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Respiratory and sniffing behaviors throughout adulthood and aging in mice

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Abstract

Orienting responses are physiological and active behavioral reactions evoked by novel stimulus perception and are critical for survival. We explored whether odor orienting responses are impacted throughout both adulthood and normal and pathological aging in mice. Novel odor investigation (including duration and bout numbers) and its subsequent habituation as assayed in the odor habituation task were preserved in adult C57BL/6J mice up to 12mo of age with <6% variability between age groups in investigation time. Separately, using whole-body plethysmography we found that both spontaneous respiration and odor-evoked sniffing behaviors were strikingly preserved in wildtype (WT) mice up to 26mo of age. In contrast, mice accumulating amyloid- β protein in the brain by means of overexpressing mutations in the human amyloid precursor protein gene (APP) showed preserved spontaneous respiration up to 12mo, but starting at 14mo showed significant differences from WT. Similar to WTs, odor-evoked sniffing was not impacted in APP mice up to 26mo. These results show that odor-orienting responses are minimally impacted throughout aging in mice, and suggest that the olfactomotor network is mostly spared of insults due to aging.

Keywords

Olfaction; Neurodegeneration; Alzheimer's disease; amyloid-beta; APP; orienting; respiration

1. Introduction

The active investigation of a stimulus normally follows its initial detection. In olfaction, detection of a novel odor evokes a modulation in respiration (sniffing) and perhaps a turning of your head to reposition your nose to facilitate inhaling the stimulus. These stimulus

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orienting responses were a focus of some of the earliest analyses of behavior, including that by Ivan Pavlov [1] who described orienting responses as:

“[The] reflex which brings about the immediate response in man and animals to the slightest changes in the world around them, so that they immediately orientate their appropriate receptor organ in accordance with the perceptible quality in the agent bringing about the change, making full investigation of it.”

More simply put, orienting responses can be considered any physiological and behavioral reactions to novel stimulus perception and can include changes in autonomic tone (e.g., heart-rate, galvanic skin resistance) or more overt changes in stimulus sampling behavior or arousal. Orienting responses, and even the habituation of these responses [2], play a vital role in ensuring appropriate environmental interactions and thus, understanding the neural control and modulation of orienting responses is of great importance.

In olfaction, odors are actively sampled with the dynamic respiratory behavior commonly referred to as ‘sniffing’. At rest, mice [3] and rats [4-6] respire between 1-4Hz. However, upon detection of a novel odor, mice and rats rapidly initiate a novel odor orienting response involving high frequency (>4Hz) stereotyped sniffing and positioning of their snout towards the perceived direction of the odor [3, 7]. In mice the fast sniffing odor-orienting response is very robust, representing an approximately 75% increase in basal inhalation frequency which is reliably displayed across odors [3]. Studies measuring odor investigation defined as snout-oriented sniffing in the context of an odor habituation task report similar ability of novel odors to evoke odor orienting responses [8]. The transition from a resting respiratory pattern into high frequency sniffing upon detection of a novel odor can occur very rapidly [9], even less than the time required for a rat or mouse to discriminate highly conditioned odors [3, 10-13]. Thus odor orienting behaviors are highly stereotyped and reflect a simple form of odor response.

Olfaction is modulated by age and throughout the course of age-related diseases [14-16]. We hypothesized that one mechanism whereby aging and age-related disorders may impact olfactory perception is through disruption in odor orienting responses. Both normal and pathological aging (such as that observed in Alzheimer’s disease, Parkinson’s disease, and Lewy-body disorder) entail normal cognitive decline which in some instances may be precipitated by abnormal protein aggregation, oxidative damage and neuroinflammation [17]. These factors contribute to widespread dysfunction of brain networks [18, 19] which may be required for proper odor orienting responses. Indeed, deficient odor orienting and/or stimulus sampling responses could contribute to the reported age-related olfactory deficits in both humans [15, 16, 20, 21] and rodents [22-25] as suggested by work showing impairments in sniffing in Parkinson’s disease [26]. Therefore, in the present study we investigated whether odor orienting responses in mice are impacted throughout both adulthood and normal and pathological aging (i.e., that co-occurring with Alzheimer’s-like Amyloid- β deposition).

2. Materials & Methods

2.1 Subjects

This study used 2 separate groups of mice. First, 42 adult male C57BL/6J (B6) mice were used in odor habituation tests to examine whether there were age-dependent changes across adulthood (2-12mo) in odor investigation behavior at 2 ($n=8$), 4 ($n=8$), 6 ($n=8$), 8 ($n=6$), 10 ($n=6$), and 12mo of age ($n=6$). Original B6 breeder stock were purchased from Jackson Laboratory (Bar Harbor, ME). Second, 44 Tg2576 mice [on the B6SJLF1/J background (WT, Jackson Laboratory), generated previously by overexpressing the human KM670/671NL mutation [27]] and 44 age-matched non-transgenic litter-mate mice (WT)

were used as controls, all in cohorts ranging from 2 to 21-26mo of age (Table 1). All mice were bred and maintained within the Nathan S. Kline Institute for Psychiatric Research animal facility and were only used at one age each. Mice were maintained on a 12:12 (light:dark) day cycle (light: 0600-1800hrs) in standard plastic cages with corn cob bedding and all testing performed during the daylight phase (0800-1700hrs). Access to food and water was maintained *ad libitum*, except during behavioral tests. Mice were genotyped by PCR analysis of tail DNA using standard methods. Experiments were conducted in accordance with the guidelines of the National Institutes of Health and were approved by the Nathan S. Kline Institute's Institutional Animal Care and Use Committee.

