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Alteration of olfactory perceptual learning and its cellular basis in aged mice

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ABSTRACT

Olfactory perceptual learning reflects an ongoing process by which animals learn to discriminate odorants thanks to repeated stimulations by these odorants. Adult neurogenesis is required for this learning to occur in young adults. The experiments reported here showed that olfactory perceptual learning is impaired with aging and that this impairment is associated with a reduction of neurogenesis and a decrease in granule cell responsiveness to the learned odorant in the olfactory bulb. Interestingly, we showed that the pharmacological stimulation of the noradrenergic system using dexefaroxan mimics olfactory perceptual learning in old mice, which is accompanied by an increase of granule cell responsiveness in response to the learned odorant without any improvement in neurogenesis. We provide the first published evidence that, in contrast to young adult mice, the improvement of olfactory performances in old mice is independent of the overall level of neurogenesis. In addition, restoring behavioral performances in old mice by stimulation of the noradrenergic system underlies the importance of this neuromodulatory system in regulating bulbar network plasticity.

1. Introduction

Among the various alterations in sensory processing that characterize normal aging, olfactory deficits are of special interest. Indeed, the olfactory system is important for an animal's survival, as it influences its perception of the environment and its behavior such as food intake, social interactions, and reproduction. The alterations of olfactory functions during aging include deficits in olfactory perception (Nakayasu et al., 2000), discrimination (Enwere et al., 2004; Patel and Larson, 2009; Prediger et al., 2005), impairment in olfactory associative learning and memory (Guan and Dluzen, 1994; Prediger et al., 2005; Roman et al., 1996; Schoenbaum et al., 2002; Terranova et al., 1994) and reduction of the beneficial effect of olfactory enrichment (Rey et al., 2012). However, no data are available regarding the effect of aging on perceptual learning, which consists of improvement in the discrimination of perceptually similar stimuli due to repeated presentation of these stimuli. This type of learning is crucial for basic olfactory functions because it sets the degree of discrimination between stimuli and thus reflects an ongoing process of sensorial environment assimilation (Gilbert et al., 2001; Mandairon and Linster, 2009; Mandairon et al., 2006c, 2006d, 2006e, 2008a; Moreno et al., 2009).

The olfactory bulb (OB), the first central relay of olfactory information transmission is involved in processing different aspects of olfactory perception including perceptual learning (Mandairon and Linster, 2009; Mandairon et al., 2006c, 2008a; Moreno et al., 2009). One of the OB specificity is that it is the target of an important adult neurogenesis. The newborn neurons originate from the subventricular zone of the lateral ventricles, migrate along the rostral migratory stream to reach the OB, and differentiate into granule and periglomerular inhibitory interneurons (Alvarez-Buylla and Garcia-Verdugo, 2002; Lledo et al., 2006; Whitman and Greer, 2009). The adult-born neurons that regulate the mitral cell activity and per consequence the output message of the OB (Shepherd et al., 2007) are necessary for olfactory perceptual learning by increasing the inhibition in the OB network (Moreno et al., 2009).

In addition, the OB is heavily innervated by the neuromodulatory noradrenergic system known to be important for olfactory learning (Fletcher and Chen, 2011), odor preferences (Moriceau and Sullivan, 2004; Sullivan and Wilson, 1994), discrimination performances (Doucette et al., 2007; Mandairon et al., 2008b), memory (Veyrac et al., 2007, 2009) and neural plasticity, including neurogenesis modulation (Bauer et al., 2003).

Because the noradrenergic system and adult neurogenesis are both involved in perceptual learning (Moreno et al., 2009, 2012) and altered during aging (Enwere et al., 2004; Luo et al., 2006; Rey et al., 2012; Tropepe et al., 1997), we expected perceptual learning to be affected in aged mice. We also studied whether and how this type of learning could be restored in aged mice and analyzed the underlying neural basis.

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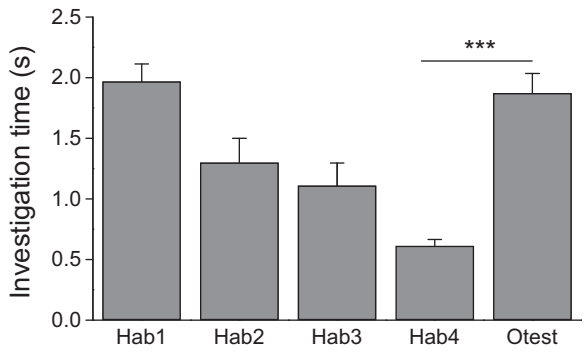

Fig. 1. Basic olfactory discrimination is not impaired in 18-month-old mice. Discrimination between dissimilar odorants (limonene and decanal) was assessed in old mice using a habituation/dishabituation test. Old mice were able to habituate, indicating that the animals detected and memorized the odorant. In addition, we observed an increase in investigation time when the second odorant of the pair was presented, compared to the last habituation trial, showing that the animals were also able to discriminate odorants. Data are expressed as mean ± SEM. *** $p < 0.0001$ (in response magnitude between Ohab4 and Otest).

