ELSEVIER

Neurobiology of Aging 30 (2009) 829–837

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Impaired olfactory discrimination learning and decreased olfactory sensitivity in aged C57Bl/6 mice

Roseanne C. Patel, John Larson *

Psychiatric Institute, Department of Psychiatry, College of Medicine, 1601 W. Taylor St., M/C 912, University of Illinois at Chicago, Chicago, IL 60612, USA

Received 16 May 2007; received in revised form 27 July 2007; accepted 13 August 2007
Available online 29 September 2007

Abstract

Young (4 months) and old (24 months) C57Bl/6J mice were tested in an automated simultaneous-cue, two-odor discrimination task. The mice were first pre-trained to execute trial-structured nose poke responses in a straight alley. They were then trained to criterion on a series of eight novel olfactory discrimination problems. Old mice required slightly more shaping sessions to acquire the nose poke response. The old mice required many more sessions and made 70% more errors than young mice before reaching criterion performance on the series of eight olfactory discrimination problems. Young and old mice did not differ in retention of the last odor discrimination when tested 2 weeks after training. Old mice had significantly higher thresholds for discriminating ethyl acetate vapor from non-odorized air. The results suggest that mice may be a good model for study of olfactory dysfunction and cognitive deficits with aging.

Keywords: Olfaction; Learning; Memory; Odor discrimination; Mouse; Olfactory learning

1. Introduction

Impairments in the sense of smell are common in human aging (Cain and Stevens, 1989; Doty et al., 1984). Age-related olfactory deficits include loss of sensitivity to odors (Stevens and Dadarwala, 1993), diminished discrimination ability, reduced recognition (Murphy et al., 1991), impaired smell identification (Doty et al., 1984), and problems with episodic memory for odors (Larsson and Backman, 1998). Whereas deficits in detecting odorants may be due to peripheral deterioration of the sensory epithelium, other impairments seem to involve degenerative processes affecting cognitive processing of odors in the brain. Age-related neurodegenerative diseases also have a negative impact on olfactory capabilities: in some cases these are probably due to general sensory and cognitive impairments; however others, including Alzheimer's and Parkinson's diseases, appear to involve selective pathology in specific brain structures involved in olfactory processing (Kovacs, 2004).

The neurobiological changes underlying age-related decline in olfactory function have not been extensively investigated. There is evidence that olfactory event-related potentials change with age (Geisler et al., 1999) and neuropathology is observed in the olfactory epithelium and olfactory bulb of elderly humans. The rodent olfactory system has served as a very useful model system for studying development and plasticity due to its anatomical organization (Brunjes and Frazier, 1986). However, relatively few studies have exploited these advantages for studies of normal aging (Mirich et al., 2002). Additionally, olfaction and odor-guided behaviors may be particularly useful for studying aspects of learning and cognition in rodents (Slotnick, 2001; Eichenbaum, 1998). The olfactory system has direct interconnections with hippocampus, prefrontal cortex, and amygdala (Lynch, 1986) and these connections are well-developed in rodents (Eichenbaum, 1998). Rats and mice readily learn large numbers of simple odor discriminations, show excellent long-term memory for odors, and show higher-order learning phenomena such as learning sets for odors (Bodyak and Slotnick, 1999; Lu et al., 1993; Slotnick et al., 1991, 2000b; Slotnick and Katz, 1974; Staubli et al., 1987; Larson

* Corresponding author. Tel.: +1 312 413 4572; fax: +1 312 413 4569.
E-mail address: jrlarson@uic.edu (J. Larson).

0197-4580/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2007.08.007

and Sieprawska, 2002). Thus odor-guided behavioral tasks may be useful for understanding age-related changes in not only the sense of smell, but cognition and memory as well. However, there have been relatively few reports of olfactory function or odor-guided learning in aging rodents. Some studies have found deficits in odor detection with aging (Nakayasu et al., 2000; Kraemer and Apfelbach, 2004) whereas others have not (Schoenbaum et al., 2002; Lasarge et al., 2007). Simple discrimination learning has been found to be impaired in some studies (Roman et al., 1996; Frick et al., 2000; Prediger et al., 2005; Lasarge et al., 2007) but not in others (Kraemer and Apfelbach, 2004; Schoenbaum et al., 2002). One study found that aging rats were selectively impaired at reversal learning (Schoenbaum et al., 2002).

In the present study, young and old mice of the C57Bl/6J strain were trained in a series of two-odor, simultaneous-cue, olfactory discrimination problems. An automated training procedure was used to measure the rate of learning. The mice were also tested for long-term memory of a learned discrimination 2 weeks after training. Finally, a task involving discrimination between clean air and varying concentrations of ethyl acetate vapor was used to determine olfactory thresholds in the young and old mice.

2. Materials and methods

2.1. Subjects

Subjects were C57Bl/6J mice aged 4 months (YOUNG) or 24 months (OLD) obtained from colonies maintained by NIA. They were housed in groups of three or four in plastic cages in a climate-controlled animal colony on a normal 14:10 light:dark cycle. The mice were maintained on a water deprivation schedule with access to 1.0–2.0 mL water once per day for at least 5 days prior to and throughout training. This schedule reduced body weight by about 20% in the first few days but maintained the mice at a stable weight throughout the study. Seven old mice were eliminated from study due to general health issues (physical inactivity or sluggishness, apparent tumors or skin lesions, poor grooming) or poor responses to water restriction (excessive weight loss). Two additional old mice failed to perform well in nose poke training and were likewise eliminated from the study. This left a total of 24 young and 15 old mice subjected to the entire odor discrimination training procedure.

All testing was done during the light phase.