2.2 Odor habituation assay

Habituation task design—B6 mice were screened for odor investigation behavior using an odor habituation test design [8, 28] as we adapted recently [25]. Testing took place during the light phase of the animals' (12:12) day:night cycle (0800-1700hrs), over two daily sessions separated by 24hrs. Home cages were cleaned with fresh corn cob bedding 24 hrs prior to behavioral testing. Odors (n=6) were applied to a cotton-applicator stick which was then enclosed in a piece of odorless plastic tubing to prevent contact of the liquid odor with the testing chamber or animal yet still allowing volatile odor delivery. Odors were delivered for 4 successive trials (1 block), 20sec each, separated by 30sec inter-trial intervals, by inserting the odor stick into a port on the side of the animal's home cage. Sequential presentation of each odor in a cross-habituation paradigm allowed habituation to each odor and provided robust novel (trial #1) odor investigation behavior measures upon the presentation of the next odor stimulus. The duration of time spent investigating, defined as snout-oriented sniffing within 1 cm of the odor presentation port, was recorded across all trials by a single observer blind to age groups (A.V.W.).

Odor selection—Stimuli included (+)-limonene, (–)-limonene, eugenol, acetic acid, propionic acid, and a 3-part mixture (MIX) [29, 30] containing 4% acetic acid, 48% eugenol, and 48% (+)-limonene. All odors were diluted 1×10^{-2} in mineral oil or ddH₂O (acetic acid). These diverse odors were selected based upon previous experience [25, 30] and with the intent that they would stimulate a variety of odorant receptor types and therefore post-synaptic neurons and reduce the probability of findings directed at particular odor-types (e.g., just acids).

Odor presentation paradigm—The odor delivery paradigm was initially designed to assess not only investigation and habituation to basic odors, but also to a 3-part mixture over repeated blocks as a rough index of intermediate-term (5-10 min) odor memory, and to structurally similar odors for a basic index of fine odor discrimination. To this end, on day 1 of testing mice were successively presented with the following odors: MIX → eugenol → MIX → (+)-limonene → (–)-limonene and on Day 2 of testing mice were presented with MIX → acetic acid → propionic acid. However, no effects of age on these measures of intermediate-term odor memory or cross-habituation during fine odor discriminations were found. Thus we simply selected to pool data across six odors, using the first presentation of MIX on day 1, eugenol, (+)-limonene, (–)-limonene, acetic acid and propionic acid as odors for all analyses.

2.3 Respiration recordings

Measurements of respiration (sniffing) were performed with a commercially available mouse plethysmograph system to provide whole body analysis (Buxco Research Systems). Medical grade breathable air (3L/min) provided refreshing of the recording chamber (~10cm diameter cylinder) air throughout all recordings. 10min of spontaneous respiration data was collected following 5min of acclimation to the recording chamber. Following this we

presented (+)-limonene (1×10^{-3} dilution in mineral oil) for an assessment of odor-evoked sniffing. A piece of filter paper was saturated with 100 μ l of liquid odor and placed into a 3ml syringe. The syringe was connected to a 3-way stop-cock to allow manual presentation of 3ml odor-saturated air which was gradually expunged into a mixing manifold next to the recording chamber. Chamber air became infiltrated with odor within 1.5 - 2sec from the onset of expunging the syringe. Odor presentation events were recorded digitally along with respiration data. Plethysmograph activity was relayed into a PC running Spike2 (Cambridge Electronic Design, Cambridge) for data acquisition and for logging stimulus events (odor presentations). Data were filtered on-line from 0.1-100Hz and later off-line filtered to eliminate high frequency artifacts (>60Hz band stop).

2.4 Data analysis

Behavior—For analysis of odor habituation task data, all raw investigatory values (sec) were pooled within animals and organized according to odor presentation (trial) number. First, as a measure of odor orientation, the durations of all trial #1 odor investigations were averaged within each subject, pooled within each age group and analyzed using 2-way ANOVAs for independent groups followed by post-hoc group comparisons with Fisher's PLSD. We performed this comparison across all trial #1 odor investigations (all inclusive) and separately following exclusion of trial#1's when the animal did not investigate at all (0sec) (nonresponders). To analyze sampling bouts of the odor (epochs separated by moments when the mouse directed its snout away from the odor port or even navigated away) we averaged the # of bouts during trial #1 (omitting nonresponders trials) within each mouse and then pooled the data within age groups for ANOVA followed by post-hoc comparisons with Fisher's PLSD. In both cases, the trial #1 values were averaged within each subject. Later, across data from trials #1-4 assuming the animal investigated to trial #1, we performed a measure of odor habituation using a repeated measured ANOVA across trials with age as a factor. % habituation scores (calculated within odors as the amount of habituation by trial #4 as a function of the maximum investigation time '1') were averaged across odors within individual subjects, pooled within age groups, and analyzed with ANOVA followed by post-hoc comparisons with Fisher's PLSD.

