Basolateral amygdala lesions attenuate safe taste memory-related c-fos expression in the rat perirhinal cortex

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ABSTRACT

Previous results indicated that damage and pharmacological inactivation of the basolateral amygdala (BLA) interfere with the attenuation of taste neophobia. A similar disruption of safe taste memories formation induced by the inhibition of protein synthesis in the perirhinal cortex (PRh) has been reported. Thus, we have assessed the effect of bilateral BLA neurotoxic lesions on PRh activity after novel and familiar taste exposure. Wistar male rats with NMDA lesions of the BLA and SHAM-operated received two consecutive exposures to a 3% cider vinegar solution. Fos-like immunoreactivity (FLI) was examined as a marker of neuronal activity in PRh. As expected the BLA lesioned group showed no evidence of neophobia attenuation. A similar number of PRh Fos-positive cells were found in SHAM and BLA groups exposed to the novel taste solution. However, the BLA-lesioned group exhibited a lower number of Fos stained cells than the SHAM-lesioned group after being exposed to the familiar taste solution. This supports the notion of BLA and PRh as components of a neural circuit involved in safe taste recognition memory and suggests a role of PRh in various forms of recognition memory.

Keywords: *Attenuation of neophobia Amygdala Fos Immediate early gene Learning Memory Perirhinal cortex Rat Taste*

Taste neophobia defined as the reluctance to consume unfamiliar tastes is used as a novelty index in rats. When the ingestion of a novel taste solution is not followed by negative consequences it becomes recognized as safe. Safe taste recognition memory leads to increased consumption of familiar tastes (habituation of neophobia) and it retards the acquisition of later aversive memories (latent inhibition).

Distinct neural circuits and underlying molecular mechanisms have been proposed for safe and aversive taste recognition memory [1]. Several brain areas seem to be involved in safe taste recognition memory. Previous studies have found that lesions and pharmacological treatments that inhibit protein synthesis or block neurotransmitter receptors impair the attenuation of taste neophobia when applied into the insular cortex [2–4], accumbens nucleus [5], hippocampus [6], basolateral amygdala [7] and perirhinal cortex [3,6,8].

Among these areas the basolateral amygdala (BLA) has been extensively investigated. The results indicate that BLA is required for safe taste memory evidenced either in attenuation of neophobia [7] or in latent inhibition [9,10]. However the BLA specific role in taste processing which is relevant for safe taste recognition memory remains unclear. Although not always found [11], the fact that BLA lesions have been reported to impair taste neophobia [12] has prompted explanations of the BLA role in safe and aversive taste memory related with taste novelty detection [13,14]. Accordingly, increased expression of the immediate early gene c-fos as an index of neuronal activity has been reported after consumption of a novel taste solution in BLA [15].

Meanwhile the perirhinal cortex (PRh) has been related with the detection of familiarity [6]. In agreement with the results supporting the PRh involvement in visual recognition memory [16–18] blockade of the perirhinal cholinergic muscarinic receptors by infusion of scopolamine [3,8] and inhibition of protein synthesis in the area by anisomycin [6] prevents the habituation of taste neophobia. Although changes in the pattern of PRh c-fos expression have been reported after object recognition memory [19–23] to the best of our knowledge no previous work has assessed Fos-like immunoreactivity (FLI) in the area after safe taste recognition memory.

In order to explore the relevance of a potential interaction between BLA and PRh for safe taste memory a behavioural procedure leading to habituation of taste

neophobia was applied to rats receiving either BLA neurotoxic lesions or SHAM lesions. FLI in PRh after drinking either a novel or a familiar taste solution was determined. In addition, the primary auditory cortex and dorsal hippocampus were examined as control areas.

Twenty male Wistar rats were assigned to two experimental groups: BLA (n = 10) and SHAM (n = 10). They were housed individually and maintained on a 12/12 h light-dark cycle with food ad libitum. All the rats were anesthetized (Dolethal: 0,05 ml/100 g) and mounted on a stereotaxic apparatus (Stoelting Co.Instruments, Word Dale, IL, USA). Injection needles (30 Gauge) connected to 10 _l Hamilton microsyringes were inserted in the BLA at the following coordinates: AP: −3; ML: ±4.8; DV: −8 [24] in order to administer 1l of either NMDA (BLA group) or vehicle (SHAM group) per hemisphere at a rate of 0.5l per min using an injection pump (Harvard, USA).