2.2. Apparatus

As described previously (Larson and Sieprawska, 2002), the testing chamber was made of black acrylic and consisted of a straight alley 60 cm long and 10 cm wide. The two side (long) walls sloped upward and outward at an angle of 15° off vertical and were 30 cm high. The end walls were vertical. At each end ("East" and "West") of the alley were two cylindrical "sniff ports" (1.5 cm i.d.) for nose poke responses (2 cm from the floor and centered 5 cm apart) and a single small cup in the floor for water delivery. The two sniff ports at the West end of the alley were connected to individual air dilution olfactometers for odor stimulus delivery; all of the sniff ports were equipped for photobeam detection of nose pokes. Water delivery was controlled by electrically driven, teflon-body solenoid valves (General Valve Co., Fairfield, NJ); a microcomputer (PC) detected infrared photobeam breaks and activated the valves under custom software control. The whole chamber was enclosed and the ceiling was equipped with an exhaust fan to remove odorants.

An air dilution system modified from that described previously (Larson and Sieprawska, 2002) was used to generate odorants (Fig. 1). Liquid odorants (50 mL) were contained in large glass test tubes (100 mL capacity) fitted with silicone stoppers with two holes drilled to accept clean air input and odorized air output tubing (teflon, 1/16 in i.d.) connected as shown. The odorant tubes were located downstream of computer-operated solenoid valves (SV) and flowmeters (FM) in order to minimize odorant contamination of these elements. The clean air supply (bottled zero air, AGA Gas Co., Lansing, IL) in each channel was run at 1.8 liter per minute (lpm); odorized air was injected into this stream at 0.2 lpm for an air dilution to 10% of the saturated vapor in the odor tube. Pinch valves (PV; Cole-Parmer, Vernon Hills, IL) located

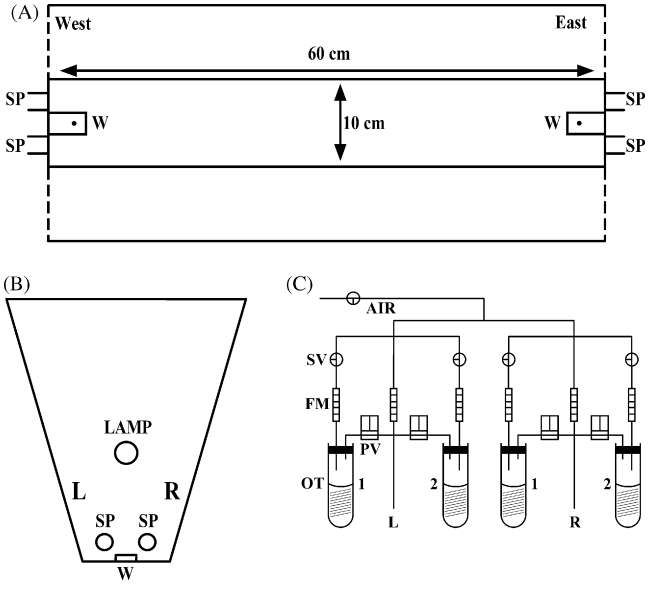

Fig. 1. Testing chamber and odor generators used for olfactory discrimination training. (A) Top view of the behavioral chamber. Two sniff ports (SP) and a single water cup (W) are located at each end of the straight alley. Drawing is to scale. Dashed lines indicate that the floor is narrower than the top of the chamber because of the outward sloping side walls. (B) View of the West end of the alley as seen from inside the chamber. Positions of sniff ports, water cup, and signal lamp are to scale. L = left; R = right. (C) Odor generator. Computer operated solenoid valves (SV) upstream and pinch valves (PV) downstream of the odor tubes (OT) control the flow of pressurized air through flow meters (FM) and the odor tubes to present either odor (1, 2) diluted with clean air to either of the West sniff ports (L, R). Drawing is not to scale.

downstream of the odorant tubes and activated and deactivated along with the appropriate upstream solenoid valves were used to minimize odor exposure between trials. The odorant tubes and tubing elements exposed to odorants were replaced as a unit for each odor pair (odor cartridge) when odor pairs were changed. Odorants used in these experiments were selected from a large stock of chemicals and flavoring essences obtained from Aldrich Chemical Co. (Milwaukee, WI) and McCormick and Co. (Baltimore, MD).

2.3. Procedures

All procedures were fully automated and controlled by computer within each training session.

2.3.1. Shaping

A shaping procedure was used to familiarize the mice to the training procedures and to reinforce nose poke responses prior to the introduction of odor cues. This proceeded in four stages. The first stage consisted of 20-trial, daily sessions in which the mice were reinforced with a small drop of water (12.5 μL) for a nose poke in either sniff port at either end of the alley. Each trial was a maximum of 120 s long and was followed by a 10 s inter-trial interval (ITI). After each reinforced response, the next reinforcement was contingent on the mouse making a nose poke in one of the sniff ports at the end of the alley opposite the last correct response, i.e., the mouse was required to traverse the alley repeatedly. If a correct response did not occur within the 120-s period, that trial was scored as incorrect and the identical contingency was in effect on the following trial. A lamp at each end of the alley was lit during the ITI and was extinguished over the ports at which reinforcement was available during the trials. Each mouse was trained in this way until it had made 90% reinforced responses in one 20-trial session. The second stage of shaping was identical to the first except that each session had 40 trials and mice were trained to criterion of 90% reinforced trials in one 40-trial session. The third stage of shaping consisted of 20-trial sessions in which each trial began with the extinguishing of the lamp at the East end of the alley. A nose poke in either of the East sniff ports (a "trial initiation" nose poke) within 120 s of the trial onset was reinforced with a drop of water, turned on the lamp, and extinguished the lamp at the West end of the alley. No response within the 120-s period terminated the trial and was followed by a 10 s ITI. A nose poke ("trial conclusion" nose poke) at either West sniff port within 60 s after the trial initiation nose poke was rewarded with water and followed by the ITI. Mice were trained to a criterion of 90% of trials rewarded at both ends in one 20-trial session. The fourth stage of shaping was identical to Stage 3 except that trial initiation nose pokes were not rewarded with water, each session was 40 trials, and mice were trained to a criterion of three sessions in which 90% of trials were rewarded.

The rate of learning in shaping sessions was assessed as the number of sessions required to reach criterion performance at each stage. Performance in shaping sessions was also assessed in young and old mice as the mean latency to initiate trials (time elapsed between the end of the ITI and the trial initiation nose poke on the next trial) in the final session of training in Stages 1 through 4 and the mean latency to conclude trials (time elapsed between trial initiation and trial conclusion nose pokes) for the final training session of Stages 3 and 4.