Respiration—Plethysmograph (respiratory or sniffing) data were analyzed off-line in Spike2 following an initial 100Hz low-pass filtering. Respiratory events were automatically detected through wavemark composition. To ensure that events were not missed, the wavemark data were manually inspected. For analysis of spontaneous and odor-evoked sniffing, the mean frequency of the data was calculated off of the wavemarks (0.5sec interpolation). The mean frequency was extracted (20Hz sampling rate) 1) across the entire data file (for spontaneous sniffing data), 2) during the 5sec prior to odor presentation, and 3) during the odor presentation (2.5sec – 7.5sec relative to odor onset). Odor response sniff magnitudes were calculated by dividing the –2.5 – 2.5sec pre odor by the 2.5 – 7.5sec during odor and converted into '% change'. These epochs were selected to best account for the approximate arrival of the odor into the recording chamber (taking ~2sec as described above). Both spontaneous and odor-evoked respiration data were pooled within age-groups (each subject contributing 1 value) and analyzed for effects with ANOVA followed by Fisher's PLSD. Inter-breath-interval values during spontaneous respiration were also extracted for additional analysis (10ms bins) and analyzed with Kolmogorov-Smirnov tests for distributions.

Statistics & Reporting—All statistical tests were performed in StatVIEW with the default level of significance set at $p < 0.05$. Unless otherwise noted, data are expressed as the mean value $\pm$ S.E.M.

3. Results

3.1 B6 mice display preserved novel odor investigation behavior from 2-12mo of age

We began our investigation into the possible age-related changes across adulthood in odor orienting responses by examining novel odor investigation behavior in 2-12mo adult B6 mice throughout performance in the odor habituation task [8]. In this task the first presentation (trial #1) of each individual odor allows it to be classified as a ‘novel’ stimulus [25]. Unlike in a different task [8] wherein the odor was puffed into a small jar or in our previous work on mice from a B6J background [25], we found variability in the tendency of B6 mice to approach the port for odor investigation. In some cases (114/250 total trials, 45.6%) mice did not investigate a given novel odor at all on trial #1. Such non-responsiveness may be attributable to the hedonics of these odors, modulating attractivity to approach. However, the display of these ‘nonresponder’ trials was not significantly greater in any adult age group ($F(5,190) = 1.774, p = 0.1201$) nor for any particular odor ($F(5,38) = 1.026, p = 0.4162$). Indeed, analysis of responses to novel (trial #1) odors in comparison to investigation times of the preceding odors’ trial #4, revealed that across all ages, mice responded to all odors, even those serving as components within the MIX with $\geq 200\%$ increase in investigation time (minimum average increase / odor). Therefore, to determine whether the amount of time investigating novel odors depends on age, we incorporated analysis of time investigating across all trial #1 odor presentations (Figure 1A) and separately, following omission of the non-responsive trials (Figure 1B). While there was slight variability in the average time spent sniffing novel odors throughout the age groups, ANOVA failed to reveal an effect of age (up to 12mo) in either the all inclusive ($F(5,25) = 0.382, p = 0.856$) or exclusive data set ($F(5,25) = 0.393, p = 0.8492$). Instead, with just 0.8sec average maximum variation between 2-12mo of age (the difference between 4 and 8mo, all inclusive), adult B6 mice spent similar amounts of time sniffing novel odors. Furthermore, this relative consistency across ages did not seem to be differentially impacted by odor identity. Investigation times of individual odors did not significantly change across adult age ($p > 0.05$, ANOVA within each odor) (Figure 1C).

In addition to the time spent investigating odors (Figure 1A-C), the number of investigation bouts (sampling epochs separated by moments when the mouse directed its snout away from the odor port or even navigated away) may be important as investigation bouts may serve as indices of arousal or activity. However, there was no effect of age (up to 12mo) on the number of investigation bouts displayed towards odors ($F(5,25) = 0.566, p = 0.7254$) (Figure 1D).

3.2 Preserved odor habituation behavior from 2-12mo of age in B6 mice

Habituation of orienting responses is an important trait reflecting adaptation to environmental stimuli [2, 31]. Therefore, we next examined whether there are differences in odor habituation behavior throughout adulthood from 2-12mo in B6 mice using the same behavioral data as above (Figure 1), but in this case examining investigation behavior across all trials (1→4). For this analysis all blocks beginning with a non-responsive trial #1 were eliminated. Repeated measures ANOVA from data across trials 1-4 ($n=136$ total blocks, mean = 22.6 ± 2.8 blocks / age group) revealed an overall effect for all groups to habituate to successive odor presentations ($F(3,405) = 243.868, p < 0.0001$) (Figure 2), with surprisingly little variation between groups. Indeed, while 6mo old mice habituated qualitatively less than the other age groups, there were no group effects in the total % habituation by trial #4 ($F(5,25) = 0.838, p = 0.5351$) (Figure 2, inset). Taken together, these results suggest that odor orienting responses and their habituation are preserved throughout adulthood (2-12mo) in B6 mice.

