Neurobiology of Learning and Memory 144 (2017) 230–234

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Short communication

Flavor and object recognition memory impairment induced by excitotoxic lesions of the perirhinal cortex

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ARTICLE INFO

Article history:
Received 27 April 2017
Revised 28 July 2017
Accepted 10 August 2017
Available online 12 August 2017

Keywords:
Attenuation of neophobia
Flavor
Memory
Object recognition
Perirhinal cortex
Rat

ABSTRACT

Recognition memory is based on the ability to assess the familiarity of a previously encountered stimulus. It can be approached using tests for different sensorial modalities. Excitotoxic lesions of the perirhinal cortex (Prh) were performed in order to assess the relevance of its integrity for object and flavor recognition memory. Object recognition memory was impaired with a 24 h retention interval. Flavor neophobia attenuation was prevented on a second encounter with the tastant. These results support a role of the perirhinal cortex in mediating the transition from novel to familiar, both in object and flavor recognition memory.

1. Introduction

The perirhinal cortex (Prh) has been extensively related to recognition memory, known as the ability to determine that an event has been previously experienced (Brown & Aggleton, 2001; Brown, Warburton, & Aggleton, 2010; Warburton & Brown, 2010). Recognition memory can be studied using different sensory modalities. In rodents, one of the most widely used behavioral test for recognition memory is the spontaneous object recognition (SOR) task (Ennaceur & Delacour, 1988) based on the innate preference of the rodents to explore novel objects compared with previously encountered ones. Several studies have shown that Prh lesions impair the spontaneous discrimination between a novel object and a familiar object (Brown, Barker, Aggleton, & Warburton, 2012; Dere, Huston, & De Souza Silva, 2007; Warburton & Brown, 2015; Winters, Saksida, & Bussey, 2008), pointing to the need for the Prh integrity for visual recognition memory.

Another approach to the study of recognition memory using a different sensory modality is flavor recognition. It refers to the ability to assess the familiarity of a previously ingested flavor. Whilst a novel flavorant induces a neophobic response, as the flavor is classified as safe (with no aversive consequences) consumption increases, thus indicating attenuation of neophobia. Lesion studies exploring the Prh involvement in flavor neophobia and its attenuation are scarce. It has been reported that Prh reversible inactivation by blocking both the protein synthesis (De la Cruz, Rodriguez-Ortiz, Balderas, & Bermudez-Rattoni, 2008) and the cholinergic neurotransmission (Gutierrez, De la Cruz, Rodriguez-Ortiz, & Bermudez-Rattoni, 2004) impairs the stabilization of taste memories, preventing the habituation of neophobia to 0.3% and 0.5% sodium saccharin solutions respectively. However, a study using Prh neurotoxic permanent lesions has found a disruption of the initial neophobic response to 0.3% and 0.5% sodium saccharin solutions, but no effect was found on its attenuation (Ramos, 2015). Thus, in spite of the consistent evidence on the effect of Prh lesions in visual recognition memory, there is scarce evidence and some controversial findings regarding flavor recognition memory.

In order to further explore the effect of Prh permanent damage on both object and flavor recognition memory we have assessed the performance of lesioned and SHAM rats in both SOR and attenuation of neophobia tasks. This has allowed us to establish comparisons between the effects of the same lesion on both tasks, thus reducing variability. In addition, we have used a vinegar solution previously in our lab (Gomez-Chacon, Gamiz, & Gallo, 2012; Gomez-Chacon, Morillas, & Gallo, 2015).

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http://dx.doi.org/10.1016/j.nlm.2017.08.002
1074-7427/

2. Method

2.1. Subjects

Twenty five adult Wistar rats were assigned to one of two surgical groups receiving i.c. bilateral infusions of either NMDA (lesioned PrhX group, n = 15) or vehicle solution (SHAM lesions group, n = 10). One SHAM rat died during surgery and one lesioned rat showed no damage in Prh and was removed from all the analyses. Thus the final number of subjects was: PrhX = 14, SHAM = 9. The rats were housed individually and maintained in a temperature controlled room (21 °C) on a 12:12 h light–dark cycle (lights on at 8:00 am) with food and water ad libitum until the behavioral procedure started. All the procedures were approved by the University of Granada Ethics Committee for Animal Research and Junta de Andalucía (17-02-15-195).

2.2. Surgery

All the rats were anaesthetized (mixture of 5 mg/ml of xylacine and 50 mg/ml of ketamine in saline solution 0.9%) and mounted on a stereotaxic apparatus (Stoelting Co. Instruments, Word Dale, IL, USA). They were randomly assigned to one of two groups. The lesioned group (PrhX) received bilateral injections (three per hemisphere) of N-methyl-D-aspartic acid (NMDA, Sigma–Aldrich, 0.077 M) through injection cannulae (30 gauge) connected to 10 μl Hamilton microsyringes, so that 0.4 μl of NMDA solution were infused in each injection site for 2 min at a rate of 0.2 μl/min using an injection pump (Harvard, USA). The cannulae were left in situ for an additional 2 min before being withdrawn. The stereotaxic coordinates from bregma according to Paxinos and Watson (2009) were: AP = −3.3, ML = ±6.6, DV = −7.5; AP = −4.8, ML = ±6.8, DV = −7.4; AP = −6.3, ML = ±7, DV = −6.8. The SHAM group received equivalent volumes of phosphate-buffered saline infused into the same coordinates.

