Behavioural Brain Research 281 (2015) 245–249

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short Communication

Altered perirhinal cortex activity patterns during taste neophobia and their habituation in aged rats

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HIGHLIGHTS

- Aged rats showed slower attenuation of neophobia.
- Opposite perirhinal cortex activity patterns were found in adult and aged groups.
- The role of the area changes during aging.

ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form 3 December 2014
Accepted 7 December 2014
Available online 20 December 2014

Keywords:
Aging
Attenuation of neophobia
Memory
Perirhinal cortex
Rat
Taste

ABSTRACT

Perirhinal cortex (PRh) pathology and chemosensory identification dysfunction are early signs of Alzheimer's disease. We have assessed the impact of normal aging on PRh activity during flavor recognition memory using c-Fos immunoreactivity as a marker for neuronal activity. Adult (5-month-old) and aged (24-month-old) Wistar male rats were exposed to a vinegar solution on a daily basis for a period of six days. Behavioral assessment indicated similar performance in both age groups but suggested slower attenuation of neophobia in aged rats. Regarding c-Fos immunoreactivity, an opposite pattern of PRh activity was found in adult and aged groups drinking the flavor solution during the first (Novel), second (Familiar I) or sixth (Familiar II) exposure as the flavor became familiar. While adult rats exhibited a higher number of PRh c-Fos-positive neurons during the presentation of the novel flavor than during the second and sixth presentation, in aged rats the number of PRh c-Fos-positive neurons was higher during the presentation of the familiar flavor in the last session than in the first and second. The results suggest that the role of the PRh changes during aging and can help to dissociate PRh dysfuntions induced by neurodegenerative diseases and normal aging.

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The perirhinal cortex (PRh) is one of the most heavily damaged cortical areas in Alzheimer's disease (AD) and the cortical focus for disease onset [1]. Impaired recognition of previously encountered stimuli is one of the earlier signs of AD. Among them, preclinical odorant identification deficits precede other sensory modalities of recognition memory impairment [2]. Given the fact that a similar dysfunction often appears in healthy aging, animal research might help to dissociate age- and disease-related changes in PRh function. However, most of the research has been centered on visual recognition memory, indicating impairment in aged rats at 24 h retention intervals and even at shorter intervals provided that complex objects are used [3,4]. In fact, it has been suggested that visual recognition memory impairments in aged rats could be linked to PRh dysfunction [3].

Flavor recognition memory refers to the ability to assess the familiarity of a previously ingested flavor that was not followed by negative consequences [5]. Concomitantly with the flavor being classified as safe consumption increases, thus showing attenuation of neophobia. Although not always found [6], it has been previously reported in rats that aging selectively induces a lower rate of neophobia attenuation [7–9] while the neophobic response is not affected [10]. It could be proposed that this impairment is also related to PRh dysfunction at advanced age since the blocking of protein synthesis [11] and cholinergic neurotransmission [12] of the PRh impairs stabilization of taste memories. Accordingly, we previously found PRh Fos activity changes associated with flavor familiarity, which are dependent on amygdala integrity [13].

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http://dx.doi.org/10.1016/j.bbr.2014.12.020
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In order to explore the effect of aging on PRh activity during flavor recognition memory we have assessed both the attenuation of the neophobic reaction to a vinegar solution during drinking sessions over a period of 6 days and the PRh c-Fos activity in adult and aged animals during the first (Novel), the second (Familiar I), and the sixth presentation (Familiar II). Assessment of c-Fos activity in the auditory cortex was used as a control area.

Twenty-one adult (5-month-old) and twenty-four aged (24-month-old) male Wistar rats were used. They had been previously subjected to an object recognition memory task not involving exposure to flavors in a different experiment. They were housed individually and maintained on a 12-hour light–dark cycle (lights on from 8:00 to 20:00 h). All the experimental procedures were performed during the light cycle at the same time each morning in the home cage. Rats were given *ad libitum* food and water until the experiment started when water access was restricted.

All the subjects in each group (adult vs aged) received the same behavioral treatment in order to assess flavor neophobia and its attenuation. Consumption (ml) was recorded after each session. In addition, the animals were randomly assigned to the following groups according to the experimental day in which they were euthanized for the immunohistochemical procedure: adult ($n = 7$) and aged ($n = 8$) rats were euthanized after drinking the novel flavor solution on day 1 (Novel); adult ($n = 7$) and aged ($n = 8$) rats were euthanized after drinking the familiar flavor solution on day 2 (Familiar I); adult ($n = 7$) and aged ($n = 8$) rats were euthanized after drinking the familiar flavor solution on day 6 (Familiar II) (Table 1). Only the consumption of the latter groups (adult and aged Familiar II) was taken into account for the behavioral analysis. All the procedures were approved by the University of Granada Ethics Committee for Animal Research and were in accordance with the European Communities Council Directive 86/609/EEC.