After a week recovery the water access was restricted to one daily 15 min drinking session in their home cages for the next 4 days. On day 5 and 6 water was substituted by a cider vinegar solution (3%) and the amount ingested was recorded. For imnunohistochemical staining half of the animals in each group were deeply anesthetized with pentobarbital (200 mg/Kg) and perfused with 4% formaldehyde 90 min after drinking the novel taste solution on day 5 and half of them after drinking the familiar taste solution on day 6. The brains were removed and postfixed for 48 h and then transferred to a 30% sucrose solution overnight. Coronal sections were cut at 20 _m in a cryostat (Leica, CM1900). Two series of sections were collected for applying Cresyl Violet staining to identify the lesion site and inmunohistochemistry for Fos-positive cell counting. After rinsed in phosphate-buffered saline (PBS), sections were incubated for 20 min with 3% hydrogen peroxide (H2O2) to block endogenous peroxidase activity. Afterwards, sections were incubated in 3% normal goat serum for 30 min and 0.4% Triton X-100 in PBS. Slices were transferred to C-Fos primary rabbit policlonal antibody (1:10000; Santa Cruz Biotechnology) for 48 h at 4 ◦C. After being rinsed with PBS several times, they were incubated in a secondary antibody containing goat anti-rabbit IgG (1:200; Vector Laboratories, Burlingame. CA) for 120 min at room temperature. Sections were rinsed, processed using the ABC kit (Vectastain, Vector Laboratories, Burlingame, CA). Reaction was visualized using peroxidase substrate kit

(DAB) (Vector Laboratories, Burlingame, CA). The Neurolucida system (Neurolucida, MicroBrightField Bioscience, USA) was used to count the number of Fos positive cells using a microscope with a motorized stage interfaced to a computer. This program allowed us to delineate, using an X4 objective, an area covering the medial PRh (mean: 1,317,763.500 μm2) and to count the number of cells identified in this area. FLI was also determined in two additional control areas of a similar extent and located at the same A–P coordinates level: primary auditory cortex (AU1) (mean of 1,911,636 μm2) and dorsal hippocampus (DH), including dentate gyrus as well as CA1, CA2 CA3 fields (mean of 1,511,431.062 μm2). Counts were taken from 3 consecutive sections. A mean of the two hemispheres per section was estimated. 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.

Fig. 1 show mean (±SEM) consumption of water during the last baseline session and cider vinegar solution during the first and second exposure session by BLA and SHAM groups. A 2(lesion) × 3 (day) ANOVA analysis yielded a significant interaction lesion × day ($F[2,16] = 4.92$, $p < 0.05$). The analysis of the interaction indicated that the BLA lesioned group drank a higher amount of the unfamiliar cider vinegar solution during the first drinking session ($F[1,18] = 5.56$, $p < 0.05$) while no significant differences were found between the groups in water intake during the baseline or the familiar taste solution consumption on the second exposure. However, the neophobic response to the cider vinegar solution was evident in both groups since a decreased intake in comparison with the water baseline was found both in BLA ($F[1,9] = 10.52$, $p < 0.01$) and SHAM ($F[1,9] = 109.90$, $p < 0.01$) groups. Nonetheless only the SHAM group exhibited attenuation of neophobia indicated by a significant increase in consumption of the familiar taste solution during the second exposure with respect to the first one ($F[1,4] = 14.27$, $p < 0.05$). Thus, consistent with previous reports the BLA lesioned group exhibited a reduced neophobic response and impaired attenuation of taste neophobia.

Histological examination evidenced amygdala damage centred in the basolateral nucleus in all the BLA lesioned subjects. The lesions were placed centrally in BLA sparing the ventral striatum and partially affecting the medial pyriform cortex in two cases (Fig. 2). However the pyriform cortex was largely intact even in these cases. Both the perirhinal cortex and the entorhinal were spared.

In accordance to the behavioural results BLA lesions reduced FLI in PRh (Fig. 3). A 2 (lesion) × 2 (familiarity) ANOVA analysis of the number of Fos-positive cells yielded a main significant lesion effect ($F[1,12] = 11.73$, $p < 0.01$), indicating a higher number of stained cells in SHAM than BLA groups. Neither the main effect familiarity ($F[1,12] = 3.47$, $p > 0.05$) nor the interaction ($F[1,12] = 2.13$, $p > 0.1$) were significant. The reduced FLI in the lesioned groups is consistent with previous reports [25]. However, post hoc planned comparisons by one-way ANOVAs showed that the difference was due to decreased c-fos expression in the BLA lesioned group after drinking the familiar cider vinegar solution ($F[1,5] = 7,43$, $p < 0.05$). However, there were no differences related with taste familiarity in the number of stained cells in the SHAM group ($F[1,5] = 2.54$, $p > 0.1$). There were no differences between BLA and SHAM groups FLI in the PRh after drinking the novel taste solution. No such differences were evident in the control areas (DH and AU1) examined. Fig. 4 displays representative microphotographs showing Fos-positive cells in each of the four experimental conditions.