We report here that olfactory perceptual learning is altered in aged (18-month-old) mice, whereas basic olfactory discrimination remains intact. We showed that the alteration of perceptual learning is associated with a reduction in adult-born neuron survival and granule cell responsiveness to the learned odorant. Interestingly, we found that the pharmacological stimulation of the noradrenergic system (using dexefaroxan injection) mimics perceptual learning in old mice. This enhancement of performances is accompanied by an increase in granule cell responsiveness in old mice without any increase in overall neurogenesis. In conclusion, upon stimulation of the noradrenergic system, old mice are able to improve their discrimination performances through an activation of the granule cell network resembling that of young adult mice.

2. Methods

Male C57Bl/6J mice (Charles River, L'arbresles, France), aged respectively 2 months (n = 50) and 18 months (n = 50), were used in this experiment. All mice were housed under a 12-hour light/dark cycle in an environmentally controlled room and with an access to food and water ad libitum. All behavioral training was conducted in the afternoon (14:00–17:00). All efforts were made to minimize the number of animals used and their suffering during the experimental procedure, in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC), and the French Ethical Committee.

2.1. Behavioral testing

2.1.1. Experimental design

At the beginning of the experiment, all mice were tested on spontaneous discrimination between pairs of chemically and perceptually similar odorants (+limonene/−limonene, butanol/pentanol, and decanal/dodecanone). Discrimination was tested using an olfactory habituation/dishabituation task. Mice were then enriched 1 hour daily during 10 days with +limonene and −limonene. After the enrichment period, mice were tested again on spontaneous discrimination among these 3 test odor pairs.

2.1.2. Enrichment

For the olfactory enrichment, swabs containing 100 μL of pure odorant (+limonene and −limonene) were placed in 2 tea balls hanging from the cover of the standard breeding cages for 1 hour daily during 10 days. Young adult mice (n = 10) and old mice (n = 10) both were subjected to the enrichment period. In the control groups (n = 10 young adult, n = 10 old mice), the mice were housed under the same conditions except that the 2 tea balls contained 100 μL of mineral oil.

To assess exploration of the tea balls during the enrichment period, additional young adult (n = 10) and old (n = 10) animals were subjected to enrichment or to empty tea balls, and the number of visits, as well as the total investigation time to the tea balls, were recorded using a video track system during the first 10 minutes of enrichment each day during 10 days. The visit was defined as the mouse's nose coming to less than 2 cm of the ball.

2.1.3. Olfactory habituation/dishabituation

In this experiment, we assessed the spontaneous discrimination among 3 pairs of chemically and perceptually similar odorants: +limonene/−limonene, butanol/pentanol, and decanal/dodecanone. These tested pairs of odorants exhibit various degrees of response overlap (as measured by 2-deoxyglucose activation maps in the OB) with enrichment odors (the enantiomers of limonene). +Limonene and pentanol are partially similar odorants, as their activation patterns in the OB are overlapping, whereas decanal is a dissimilar odorant with no overlapping with +limonene (Mandairon et al., 2006c). The odorants were all diluted in mineral oil proportionally to their vapor pressure to reach a pressure of 1 Pa (Table 1) (Cleland et al., 2002; Mandairon et al., 2006d). Habituation experiments were performed in standard home cages, and odorants were presented by placing 60 μL of odor stimulus onto a filter paper (Whatman). The filter paper was presented in a tea ball hanging from the cover of the cage. Each mouse was tested on the 3 odor pairs; the odor pairs were tested in a random order. A test session consisted of one 50-second presentation of mineral oil, then four 50-second odor presentations of a first odorant (Ohab) at 5-minute intervals, followed by one 50-second presentation of the second odorant of the pair (Otest). Investigation was defined as active sniffing within 1 cm of the tea ball. Each odorant of each pair was alternatively used as Ohab or Otest.

2.1.4. Data analysis

Data analysis was performed using Systat statistical software (SSI, Richmond, CA). Only the mice that investigated for at least 1 second during the first presentation of the Ohab were included in the analysis. Outlier trials that deviated from the mean by more than 2 standard deviations were also excluded from analysis to exclude trials during which mice may have been distracted by other stimuli (4.23% of trials, no difference between young and aged animals). An analysis of variance (ANOVA) followed by a paired *t* test was performed to determine whether mice exhibited habituation (ANOVA trial effect), and the discrimination abilities by comparing Ohab4 and Otest. Discrimination was indicated by a significant increase in investigation time during the test trial. The criterion for significance was set at $p = 0.05$.

2.2. Adult-born cells

2.2.1. Bromodeoxyuridine (BrdU) administration

To determine the fate of newborn cells in the OB, BrdU (Sigma, St. Louis, MO) was injected intraperitonally 8 days before the enrichment period (25 days before sacrifice). Three injections of BrdU (50 mg/kg in saline solution) at 2-hour intervals were performed.