3.3 APP, but not WT mice show changes in spontaneous sniffing behavior throughout aging

While our investigations into odor orienting behaviors in the odor habituation task failed to reveal an effect of age during adulthood (up to 12mo), we wished to explore odor orienting behaviors in older mice and also mice expressing pathological accumulation of the A β protein – a common feature of aging in humans, especially in the context of Alzheimer's disease [32]. We also wanted to provide a more direct measure of odor orienting behaviors, versus that supplied by manually counting snout-oriented investigatory sniffing as done in the odor habituation task. Therefore, here we measured spontaneous and odor-evoked sniffing behavior of mice overexpressing human mutations of the amyloid precursor protein gene (APP) [27] and their wild-type (WT) littermates (BJL background), from 2-26mo of age, in a whole-body plethysmograph. Recent work from our group [25] has shown that these APP mice first display non-fibrillar A β deposition within the olfactory bulb at 3mo of age, where after 6mo of age, fibrillar A β deposits are evident. After 9mo of age, fibrillar and non-fibrillar A β deposits are found in the hippocampus, neocortex (including the olfactory cortex), orbitofrontal cortex, and entorhinal cortex [25]. The spatiotemporal pattern of A β deposition across these structures correlates with the magnitude of olfactory behavioral dysfunction as measures in the odor habituation test [25]. Given the detrimental impact of A β on synaptic and neural network function and behavior [33, 34], we predicted that especially old APP mice (≥ 14 mo), corresponding with known elevations in A β deposition, should show atypical sniffing behavior in comparison to young APP mice or age-matched WT mice.

We started with a measure of spontaneous respiration in wildtype (WT) and APP mice between 2-26mo of age (Table 1). We recorded 10min of spontaneous respiration while mice explored within the plethysmograph recording chamber. As described previously [3], we found the respiratory behavior of mice in this context to be highly dynamic, consisting of rapid transitions between slow respiration (2-5Hz) and fast, exploratory sniffing bouts (6-14Hz) lasting at times for several seconds (see Figure 3A). Extracting the mean frequency of all respiratory events for each mouse throughout this spontaneous data, we found that the display of spontaneous respiration is preserved within a fairly narrow frequency band (~ 5.5 - 6.5 Hz, group averages) throughout aging (Figure 3B). We failed to find an effect of age on spontaneous respiration frequency in WT mice ($F(7,28) = 1.998$, $p = 0.0912$), with the average frequency of 2mo mice only differing from that of 21-26mo mice by 0.37Hz (Figure 3B). In contrast, APP mice displayed a significant effect of age on spontaneous respiration frequency ($F(7,35) = 2.564$, $p = 0.0305$) (Figure 3C). While only within the 10mo age group were there enough mice ($n \geq 3$) of each sex to perform a sex comparison, no effect of sex on spontaneous respiratory frequency was observed within either WT ($F(1,4) = 0.180$, $p = 0.6934$) or APP mice ($F(1,6) = 3.984$, $p = 0.093$) at this age. Interestingly, the respiration of APP mice throughout age formed a quasi u-shaped distribution reflecting fast sniffing (~ 6.25 Hz) between 2-6mo, slower (~ 5.75 Hz) sniffing between 8-12mo, and then a return to faster spontaneous respiratory rates later in life (>14 mo).

We performed a closer inspection of respiration frequency dynamics in these groups by analyzing the percentage of respiratory events falling within different frequency bands (Figure 3D). We took advantage of previous work in rats [6, 35] which showed dramatic modulation of olfactory bulb responses during respiratory frequencies <4 Hz or >4 Hz to allow simple classification of respiratory behavior as either slow or fast sniffing respectively. Across all ages, WT mice spent 88.4 – 91.0% of their time respiring >4 Hz. APP mice spent 84.8 – 97.1% time respiring >4 Hz, showing slightly greater variability throughout age in comparison to WT mice. The display of slow frequency respirations was highly

uncommon in both genotypes across ages, with no group showing more than 0.44% of their respirations <2Hz in this paradigm.

While respiration was mostly consistent throughout aging, the % time analysis uncovered changes in spontaneous respiration frequency distributions between WT and APP mice starting at 14mo and continuing into 21-26mo ($p < 0.05$, Kolmogorov-Smirnov tests of distributions within individual age cohorts) (Figure 3D). Particularly, in 21-26mo mice this genotype effect is attributable to a heightened occurrence of high frequency (>4Hz) sniffing (>97% of all sniffs were >4Hz) in APP mice which resulted in significantly less sniff events between 2-4Hz ($p < 0.005$, 2-tailed t-test) and <2Hz ($p < 0.005$, 2-tailed t-test). Thus, while the frequency of spontaneous respiration is mostly preserved in WT and APP mice throughout the course of aging, subtle differences between genotypes emerge later in life (>14mo).

3.4 Odor-evoked sniffing frequency is maintained throughout aging in both WT and APP mice

Immediately following spontaneous respiration recordings, we administered a single presentation of the odorant (+)-limonene (see Methods) and measured odor-evoked sniffing behavior. As described previously [3], novel odor presentation evoked an abrupt increase in respiration frequency from spontaneous levels to a high frequency, stereotyped sampling behavior called sniffing (Figure 4A). As a simple index of odor-evoked sniffing, we compared the % change (relative magnitude) in sniffing frequency during odors (5sec stimulus compared to 5sec baseline, see Methods) across ages and between WT vs. APP mice (Figure 4B). We found that the magnitude of odor evoked sniffing was independent of age in both WT ($F(7,36) = 0.575$, $p = 0.7712$) and APP mice ($F(7,36) = 1.154$, $p = 0.3526$), however, with perhaps subtle differences occurring between particular groups (e.g., 2 v. 4mo and 14 v. 21-26mo APPs, Figure 4B). Further, there was no effect of genotype on odor evoked sniffing behavior ($F(1,86) = 0.456$, $p = 0.5013$). Additionally, while only within the 10mo age group were there enough mice ($n \geq 3$) of each sex to perform a sex comparison, no effect of sex on odor-evoked sniffing was observed within either WT ($F(1,4) = 0.778$, $p = 0.427$) or APP mice ($F(1,6) = 0.901$, $p = 0.379$) at this age. Thus, while spontaneous respiratory frequency in APP mice may differ with age, these data demonstrate the relative preservation of odor-evoked sniffing behavior in both APP and WT mice.