All data are presented as means and standard errors.

2.3.2. Olfactory discrimination

Olfactory discrimination training used a simultaneous-cue, two-odor, forced-choice paradigm. The trial procedures and timing were similar to those of shaping Stage 4 except that a trial initiation nose poke at the East end also activated the delivery of the two discriminative odors to the West sniff ports. The spatial position of the two odors (S+ and S−) on any given trial was randomly determined except that no more than three identical trials could occur in succession. A nose poke in the port containing the S+ stimulus was rewarded with a drop of water (12.5 μL) and scored as a correct trial; a nose poke in the S− port was not rewarded and was scored as an error. S+ nose pokes were followed by a 60 s ITI; S− nose pokes were followed by a 60 s ITI. No response within 60 s after trial initiation also terminated the trial but no-response trials were not scored as errors and were followed by a 60 s ITI.

All mice were trained on a series of eight different two-odor discrimination problems. Each mouse was given a single session of 40 training trials per day. Training on a given discrimination problem continued until the mouse achieved a performance criterion of 80% correct responses or better in the last 20 trials of a session. Training on the next problem then commenced with the following session. If a mouse failed to reach criterion on a discrimination problem after eight training sessions, it was determined to have "failed" that problem and advanced to the next. This occurred only once in the present study: one old mouse failed on the eighth discrimination problem. The odor pairs were as follows: strawberry and banana, propionic acid and hexyl octanoate, ethyl lactate and methyl salicylate, almond and root beer, pineapple and cherry, maple and coconut, dihydrojasmone and *cis*-3-hexen-1-ol, walnut and butter. One subset of each group learned the discriminations in the order listed; the other subsets learned the discriminations in the reverse order. Valences of odors (S+, S−) in each pair were counter-balanced across mice. In previous studies, these odor discriminations were acquired with comparable facility by mice (Larson and Sieprawska, 2002; Larson et al., 2003).

The number of errors committed before reaching the learning criterion and the number of sessions required for each discrimination was tabulated for each mouse. Response latencies were computed as the time that elapsed between the trial initiation nose poke and the odor choice nose poke on each trial.

2.3.3. Memory after 2 weeks

After each mouse reached criterion performance on the eighth (final) discrimination, it was not tested for a 2-week period. After this delay, it was given a series of 10 probe trials, each of which was identical to a training trial in the final discrimination problem except that no differential reinforcement was provided. Responses at the port containing the odor trained as S+ were scored as correct and responses to the trained S− were scored as incorrect. However, no water was given on either correct or incorrect trials. The ITI was 60 s for all trials. A retention score was calculated for each mouse as the percentage of correct responses on the probe trials.

2.3.4. Olfactory sensitivity

Young and old mice were tested for threshold detection of ethyl acetate. Fifteen of the mice described above (11 young and 4 old) and 20 other mice trained similarly in odor discriminations as part of another experiment (12 young and 8 old) were the subjects. The mice were first trained to discriminate between a 0.001% (10^{-3}%) ethyl acetate stimulus (S+) and a clean air stimulus (S−). The S+ odor tube contained a 50 mL solution of 0.02% ethyl acetate in ultrapure water; the S− odor tube contained ultrapure water alone. A final air dilution in the odor generator diluted the vapor in each S+ and S− odor tube by 20-fold (0.1 lpm through the odor tubes and 1.9 lpm in the main airstream). Lower concentrations of ethyl acetate in subsequent tests were generated by further dilution of ethyl acetate in water in the odor tubes. The final (and only) air dilution was always 20-fold and was the same for the ethyl acetate and clean air stimuli. The odor tubes and odor-exposed tubing for each concentration of ethyl acetate (S+) and water (S−) tested were prepared as separate odor cartridges that were inserted in the odor generator as required.

Mice were trained in two 20-trial sessions each day. In the second session each day, the connections between the odor tubes and the control valves for each channel in the olfactometer were reversed and the software controls for the valves were also reversed in order to ensure that mice could not use the sounds of the valves as cues in the task (this maneuver switched the valves controlling S+ and S− but did not alter the reward contingencies). Mice were trained on the "high intensity" ethyl acetate (10^{-3}%):water discrimination until all had performed at least two sessions at criterion (80% correct) on 1 day. They were then trained in two daily 20-trial sessions on a descending concentration series consisting of 10^{-4}%, 10^{-5}%, 10^{-6}%, 10^{-7}%, and 10^{-8}% ethyl acetate, with 1 day training on the high concentration (10^{-3}%) interleaved between the lower concentration days. This was followed by a second descending series consisting of $10^{-3.5}$%, $10^{-4.5}$%, $10^{-5.5}$%, $10^{-6.5}$%, and $10^{-7.5}$%, again with high concentration days interleaved. S+ was always ethyl acetate and S− was always ultrapure water (no odor).

The percentage of correct trials was calculated for each session. Response latencies were also computed for each correct trial and averaged across trials within a session.

3. Results

3.1. Nose poke training

Young mice required 7–12 sessions (mean = 8.33 ± 0.32, $n = 24$) to satisfy shaping criteria; old mice required 7–14 sessions (9.87 ± 0.54, $n = 15$), a statistically significant difference ($t_{37} = 2.60$, $p < .02$). The mean number of sessions required to reach criterion at each stage of shaping are provided in Table 1. The mean latencies to perform nose pokes as required at the different stages of shaping are also shown in Table 1. There was no main effect of age on latency scores ($F_{1,37} = 0.07$, $p > .5$); however, there was a significant interaction between age and shaping stage ($F_{5,185} = 5.27$, $p < .001$). Old mice had shorter latencies in the first stage of shaping (Newman–Keuls test, $p < .01$) and longer latencies to initiate trials in the final stage of nose poke training ($p < .05$).