For five days water intake was recorded in the morning 15 minutes drinking period during the acclimation to the deprivation schedule. Once the water intake baseline (BL) was stabilized, both adult and aged rats belonging to Familiar II group received access to a 3% cider vinegar solution during the daily drinking session for 6 consecutive days (Table 1).

For the immunohistochemical procedure, all the animals were subjected to the behavioral procedure described above but they were euthanized at different time points depending on the c-Fos immunohistochemical group they were assigned to (Table 1). They were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde 90 min after drinking the vinegar solution during the first (Novel), the second (Familiar I), and the sixth day (Familiar II). The brains were removed and placed in 4% paraformaldehyde solution for 4 h before being transferred to 30% sucrose solution until they sank for cryoprotection. Coronal sections were cut at 20 μm in a cryostat (Leica CM1900).

Tissue sections were rinsed in phosphate-buffered saline (PBS; 0.01 M, pH 7.4), incubated for 15 min with 3% hydrogen peroxide, rinsed again, and incubated in a solution of 3% normal goat serum and 0.4% Triton X-100 in PBS for 30 min. Slices were transferred to c-fos primary antibody (1:10,000; Calbiochem) for 48 h at 4 °C. After being rinsed with PBS, they were incubated in a secondary antibody (biotinylated goat anti-rabbit IgG, 1:500; Calbiochem) for 120 min at room temperature. Primary and secondary antibody solutions were mixed in a solution of 2% normal goat serum, 0.4% Triton X-100, and PBS. The sections were rinsed, then processed using the ABC kit (Vector Laboratories, Burlingame, CA) and the reaction was visualized using the peroxidase substrate kit (DAB) (Vector Laboratories, Burlingame, CA). Finally, they were rinsed, mounted on gelatine-subbed slides, rehydrated with ethanol and xylenes, and cover-slipped.

In order to quantify c-Fos positive cells, four digital images of the section in PRh and dorsal auditory cortex (AuD) per hemisphere were captured in each brain using a light microscope (Olympus BX41) at 40× magnification (Fig. 1A). PRh and AuD were identified at −3.00 mm relative to bregma according to Paxinos and Watson [14]. The number of Fos-positive cells was counted using the Image J software (National Institute of Mental Health). Threshold objects having area (300–3600) and circularity (0.10–1.00) values matching those of c-Fos positive nuclei were automatically counted for each image. Mean values were calculated for each brain area.

Fig. 2A shows the mean (±SEM) intake of vinegar solution by adult and aged groups. As mentioned above the statistical analyses are based on the data of Familiar II groups since they were euthanized after the end of the behavioral procedure. The groups did not differ in water intake during the last baseline day [$F(1,12) = 0.20$; $p > 0.66$]. A 2 × 6 (age × day) mixed ANOVA analysis of the amount ingested by the different groups during the vinegar sessions revealed a significant effect of the within-subject factor days [$F(5,65) = 14.20$; $p < 0.001$] but no effect of the between-groups factor age, [$F(1,13) = 2.13$; $p > 0.16$] or the interaction age × day [$F(5,65) = 0.95$; $p > 0.45$]. This indicated habituation of neophobia in both age groups. Nevertheless, further planned comparisons suggested slower habituation of flavor neophobia in the older group. While adult rats drank lesser amounts of vinegar in the first ($p < 0.001$) and second ($p < 0.05$) sessions compared with the last drinking session, aged rats drank significantly less in the first ($p < 0.001$), second ($p < 0.001$), third ($p < 0.05$), and fourth ($p < 0.05$) sessions compared with the last session thus not showing attenuation of neophobia until the fifth session. In addition, the aged group drank a significantly lower amount of vinegar solution than the adult group during the drinking session of the fourth exposure day ($p < 0.05$).

Mean (±SEM) PRh Fos-positive cells for both age groups during the vinegar drinking sessions is shown in Fig. 2B. A 2 × 3 (age × familiarity) ANOVA analysis indicated a significant effect of the interaction of both between-group factors [$F(2,36) = 6.53$; $p < 0.01$] but no effect of the main factors age [$F(1,36) = 0.15$; $p > 0.69$] and familiarity [$F(2,36) = 1.78$; $p > 0.18$]. Analyses of the interaction by Fisher LSD tests showed a higher number of Fos-positive cells in the adult than in the aged group during the first exposure (Day 1) to the novel vinegar solution ($p < 0.05$). However, a higher number of Fos-positive cells was evident in the aged group compared with the adult group during exposure to a familiar

Table 1
Timeline depicting the experimental procedure.

	Groups	Days	1	2	3	4	5	6
Novel	Adult ($n = 7$) Aged ($n = 8$)		VIN[a]					
Familiar I	Adult ($n = 7$) Aged ($n = 8$)		VIN	VIN[a]				
Familiar II	Adult ($n = 7$) Aged ($n = 8$)		VIN	VIN	VIN	VIN	VIN	VIN[a]

n: number of animals per group; VIN: 3% vinegar solution.
[a] Sacrifice 90 min after the drinking period.

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