4. Discussion

In our previous work [25] we found that APP mouse odor habituation behavior was significantly impaired early in life (at just 6mo of age). While not the focus of that study, age-matched WT mice showed no age-dependent changes in odor investigation or habituation. Here we sought to provide a more detailed analysis of odor orienting behaviors throughout adulthood and aging in mice, focusing mostly on WT behavior. We used two different paradigms and found, surprisingly, that odor orienting behaviors including snout-directed odor investigation, the habituation of snout-directed odor investigation, spontaneous respiration and odor-evoked sniffing are all preserved throughout normal aging in B6 and B1J mice. The frequencies of spontaneous respiration and odor-evoked sniffing were also mostly preserved during aging in APP transgenic mice, however with slight differences between WT and APP mice observed starting at 14mo of age. These findings show that the basic display of odor-orienting responses is not as vulnerable to age-related insults as more cognitively-demanding olfactory behaviors (e.g., odor-learning and discrimination) and suggest a preservation of the necessary olfactomotor network function during adulthood and aging.

Sniffing behavior overcomes known age-related synaptic alterations

Sniffing behaviors are considered critical in modulating the coding of odors in the brain [36] and ultimately to olfactory perception itself [37, 38]. Specifically, the frequency of sniffing has received considerable attention in terms of how frequency shifts during odor investigation may shape odor information processing [6, 35, 39–41] and aid in decision making [42]. Thus, age-dependent changes in sniffing frequency may entail serious consequences for olfactory behaviors. Here we found that the frequency of both spontaneous exploratory and odor-evoked sniffing itself is preserved throughout normal aging in mice up to 26mo. These results suggest that differences in sniffing itself does not contribute to previous reports of olfactory behavior deficits in aged rodents [23, 43–49], even in the context of age-related pathological accumulation [22, 24, 25]. Thus, the olfactory deficits in most rodent models studied to date likely stem from central impairments in processing, rather than changes in peripheral stimulus sampling behaviors. This is further supported by recent work in humans with Alzheimer's disease [50] and Parkinson's disease [51, 52] and in work from our group in APP mouse models [25].

The present results show that, despite the likely anatomical changes occurring in the B6 and B7L mice used herein, and the known pathological accumulation of A β throughout the olfactory system in the APP mice [25], adult and aged mice can still perform the most basic act of odor investigation with only slight differences in comparison to young, near-adolescent mice (at 2mo). When combined with work from other labs studying early development [53], these results suggest a robust maintenance of odor-evoked sniffing (sampling behavior) from birth through senescence. Relatedly, our results also suggest that aging does not seriously impact arousal or attraction for odors – an essential requirement in orienting responses. Indeed, all age-groups equally investigated a variety of novel odors, including terpenes, acids, and phenylpropanoids within similar durations and with similar numbers of bouts. It is interesting to consider that the shift in sniff frequency distributions observed in mice >14mo of age (Fig 4) may reflect a modulation in sampling strategies with age, perhaps due to changes in odor hedonic coding. More precise examinations of odor hedonics in aged rodents, including disease models, will be interesting in light of understanding whether reward circuitry function becomes impacted throughout age or disease progression. One possibility would be to examine habituation to biologically-relevant odors (food, conspecific urine) or odors without direct biological relevance (e.g., isoamyl acetate or methyl valerate) among young and aged groups. Alternatively, more precision may be offered within operant tasks wherein odor-directed motivation can be experimentally controlled and response latencies towards odors can be analyzed.

The present work did not provide an analysis of how the odor investigation behaviors of the sexes may be differentially impacted throughout aging. Indeed, olfactory perception (detection and discrimination) is differentiated in mice [54, 55] and in humans [56, 57] based upon sex and endocrine status. Here, only within one age group (10mo old mice) were there sufficient numbers of both sexes to perform a sex comparison. While in this case no sex differences were uncovered in odor investigation, future work employing larger sample sizes of both sexes throughout adulthood and aging will be important in understanding the impact of endocrine status and sex on odor investigation and habituation.

Conclusion

These results show that aging, even up to 26mo of age in a disease model, does not seriously impact odor-evoked orienting responses in mice. Future studies exploring the olfactomotor circuit underlying odor-orienting responses in mice may therefore provide unique understandings regarding how these behaviors can normally occur despite aging and pathological accumulation.