3.2. Olfactory discrimination learning

The results for odor discrimination learning are summarized in Fig. 2. All mice were trained to criterion on the eight discrimination problem series. Old mice made significantly more errors in the entire problem series than did young mice (Fig. 2A; $t_{37} = 6.42$, $p < .0001$). The total number of sessions

Table 1
Performance of young and old mice in shaping sessions

	Sessions to criterion				
	Stage 1	Stage 2	Stage 3	Stage 4	Total
Young	2.75 ± 0.18	1.00 ± 0.00	1.00 ± 0.00	3.58 ± 0.26	8.33 ± 0.32
Old	3.27 ± 0.25	1.07 ± 0.07	1.33 ± 0.19	4.20 ± 0.55	9.87 ± 0.54*

	Mean latency in last session					
	Stage 1	Stage 2	Stage 3 (I)	Stage 3 (C)	Stage 4 (I)	Stage 4 (C)
Young	42.38 ± 2.31	21.49 ± 1.43	10.83 ± 0.91	20.57 ± 1.14	23.40 ± 1.38	5.98 ± 0.23
Old	33.35 ± 3.14*	21.44 ± 2.18	12.96 ± 1.62	22.24 ± 1.70	29.03 ± 2.96*	7.96 ± 0.65

Latencies were recorded for the final training session at each stage when performance was at criterion. In Stages 3 and 4, separate latencies were recorded for trial initiation (I) nose pokes and trial conclusion (C) nose pokes. *$p < .05$, comparing young with old.

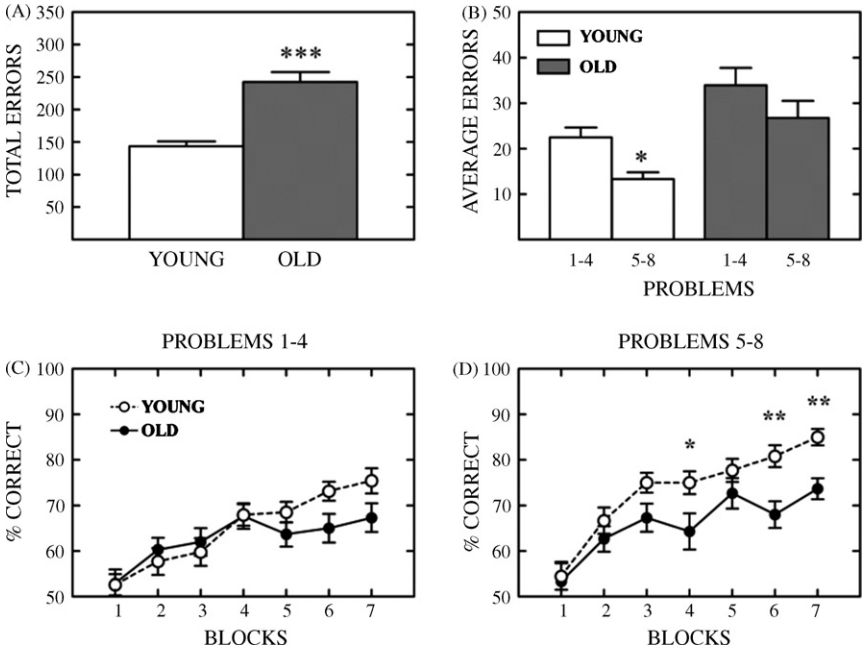

Fig. 2. Acquisition of olfactory discriminations is slower in old than in young mice. (A) Total errors (mean ± S.E.M.) committed during training to criterion performance on all eight discrimination problems. Old mice made significantly more errors than young mice. (B) The average number of errors (mean ± S.E.M.) committed before achieving criterion performance on discrimination problems 1–4 and 5–8 for young (open bars) and old (shaded bars) mice. Young mice made significantly fewer errors on the second block of problems than on the first block of problems. Old mice did not make significantly fewer errors on problems 5–8 than on problems 1–4. (C) Average percent correct responding (mean ± S.E.M.) on the initial training (blocks of five trials each) for problems 1–4 for young (open circles) and old (filled circles) mice. Mice occasionally did not respond on one to a few trials in a session; these were not counted as correct or incorrect and only the first 35 trials in which an odor choice was made were used in the figure. (D) Average percent correct responding (mean ± S.E.M.) on the initial training (blocks of five trials each) for problems 5–8 for young (open circles) and old (filled circles) mice. Young mice made significantly more correct responses than old mice for blocks 4, 6, and 7. *$p < .05$; **$p < .01$; ***$p < .001$.

required was also higher for old than for young mice (young: 14.21 ± 0.50; old: 19.87 ± 0.89; $t_{37} = 5.96$, $p < .0001$). The problem series was divided into two blocks of four discrimination problems each in order to determine if learning rate improved across problems (Fig. 2B). Repeated measures analysis of variance indicated a significant main effect of age ($F_{1,37} = 41.22$, $p < .0001$) and a significant interaction between age and problem block ($F_{1,37} = 6.23$, $p < .02$) on the average number of errors committed per problem. Young mice showed a significant reduction in errors from the first block to the second block of problems ($p < .05$, Newman–Keuls test); old mice did not ($p > .05$).

The total number of "no-response" trials throughout the problem series was not larger in the old group than in the young (young mean: 13.17 ± 3.47; old mean: 8.80 ± 1.40; $t_{37} = 0.96$, $p > .30$), even though the old mice had significantly more training sessions. This suggests that the old mice were neither insufficiently motivated nor inadequately trained in nose poking; rather they appear to be impaired either in discrimination ability or in the formation of odor-reward associations. In both groups the number of no-response trials per 40-trial session was less than one (young: 0.86 ± 0.19; old: 0.43 ± 0.06; $t_{37} = 1.74$, $p > .05$).

Fig. 2C shows the percent correct responses in the first session of training in blocks of five trials, averaged across problems 1–4. Both young and old mice demonstrated an increase in choice accuracy during the first session of training for these discrimination problems. Analysis of variance indicated no main effect of age ($F_{1,37} = 0.72$, $p > .40$). There was a highly significant effect of trial block ($F_{6,222} = 17.47$, $p < .0001$) and a marginally significant interaction between age and trial block ($F_{6,222} = 2.18$, $p < .05$). However, none of the old to young comparisons were significant (Newman–Keuls tests). In problems 5–8 (Fig. 2D), there was a significant effect of age on percent correct ($F_{1,37} = 6.82$, $p < .02$) and a significant effect of trial block ($F_{6,222} = 28.70$, $p < .0001$) but the interaction was not significant ($F_{6,222} = 1.91$, $p > .05$). The young mice made more correct responses than the old mice in the first session trials for problems 5–8 (blocks 4, 6, and 7; Newman–Keuls tests).