2.3. Behavioral procedure

2.3.1. Spontaneous object recognition

Two weeks after surgery, a standard SOR task was performed in an open box made of black-painted wood (52 × 52 × 40 cm) placed in a dimly lit room. Three geometrical objects (duplicated copies) with different shapes and colors were used: a blue pentagonal-based pyramid, a green triangular prism and a yellow cube. The objects were fixed to the floor with Velcro and thoroughly cleaned with 70% ethanol before each trial to avoid olfactory cues. Objects and their relative positions were counterbalanced.

On days 1, 2 and 3 all the rats were given a daily habituation session in which they were placed individually into the empty box for 5 min. In the sample phase, rats were placed in the box facing the wall opposite to two identical objects (A1 and A2) and they were allowed to freely explore them. This phase ended either after the time spent exploring both objects reached a total of 40 s duration or after 4 min had elapsed. Test 1 took place 1 min after the sample phase. The rats were placed again in the box and one copy of the object from the sample phase (A) together with a new object (B) were presented for 2 min. Twenty-four hours later a second test (Test 2) was applied. The rats were placed once again in the box containing one copy of the familiar object (A) together with a different new object (C) for 2 min in order to test memory consolidation. Both the sample phase and tests were video recorded and the total time spent exploring each of the two objects was scored by the experimenter with two stop watches. The experimenter was unaware of the group identity of each rat, therefore scored blind.

2.3.2. Attenuation of neophobia

The attenuation of neophobia procedure started one week after concluding the SOR task. For this task the number of animals was reduced since some of them were assigned to a preliminary inmunohistochemical experiment. Thus, 8 lesioned and 7 SHAM rats were used. Animals were water deprived and subjected to daily 15 min drinking sessions. After 6 days of water baseline habituation, a 3% (vol/vol) cider vinegar solution was available instead of water during the following four days. The drinking solutions were presented using inverted 50-ml plastic tubes equipped with stainless steel ball-bearing-tipped spouts. Consumption (ml) was recorded after each session.

2.4. Histology

Once the behavioral testing was completed, the rats were deeply anaesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde. The brains were then removed and placed in 4% paraformaldehyde solution for 4 hours before being transferred for cryoprotection to 30% sucrose solution until they sank. Coronal sections (40 μm) were cut on a cryostat (Leica CM 1900) and stained with cresyl violet. The Neurolucida system (Micro Bright Field Inc., Williston, USA) was used to quantify the extension of the damage in each lesioned rat using a light microscope (Olympus BX41) with a motorized stage interfaced to a computer. Regions of cell loss and gliosis were identified and every coronal section was digitalized.

3. Results

3.1. Histology

As shown in Fig. 1, the histological analysis confirmed that NMDA infusion induced neuronal loss as well as large changes in Prh cytoarchitecture in the lesioned group. Most of the lesions involved the entire rostro-caudal extent of the perirhinal cortex bilaterally, although in one case most of the damage was found unilaterally. In another case there was limited sparing of the perirhinal cortex at its most rostral border.

Adjacent regions to the Prh were largely preserved. Nonetheless, in the two largest lesions the cell loss extended ventrally to involve dorsal and superficial parts of the piriform cortex, in one of them the basolateral amygdala was also damaged at its dorsal part unilaterally. The CA1 field of the hippocampus was also, affected minimally and always unilaterally in three cases. The insular cortex was intact in all the brains.

3.1. Object recognition

During the sample phase of the SOR task, the rats explored the two copies of an object equally and there were no differences in exploration time (ET) among groups [$F < 1$]. A 2 × 2 (Lesion × Novelty) ANOVA analysis of the ET in Test 1 (1 min retention interval) yielded a significant main effect of Novelty [$F(1, 21) = 16.44$, $p < 0.001$; $\eta_p^2 = 0.44$], revealing a longer exploration of the novel object for both groups (Fig. 2a). That is, with a short retention interval, both groups performed the task equally, exploring a novel object longer than a familiar one, thus showing intact short-term memory in the lesioned group. The same ANOVA analysis of the performance in test 2 after a 24-h retention interval revealed a significant effect of Novelty [$F(1, 21) = 4.78$, $p < 0.05$; $\eta_p^2 = 0.19$] and a Lesion × Novelty interaction [$F(1, 21) = 4.78$, $p < 0.05$; $\eta_p^2 = 0.19$].