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References

- [1]. Pavlov, IP. *CONDITIONED REFLEXES: AN INVESTIGATION OF THE PHYSIOLOGICAL ACTIVITY OF THE CEREBRAL CORTEX*. Oxford University Press; London: 1927.
- [2]. Sokolov, EN.; Vinogradova, OS. *Neuronal mechanisms of the orienting reflex*. L. Erlbaum Associates; Hillsdale, N.J.: 1975. distributed by the Halsted Press Division of Wiley
- [3]. Wesson DW, Donahou TN, Johnson MO, Wachowiak M. Sniffing behavior of mice during performance in odor-guided tasks. *Chem Senses*. 2008; 33:581–96. [PubMed: 18534995]
- [4]. Kepecs A, Uchida N, Mainen ZF. Rapid and precise control of sniffing during olfactory discrimination in rats. *J Neurophysiol*. 2007; 98:205–13. [PubMed: 17460109]
- [5]. Youngentob SL, Mozell MM, Sheehy PR, Hornung DE. A quantitative analysis of sniffing strategies in rats performing odor discrimination tasks. *Physiol Behav*. 1987; 41:59–69. [PubMed: 3685154]
- [6]. Verhagen JV, Wesson DW, Netoff TI, White JA, Wachowiak M. Sniffing controls an adaptive filter of sensory input to the olfactory bulb. *Nat Neurosci*. 2007; 10:631–9. [PubMed: 17450136]
- [7]. Welker WI. Analysis of sniffing in the albino rat. *Behavior*. 1964; 22:223–44.
- [8]. Sundberg H, Doving K, Novikov S, Ursin H. A method for studying responses and habituation to odors in rats. *Behavioral and Neural Biology*. 1982; 34:113–9. [PubMed: 7073634]
- [9]. Wesson DW, Carey RM, Verhagen JV, Wachowiak M. Rapid encoding and perception of novel odors in the rat. *PLoS Biol*. 2008; 6:e82. [PubMed: 18399719]
- [10]. Rinberg D, Koulakov A, Gelperin A. Speed-accuracy tradeoff in olfaction. *Neuron*. 2006; 51:351–8. [PubMed: 16880129]
- [11]. Abraham NM, Spors H, Carleton A, Margrie TW, Kuner T, Schaefer AT. Maintaining accuracy at the expense of speed: stimulus similarity defines odor discrimination time in mice. *Neuron*. 2004; 44:865–76. [PubMed: 15572116]
- [12]. Slotnick B. Odor-Sampling Time of Mice under Different Conditions. *Chem Senses*. 2007; 32:445–54. [PubMed: 17426048]
- [13]. Uchida N, Mainen ZF. Speed and accuracy of olfactory discrimination in the rat. *Nat Neurosci*. 2003; 6:1224–9. [PubMed: 14566341]
- [14]. Doty RL, Shaman P, Applebaum SL, Giberson R, Sikorski L, Rosenberg L. Smell identification ability: Changes with age. *Science*. 1984; 226:1441–3. [PubMed: 6505700]
- [15]. Meshulam RI, Moberg PJ, Mahr RN, Doty RL. Olfaction in Neurodegenerative Disease: A Meta-analysis of Olfactory Functioning in Alzheimer's and Parkinson's Diseases. *Arch Neurol*. 1998; 55:84–90. [PubMed: 9443714]
- [16]. Murphy C. Loss of Olfactory Function in Dementing Disease. *Physiology & Behavior*. 1999; 66:177–82. [PubMed: 10336141]
- [17]. Herrup K. Reimagining Alzheimer's Disease--An Age-Based Hypothesis. *J Neurosci*. 2010; 30:16755–62. [PubMed: 21159946]
- [18]. Andrews-Hanna JR, Snyder AZ, Vincent JL, Lustig C, Head D, Raichle Marcus E, et al. Disruption of Large-Scale Brain Systems in Advanced Aging. *Neuron*. 2007; 56:924–35. [PubMed: 18054866]
- [19]. Sperling R, Laviolette P, O'Keefe K, O'Brien J, Rentz D, Pihlajamaki M, et al. Amyloid deposition is associated with impaired default network function in older persons without dementia. *Neuron*. 2009; 63:178–88. [PubMed: 19640477]
- [20]. Knupfer L, Spiegel R. Differences in olfactory test performance between normal aged, Alzheimer and vascular type dementia individuals. *International Journal of Geriatric Psychiatry*. 1986; 1:3–14.
- [21]. Wilson RS, Schneider JA, Arnold SE, Tang Y, Boyle PA, Bennett DA. Olfactory identification and incidence of mild cognitive impairment in older age. *Arch Gen Psychiatry*. 2007; 64:802–8. [PubMed: 17606814]