The mean latency from the "trial initiate" nose poke to the "odor choice" nose poke was calculated for the correct trials in the first and last blocks of 20 trials for each discrimination problem for each mouse. This latency includes the time needed to run the length of the alley, sample the odors, and make the choice; it thus should be sensitive to these "performance" variables. As shown in Fig. 3, there were only small differences in response latency between young and old mice. In the data for the first training block, there was no significant main effect of age ($F_{1,37} = 0.01$, $p > .90$), there was a significant main effect of discrimination problem ($F_{7,259} = 43.40$, $p < .0001$), but there was no interaction

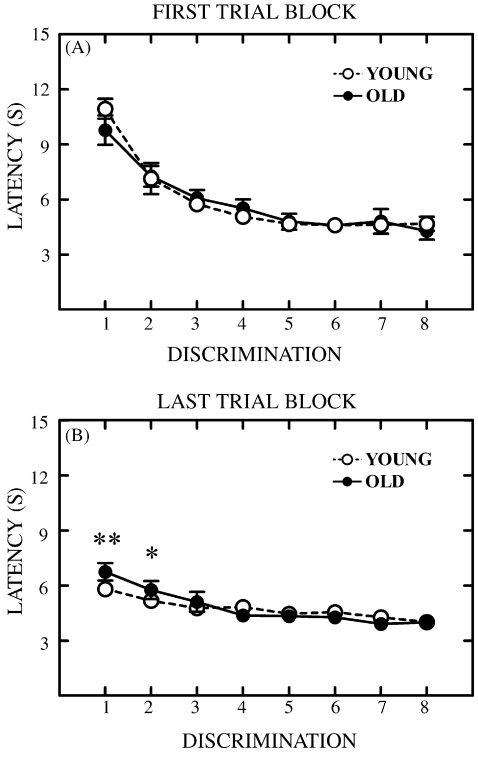

Fig. 3. Response latency comparison between young and old mice across discrimination problems. (A) Mean latency (±S.E.M.) for correct trials in the first block of 20 training trials for each discrimination problem in young (open circles) and old (filled circles) mice. There were no significant differences in response latency due to age. (B) Mean latency (±S.E.M.) for correct trials in the last block of training trials for each discrimination problem. Old mice were significantly slower to respond in these trials for the first two problems of the series. *$p < .05$; **$p < .01$.

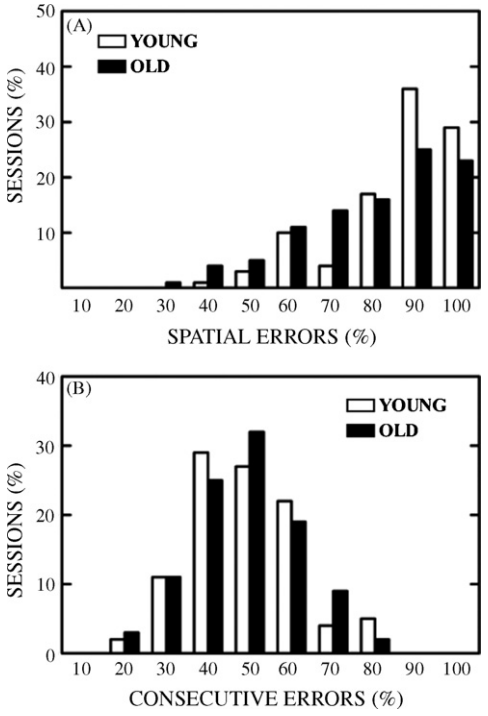

Fig. 4. Error patterns in discrimination learning by young and old mice. (A) Errors were classified as spatial errors if the response on the trial preceding the error trial was at the same sniff port, regardless of the odor present. Sessions with 15 or more errors had high percentages of spatial errors as shown by the rightward skew in the histograms. Young and old mice were not significantly different. (B) The distribution of consecutive errors were centered at 50% for both young and old mice, indicating that perseverative responding to the negative cue was no more or less likely than expected by chance in both groups of mice.

between age and problem on response latency ($F_{7,259} = 0.73$, $p > .60$). For the last training block, there was no main effect of age ($F_{1,37} = 0.07$, $p > .75$), there was again a significant effect of discrimination problem ($F_{7,259} = 31.62$, $p < .0001$), and there was a significant interaction between age and problem ($F_{7,259} = 3.24$, $p < .01$). Old mice had significantly longer latencies on correct trials of the final block of training on the first and second discrimination problems.

Training sessions with 15 or more errors were analyzed for error patterns. The mean number of such sessions per mouse was 3.92 (±0.38) for young mice and 7.60 (±0.65) for old mice ($t_{37} = 5.26$, $p < .0001$). Errors in these sessions were classified into four types, based on spatial position of the error trial and the spatial position and odor response on the preceding trial (Larson and Sieprawska, 2002). Spatial bias was manifest in sessions in which the number of errors preceded by same-side responses (spatial errors) was greater than that predicted by the binomial distribution for chance responding. This occurred in 76.6% of the sessions for young mice and 60.5% of the sessions for old mice. As can be seen in Fig. 4A, the distributions of spatial errors were clearly skewed to the right for both young and old mice; the two groups did not differ in this regard (χ^2 test, $p > .05$). The most common error pattern in both groups was consistent responding at one of the sniff ports throughout a session. The distributions of consecutive errors (responses to the S− odor twice in a row, regardless of spatial position) are shown in Fig. 4B. The percentages of consecutive errors were centered at 50%, indicating that mice were not consistently responding to the negative odor. Only a few sessions had more consecutive errors than would be predicted to occur by chance (five sessions in young mice, two sessions in old mice).

