O** individual subjects are represented by circles that are plotted immediately to the right of each group mean. The example subjects shown in **A-D** and **G-I** are represented by red and yellow dots, respectively.

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