- [22]. Montgomery KS, Simmons RK, Edwards Iii G, Nicolle MM, Gluck MA, Myers CE, et al. Novel age-dependent learning deficits in a mouse model of Alzheimer's disease: Implications for translational research. *Neurobiology of Aging*. 2009 In Press, Corrected Proof.
- [23]. Schoenbaum G, Nugent S, Saddoris MP, Gallagher M. Teaching old rats new tricks: age-related impairments in olfactory reversal learning. *Neurobiology of Aging*. 2002; 23:555–64. [PubMed: 12009505]
- [24]. Guérin D, Sacquet J, Mandairon N, Jourdan F, Didier A. Early locus coeruleus degeneration and olfactory dysfunctions in Tg2576 mice. *Neurobiology of Aging*. 2009; 30:272–83. [PubMed: 17618708]
- [25]. Wesson DW, Levy E, Nixon RA, Wilson DA. Olfactory Dysfunction Correlates with β -Amyloid Plaque Burden in an Alzheimer's Disease Mouse Model. *J Neurosci*. 2010; 30:505–14. [PubMed: 20071513]
- [26]. Sobel N, Thomason ME, Stappen I, Tanner CM, Tetrad JW, Bower JM, et al. An impairment in sniffing contributes to the olfactory impairment in Parkinson's disease. *Proc Natl Acad Sci U S A*. 2001; 98:4154–9. [PubMed: 11259673]
- [27]. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Yonkin S, et al. Correlative memory deficits, A β elevation, and amyloid plaques in transgenic mice. *Science*. 1996; 274:99–102. [PubMed: 8810256]
- [28]. Cleland TA, Morse A, Yue EL, Linster C. Behavioral models of odor similarity. *Behav Neurosci*. 2002; 116:222–31. [PubMed: 11996308]
- [29]. Laing DG, Francis GW. The capacity of humans to identify odors in mixtures. *Physiol Behav*. 1989; 46:809–14. [PubMed: 2628992]
- [30]. Kadohisa M, Wilson DA. Separate encoding of identity and similarity of complex familiar odors in piriform cortex. *Proc Natl Acad Sci U S A*. 2006; 103:15206–11. [PubMed: 17005727]
- [31]. Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, et al. Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. *Neurobiology of Learning and Memory*. 2009; 92:135–8. [PubMed: 18854219]
- [32]. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol*. 1991; 82:239–59. [PubMed: 1759558]
- [33]. Wesson DW, Nixon RA, Levy E, Wilson DA. Mechanisms of Neural and Behavioral Dysfunction in Alzheimer's disease. *Molecular neurobiology*. in press.
- [34]. Palop JJ, Mucke L. Amyloid- β -induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. *Nat Neurosci*. 2010; 13:812–8. [PubMed: 20581818]
- [35]. Carey RM, Verhagen JV, Wesson DW, Pirez N, Wachowiak M. Temporal Structure of Receptor Neuron Input to the Olfactory Bulb Imaged in Behaving Rats. *J Neurophysiol*. 2009; 101:1073–88. [PubMed: 19091924]
- [36]. Wachowiak, M. *The Neurobiology of Olfaction*. CRC Press; 2009. Active Sensing in Olfaction; p. 305-28.
- [37]. Mainland J, Sobel N. The sniff is part of the olfactory percept. *Chem Senses*. 2006; 31:181–96. [PubMed: 16339268]
- [38]. Laing DG. Natural sniffing gives optimum odour perception for humans. *Perception*. 1983; 12:99–117. [PubMed: 6657430]
- [39]. Wesson DW, Verhagen JV, Wachowiak M. Why Sniff Fast? The Relationship Between Sniff Frequency, Odor Discrimination, and Receptor Neuron Activation in the Rat. *J Neurophysiol*. 2009; 101:1089–102. [PubMed: 19052108]
- [40]. Cury KM, Uchida N. Robust Odor Coding via Inhalation-Coupled Transient Activity in the Mammalian Olfactory Bulb. *Neuron*. 2010; 68:570–85. [PubMed: 21040855]
- [41]. Bathellier B, Buhl DL, Accolla R, Carleton A. Dynamic Ensemble Odor Coding in the Mammalian Olfactory Bulb: Sensory Information at Different Timescales. *Neuron*. 2008; 57:586–98. [PubMed: 18304487]
- [42]. Uchida N, Kepecs A, Mainen ZF. Seeing at a glance, smelling in a whiff: rapid forms of perceptual decision making. *Nat Rev Neurosci*. 2006; 7:485–91. [PubMed: 16715056]