3.3. Long-term memory

Retention of the eighth discrimination problem was tested 2 weeks after the last training session. All of the young mice and all but one of the old mice reached the 80% learning criterion for this problem. As shown in Fig. 5, both young and old mice showed good memory for the discrimination; both groups had retention scores significantly above chance (one-sample t-tests—young: $t_{23} = 7.53$, $p < .0001$; old: $t_{13} = 6.33$, $p < .0001$); the two groups were not significantly different ($t_{36} = 0.08$, $p > .90$).

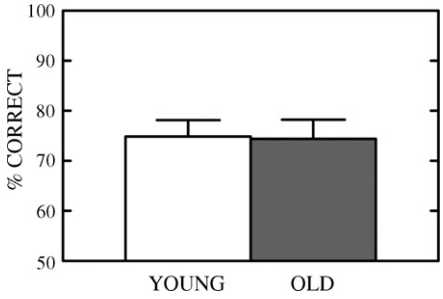

Fig. 5. Long-term memory. Histogram shows percent correct responding (mean ± S.E.M.) in a retention test 2 weeks after training on the last discrimination problem. Young (open bar) and old (shaded bar) mice were not different.

3.4. Olfactory sensitivity

An odor detection task was used to assess olfactory sensitivity in young and old mice. Mice were first trained to discriminate ethyl acetate (S+, 0.001%) from clean air (S−). They were then tested on the same discrimination using decreasing concentrations of ethyl acetate. Tests with the original ethyl acetate concentration (0.001%) were interleaved with lower ones to maintain behavioral performance. The results are shown in Fig. 6. The relationship between stimulus intensity (concentration) and accuracy of detection was shifted to the left in old mice (Fig. 6A). Analysis of variance indicated no significant main effect of age ($F_{1,33} = 1.98$, $p > .15$) but a significant interaction of age and concentration ($F_{10,330} = 3.56$, $p < .001$) as well as a significant main effect of concentration ($F_{10,330} = 76.53$, $p < .0001$). Aged mice had significantly poorer performance only on the discrimination between clean air and 0.00003% (−4.5log%) ethyl acetate ($p < .01$, Newman–Keuls tests). The leftward shift could also be seen in a plot of the percentage of mice in each group that had significantly above-chance performance (at least 80% correct) at each of the concentrations tested (Fig. 6B).

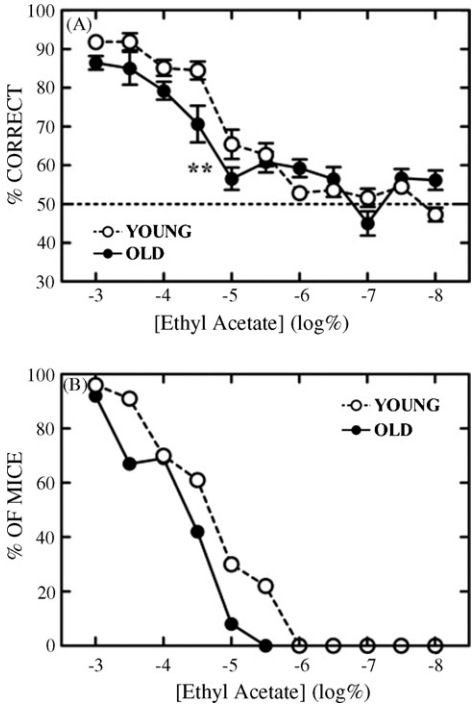

Fig. 6. Detection thresholds for ethyl acetate vapor in young and old mice. (A) Percent correct responding (mean ± S.E.M.) as a function of ethyl acetate concentration in sessions involving discrimination of ethyl acetate (S+) from clean air (S−). There was a significant interaction between age and ethyl acetate concentration on percent correct responses. Old mice performed significantly more poorly than young mice at discriminating 0.00003% (log% = −4.5) ethyl acetate from clean air. $^{**}p < .01$. (B) Percent of mice that showed above-chance correct responding (80% or more) in tests at each concentration of ethyl acetate vs. clean air. Since the highest concentration (0.001% ethyl acetate) was tested repeatedly on alternate days, the average of all days was used. One mouse in each group was below 80% correct on the highest concentration. All mice failed to discriminate between clean air and ethyl acetate concentrations below 0.000003% (log% = −5.5).

4. Discussion

Mice at 24 months of age required significantly more training sessions and made more errors in acquisition of a series of two-odor discrimination problems than did mice at 4 months of age. The differences between old and young mice were quite substantial: old mice made an average of nearly 70% more errors before reaching criterion than did young mice. Old mice did eventually reach criterion performance on each problem, demonstrating that they could discriminate between the odors.

Old mice were only mildly impaired in acquiring the sequence of nose poking responses prior to odor discrimination training. Since it is possible that a deficit in nose poke responding could be responsible for poorer performance in the odor discrimination sessions, the number of trials in which no odor choice nose poke was made was determined for both young and old mice. The average number of "no-response" trials was actually (but non-significantly) higher in the young mice than in the old mice. This suggests that the old mice were sufficiently motivated and adequately trained to make the required responses. Furthermore, an analysis of response latency only revealed small differences in response speed between the two ages: old mice were slightly slower to respond on correct trials in the last block of training on the first two discrimination problems. These results suggest that performance variables are not likely to explain the large differences between old and young mice in acquiring the two-odor discriminations.

Old mice appear to require more training either to learn to discriminate between two odors or to make odor-reward associations. Young mice made significantly fewer errors in acquisition of the last four discrimination problems compared to the first four problems. This increase in learning rate across problem blocks is consistent with acquisition of a "learning set" (Slotnick and Katz, 1974) or "rule learning" (Quinlan et al., 2004). Old mice showed no significant change in error

rates from the first block of four discrimination problems to the last four.

Both young and old mice showed good retention of the final discrimination problem in unrewarded probe trials 2 weeks after the last training session. Long-term memory for odors appears to be unimpaired in old mice. All of the young and all but one of the old mice had achieved criterion performance in acquisition of that discrimination. It remains to be seen whether or not more limited training or longer retention delays would reveal memory deficits in old mice.

Relatively strong odors were used in the discrimination learning study: each of the odorants was only diluted to 20% of full strength in solvent and by a further factor of 10 in the olfactometer. They were all well above threshold for human observers. Odor sensitivity of young and old mice was tested using successive dilutions of ethyl acetate, an odorant commonly used in prior studies of odor sensitivity in rats and mice (Bodyak and Slotnick, 1999; Slotnick et al., 2000a). Old mice showed significantly poorer performance on discrimination of 0.00003% ethyl acetate versus clean air than did young mice. The stimulus strength—performance curves suggest that the threshold for ethyl acetate detection is about threefold lower for young mice than for old mice. These results indicate that old mice are impaired in odor detection. However, this deficit is unlikely to explain the impairments in discrimination learning observed with relatively strong odors.

Several studies have examined odor discrimination learning in aged rats. Two types of task have been employed. In the first, odors are presented in the form of ground spices mixed with sand in a small pot; the rats are trained to associate the smell of the pot with the presence or absence of a food reward buried in the pot and indicate their choice by digging for the reward. In this digging task, old (24 months) rats were found to be impaired in acquiring two-odor discriminations (Lasarge et al., 2007). Importantly, when the old rats were divided into two groups based on their performance on a spatial learning task, it was found that the spatially impaired rats were also impaired on the olfactory discrimination task while the spatially unimpaired rats were not. These results suggest that the impairment in olfactory discrimination learning may reflect a more general impairment of complex learning (Lasarge et al., 2007). In the second task, an olfactometer is used to generate an odorized airstream in an operant chamber; on any given trial one of two odors is present; responses in the presence of one odor are rewarded and responses in the presence of the other are not. In one version of this successive-cue, go-no go task, a subset of old rats was severely impaired while other old rats learned normally (Roman et al., 1996). The unimpaired old rats were found to have more rapid forgetting of the odor-reward association than young rats. In a second version of this task, old rats were found to learn three discriminations as quickly as young rats; the old rats were, however, impaired at acquiring a discrimination reversal (Schoenbaum et al., 2002). In the same study, old rats were found to be no different from young rats in a task involving threshold detection of odorants (Schoenbaum et al., 2002). On the other hand, in another experiment the threshold for detection of ethyl acetate was found to be about 10-fold lower in 3-month old than in 25-month old animals (Kraemer and Apfelbach, 2004). Finally, Schoenbaum et al. (2006) recorded from prefrontal cortical neurons in aged rats that showed normal acquisition of olfactory discriminations but impaired reversal learning. They found that odor-selective firing was diminished compared to young controls or aged rats unimpaired in reversal learning: fewer neurons developed odor-selectivity during initial acquisition, and even fewer changed odor-selectivity during reversal learning (Schoenbaum et al., 2006). These findings indicate that aging affects neural processing of odors in ways that are not necessarily expressed in simple behavioral measures.

In the only prior study of the effects of aging on discrimination learning in mice, Frick et al. (2000) report an experiment comparing young (5 months old), middle-aged (17 months), and old (25 months) mice (C57Bl/6 strain) on the sand digging task. The mice were trained in blocks of four trials each for 3 days. Choice accuracy did not improve across sessions in the old (25 months) as in young mice; more errors were made across all sessions in the old mice. Finally, the old mice responded much faster in this task than did the young mice. Tests showed that the mice were not anosmic in that they could detect spices mixed in various proportions with sand; however the comparisons between young and old mice did not approach threshold sensitivity (Frick et al., 2000).

The decrease in odor sensitivity seen in the present study could be due to degenerative changes in the olfactory epithelium. In both humans and rodents (Hinds and McNelly, 1981; Menco and Morrison, 2003), the number of olfactory sensory neurons declines with age, partly due to decreased regeneration (Weiler and Farbman, 1997). The decreased sensitivity was, however, relatively small, and probably cannot account for the impairment in discrimination learning. A recent study (Enwere et al., 2004) found that old mice were impaired at fine discrimination of binary mixtures of odorants when the two components approached 50%; discrimination of the unitary components was equivalent in young and old mice. These investigators provided evidence that impaired discrimination ability may result from decreased neurogenesis of olfactory bulb granule cells in the aging mice.

The major effect of aging observed in the present study was a retardation in the rate of establishment of differential responding to the rewarded and non-rewarded cues in two-odor discriminations. Aged mice required many more trials to reach a performance criterion of 80% correct responses in the discrimination problems. Since acquisition of olfactory discriminations is accompanied by synaptic plasticity in primary olfactory cortex (Roman et al., 1987, 1993; Barkai, 2005), it will be of interest to determine if synaptic mechanisms such as long-term potentiation are altered in olfactory cortex of aging mice. Beyond this, output pathways from piriform cortex to hippocampus, amygdala, and prefrontal cortex (Shipley

et al., 1995) are also candidates for age-related deterioration of olfactory learning.

Disclosure statement

The authors declare no actual or potential conflicts of interest.

Acknowledgments

This work was supported by grants from the NIA (AG16508) and NIDCD (DC5793). We are grateful for the assistance of Mr. Daniel Kim.

References

Barkai, E., 2005. Dynamics of learning-induced cellular modifications in the cortex. Biol. Cybern. 92, 360–366.

Bodyak, N., Slotnick, B., 1999. Performance of mice in an automated olfactometer: odor detection, discrimination and odor memory. Chem. Senses 24, 637–645.

Brunjes, P.C., Frazier, L.L., 1986. Maturation and plasticity in the olfactory system of vertebrates. Brain Res. 396, 1–45.

Cain, W.S., Stevens, J.C., 1989. Uniformity of olfactory loss in aging. Ann. N.Y. Acad. Sci. 561, 29–38.

Doty, R.L., Shaman, P., Applebaum, S.L., Giberson, R., Siksorski, L., Rosenberg, L., 1984. Smell identification ability: changes with age. Science 226, 1441–1443.

Eichenbaum, H., 1998. Using olfaction to study memory. Ann. N.Y. Acad. Sci. 855, 657–669.