- [43]. Nakayasu C, Kanemura F, Hirano Y, Shimizu Y, Tonosaki K. Sensitivity of the olfactory sense declines with the aging in senescence-accelerated mouse (SAM-P1). *Physiology & Behavior*. 2000; 70:135–9. [PubMed: 10978488]
- [44]. Apfelbach R, Russ D, Slotnick B. Ontogenetic changes in odor sensitivity, olfactory receptor area and olfactory receptor density in the rat. *Chem Senses*. 1991; 16:209–18.
- [45]. Patel RC, Larson J. Impaired olfactory discrimination learning and decreased olfactory sensitivity in aged C57Bl/6 mice. *Neurobiology of Aging*. 2009; 30:829–37. [PubMed: 17904696]
- [46]. Mandairon N, Peace ST, Boudadi K, Boxhorn CE, Narla VA, Suffis SD, et al. Compensatory responses to age-related decline in odor quality acuity: Cholinergic neuromodulation and olfactory enrichment. *Neurobiology of Aging*. 2010 In Press, Corrected Proof.
- [47]. Rui DSP, Luciano CB, Reinaldo NT. Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats: Involvement of adenosine A1 and A2A receptors. *Neurobiology of Aging*. 2005; 26:957–64. [PubMed: 15718055]
- [48]. Gilbert PE, Pirogovsky E, Brushfield A, Luu TT, Tolentino J, Renteria A. Age-related Changes in Associative Learning for Olfactory and Visual Stimuli in Rodents. *Annals of the New York Academy of Sciences*. 2009; 1170:718–24. [PubMed: 19686218]
- [49]. LaSarge C, Montgomery K, Tucker C, Slaton GS, Griffith W, Setlow B, et al. Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. *Neurobiology of Aging*. 2007; 28:928–36. [PubMed: 16806587]
- [50]. Li W, Howard JD, Gottfried JA. Disruption of odour quality coding in piriform cortex mediates olfactory deficits in Alzheimer's disease. *Brain*. 2010; 133:2714–26. [PubMed: 20724290]
- [51]. Wattendorf E, Welge-Lussen A, Fiedler K, Bilecen D, Wolfensberger M, Fuhr P, et al. Olfactory Impairment Predicts Brain Atrophy in Parkinson's Disease. *J Neurosci*. 2009; 29:15410–3. [PubMed: 20007465]
- [52]. Moessnang C, Frank G, Bogdahn U, Winkler J, Greenlee MW, Klucken J. Altered Activation Patterns within the Olfactory Network in Parkinson's Disease. *Cereb Cortex*. 2011 Epub ahead of print.
- [53]. Seelke AM, Blumberg MA. Sniffing in infant rats during sleep and wakefulness. *Behavioral Neuroscience*. 2004; 118:267–73. [PubMed: 15113250]
- [54]. Wesson DW, Keller M, Douhard Q, Baum MJ, Bakker J. Enhanced urinary odor discrimination in female aromatase knockout (ArKO) mice. *Horm Behav*. 2006; 49:580–6. [PubMed: 16448653]
- [55]. Sorwell KG, Wesson DW, Baum MJ. Sexually dimorphic enhancement by estradiol of male urinary odor detection thresholds in mice. *Behav Neurosci*. 2008; 122:788–93. [PubMed: 18729632]
- [56]. Dalton P, Doolittle N, Breslin PA. Gender-specific induction of enhanced sensitivity to odors. *Nat Neurosci*. 2002; 5:199–200. [PubMed: 11865309]
- [57]. Good PR, Geary N, Engen T. The effect of estrogen on odor detection. *Chemical Senses and Flavor*. 1976; 2:45–50.

Research Highlights

- We explored whether odor orienting responses are impacted throughout aging.
- B6 mice showed <6% variability with age in odor investigation duration.
- Spontaneous sniffing frequency was impacted starting at 14mo in APP but not WT.
- Odor-evoked sniffing frequency was conserved up to 29mo of age in WT and APPs
- The olfactomotor network is likely spared of age-related insults in these models.

Fig. 1. Adult B6 mice display preserved novel odor investigation behavior from 2-12mo of age (A) Average time B6 mice ($n \geq 6$ /group) spent investigating novel odors when accounting for all trials ('all inclusive', $n=35-48$ trials/group) and (B) after excluding trials wherein the mouse did not investigate the odor at all ('nonresponders', $n=17-36$ trials/group). (C) Average time investigating within age groups (same data as in (A)) organized by odor identity. (D) Average number of sniff bouts during the course of each odor presentation (same trials as in (B)). No effects of age were found in any of the above measures of odor investigation.

Fig. 2. Preserved odor habituation behavior in B6 mice from 2-12mo of age

Odor habituation behavior in B6 mice (same data as used in Fig 1), but plotting all 4 trials throughout the stimulus block). Also displayed (inset) is the average ‘% habituation’ by trial #4 as a function of the maximum investigation duration to the odor. While all groups significantly habituated to novel odors ($p < .0001$, repeated measures ANOVA), no significant differences in % habituation were found between any age-group.

Fig. 3. Spontaneous respiratory behavior in WT and APP mice from 2-26mo of age
(A) Example respiration traces from plethysmograph recordings in WT (wildtype) and APP (amyloid precursor protein) transgenic mice. Traces from young (2mo, top) and old (26mo, bottom) mice of the same sex are shown (band pass filtered 0.1-55Hz). **(B)** Mean spontaneous respiration in WT and **(C)** APP mice (separate age cohorts, not longitudinal). * $p < 0.05$, ANOVA, WT vs. APP group effect. Dashed line = major gridline. **(D)** Respiration frequency distributions showing the % of respiration events within frequency bands (same mice as in (B) and (C)). Values above distributions represent mode frequencies in WT (black) and APP mice (gray). * $p < 0.05$, WT v. APP, 2-sample Kolmogorov-Smirnov tests).

Fig. 4. Odor-evoked respiration ('sniffing') behavior is not impacted by aging in WT or APP mice up to 26mo of age

(A) Example odor-evoked sniffing in a 26mo WT mouse and mean sniffing frequency relative to odor onset. Raster represents individual sniffs detected in Spike2. Gray horizontal line = time of odor presentation (solid portion represents time period 2.5 – 7.5sec post odor onset used for sniff magnitude analysis in (B)). (B) Odor-evoked sniff magnitudes (% change relative to pre-odor) in WT (wildtype) and APP (amyloid precursor protein) transgenic mice.

Table 1

Mice for sniffing recordings

Age (mo.)	WT (M:F)	APP (M:F)
2	5 (3:2)	5 (1:4)
4	5 (2:3)	5 (4:1)
6	5 (4:1)	7 (5:2)
8	8 (1:7)	5 (3:2)
10	6 (3:3)	8 (4:4)
12	4 (0:4)	6 (1:5)
14	5 (2:3)	5 (2:3)
21-26	6 (2:4)	3 (3:0)

All mice were on a B6J background.

WT = Wildtype

APP = Amyloid precursor protein (Tg2576).

+/- 1 weeks of age (mo.) specified.

(M:F) Male:Female ratio.