Enwere, E., Shingo, T., Gregg, C., Fujikawa, H., Ohta, S., Weiss, S., 2004. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. J. Neurosci. 24, 8354–8365.

Frick, K.M., Burlingame, L.A., Arters, J.A., Berger-Sweeney, J., 2000. Reference memory, anxiety and estrous cyclicity in C57BL/6NIA mice are affected by age and sex. Neuroscience 95, 293–307.

Geisler, M.W., Morgan, C.D., Covington, J.W., Murphy, C., 1999. Neuropsychological performance and cognitive olfactory event-related brain potentials in young and elderly adults. J. Clin. Exp. Neuropsychol. 21, 108–126.

Hinds, J.W., McNelly, N.A., 1981. Aging in the rat olfactory system: correlation of changes in the olfactory epithelium and olfactory bulb. J. Comp. Neurol. 203, 441–453.

Kovacs, T., 2004. Mechanisms of olfactory dysfunction in aging and neurodegenerative disorders. Ageing Res. Rev. 3, 215–232.

Kraemer, S., Apfelbach, R., 2004. Olfactory sensitivity, learning and cognition in young adult and aged male Wistar rats. Physiol. Behav. 81, 435–442.

Larson, J., Hoffman, J.S., Guidotti, A., Costa, E., 2003. Olfactory discrimination learning deficit in heterozygous reeler mice. Brain Res. 971, 40–46.

Larson, J., Sieprawska, D., 2002. Automated study of simultaneous-cue olfactory discrimination learning in adult mice. Behav. Neurosci. 116, 588–599.

Larsson, M., Backman, L., 1998. Modality memory across the adult life span: evidence for selective age-related olfactory deficits. Exp. Aging Res. 24, 63–82.

Lasarge, C.L., Montgomery, K.S., Tucker, C., Slaton, G.S., Griffith, W.H., Setlow, B., Bizon, J.L., 2007. Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. Neurobiol. Aging 28, 928–936.

Lu, X.C., Slotnick, B.M., Silberberg, A.M., 1993. Odor matching and odor memory in the rat. Physiol. Behav. 53, 795–804.

Lynch, G., 1986. Synapses, Circuits and the Beginnings of Memory. MIT Press, Cambridge, MA.

Menco, B.Ph.M., Morrison, E.E., 2003. Morphology of the mammalian olfactory epithelium: form, fine structure, function, and pathology. In: Doty, R.L. (Ed.), Handbook of Olfaction and Gustation. Marcel Dekker, Inc., New York, pp. 17–49.

Mirich, J.M., Williams, N.C., Berlau, D.J., Brunjes, P.C., 2002. Comparative study of aging in the mouse olfactory bulb. J. Comp. Neurol. 454, 361–372.

Murphy, C., Cain, W.S., Gilmore, M.M., Skinner, R.B., 1991. Sensory and semantic factors in recognition memory for odors and graphic stimuli: elderly versus young persons. Am. J. Psychol. 104, 161–192.

Nakayasu, C., Kanemura, F., Hirano, Y., Shimizu, Y., Tonosaki, K., 2000. Sensitivity of the olfactory sense declines with the aging in senescence-accelerated mouse (SAM-P1). Physiol. Behav. 70, 135–139.

Prediger, R.D., Batista, L.C., Takahashi, R.N., 2005. Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats. Involvement of adenosine A1 and A2A receptors. Neurobiol. Aging 26, 957–964.

Quinlan, E.M., Lebel, D., Brosh, I., Barkai, E., 2004. A molecular mechanism for stabilization of learning-induced synaptic modifications. Neuron 41, 185–192.

Roman, F., Staubli, U., Lynch, G., 1987. Evidence for synaptic potentiation in a cortical network during learning. Brain Res. 418, 221–226.

Roman, F.S., Alescio-Lautier, B., Soumireu-Mourat, B., 1996. Age-related learning and memory deficits in odor-reward association in rats. Neurobiol. Aging 17, 31–40.

Roman, F.S., Chaillan, F.A., Soumireu-Mourat, B., 1993. Long-term potentiation in rat piriform cortex following discrimination learning. Brain Res. 601, 265–272.

Schoenbaum, G., Nugent, S., Saddoris, M.P., Gallagher, M., 2002. Teaching old rats new tricks: age-related impairments in olfactory reversal learning. Neurobiol. Aging 23, 555–564.

Schoenbaum, G., Setlow, B., Saddoris, M.P., Gallagher, M., 2006. Encoding changes in orbitofrontal cortex in reversal-impaired aged rats. J. Neurophysiol. 95, 1509–1517.

Shipley, M.T., McLean, J.H., Ennis, M., 1995. Olfactory system. In: Paxinos, G. (Ed.), The Rat Nervous System. Academic Press, San Diego, pp. 899–926.

Slotnick, B., 2001. Animal cognition and the rat olfactory system. Trends Cogn. Sci. 5, 216–222.

Slotnick, B., Glover, P., Bodyak, N., 2000a. Does intranasal application of zinc sulfate produce anosmia in the rat? Behav. Neurosci. 114, 814–829.

Slotnick, B., Hanford, L., Hodos, W., 2000b. Can rats acquire an olfactory learning set? J. Exp. Psychol. Anim. Behav. Process 26, 399–415.

Slotnick, B.M., Katz, H.M., 1974. Olfactory learning-set formation in rats. Science 185, 796–798.

Slotnick, B.M., Kufera, A., Silberberg, A.M., 1991. Olfactory learning and odor memory in the rat. Physiol. Behav. 50, 555–561.

Staubli, U., Fraser, D., Faraday, R., Lynch, G., 1987. Olfaction and the "data" memory system in rats. Behav. Neurosci. 101, 757–765.

Stevens, J.C., Dadarwala, A.D., 1993. Variability of olfactory threshold and its role in assessment of aging. Percept. Psychophys. 54, 296–302.

Weiler, E., Farbman, A.I., 1997. Proliferation in the rat olfactory epithelium: age-dependent changes. J. Neurosci. 17, 3610–3622.