Therefore, the results indicate that BLA bilateral lesions reduced FLI induced by the familiar cider vinegar solution in the PRh. This supports the notion that BLA and PRh are interdependent components of a neuronal network involved in safe taste memory. A FLI non-specific increase related with drinking behaviour or taste processing in the PRh can be discarded because there were no differences between the groups after being exposed to the novel taste during the first drinking session.

To our knowledge this is the first report relating immediate early gene expression and taste in the PRh. Previous studies in rats have found increased fos expression in the PRh when presented with novel visual objects [19,20,22,23]. Our results point to an involvement of the medial PRh also in the detection of taste familiarity. This is in accordance with previous studies demonstrating that protein synthesis [6] and cholinergic neurotransmission [3,8,26] in the same PRh portion are required for stabilization of safe taste memories. Hence, the present data represents additional evidence to previous research that has established the crucial role of the PRh in object recognition memory [16–18] and support an additional involvement of the area in taste recognition memory. Since similar increments of the Arc protein expression are induced by familiar rather than novel taste solutions in the insular cortex [27], it is conceivable

that the PRh might contribute to a widespread neural network involved in safe taste memory consolidation.

The approach applied, which combines lesion and FLI assessment used as a neuronal activity marker, has proven to be fruitful in order to delineate interdependent components of neural circuits involved in aversive taste memory [28–30]. Given the wide anatomical afferent projections relaying highly processed multisensory information to the PRh and its connections with the hippocampus, a similar approach has been used to map neuronal networks involved in spatial working memory [30]. Also, a comparable design has been applied to explore the effect of hippocampal inactivation by anisomycin on the reported taste familiarity-induced Arc increase in the insular cortex [27]. To this respect a main finding of the present study is the fact that amygdala lesions affect the fos expression in PRh reducing significantly FLI during detection of taste familiarity. This stands by the PRh as being a component of the neural circuit required for safe taste memory. The extensive anatomical reciprocal connections of the perirhinal cortex, the hippocampal formation, the insular cortex and BLA [17] might be the substrate underlying its role in recognition memory. In fact, functional efferent projections from to the perirhinal cortex to BLA have been shown by electrophysiological studies [17]. The perirhinal cortex in turn receives reciprocal connections from BLA.

The fact that BLA lesions interfere with the formation of safe taste memories is consistent with previous results applying permanent lesions or temporary inactivation of the area. Consistent with previous data the behavioral results obtained in the present experiment do not allow us to clearly dissociate the effect of the lesion either on the neophobic response [12,14] or the formation of the safe memory trace. Two main findings should be taken into account. First, BLA lesioned rats drank a higher amount of the novel cider vinegar solution than SHAM-lesioned animals, thus evidencing a reduced neophobic response. This is consistent with previous reports showing that BLA lesions affected the neophobic response [31]. However, these authors found absence of the neophobic response while the BLA group in the present study reduced their intake in comparison with the water baseline, thus indicating taste neophobia. This discrepancy could be due to the high neophobic response induced by the vinegar solution used in the present study while Kesner et al. applied grape juice [31]. Second, the reduced

neophobic response cannot explain the absence of the attenuation of neophobia since the neophobic response was evident and there are no reasons for a ceiling effect. In addition, the disruption of the attenuation of neophobia found in BLA group is consistent with ample evidence supporting a role of the area in the formation of the safe taste memory [7,9,10].

Whatever the contribution of BLA lesion-induced deficits in processing taste novelty and familiarity might be, the results indicate that they are interfering with the PRh activity related with safe taste recognition memory. Thereby, the requirement of the BLA functional integrity for both the detection of taste familiarity and FLI in the PRh supports the involvement of medial temporal lobe mechanisms in this type of memory. It has previously been proposed that different medial temporal lobe networks might be involved in safe an aversive recognition memory [6]. We have previously shown the hippocampal role in aversive taste memory provided that complex learning tasks are used [32,33]. The present results support a distinct mechanism relaying on the amygdala and perirhinal cortex for safe taste memory. Since the amygdala is known to be a component of the neural circuits involved in the formation of safe taste memories [1] it is conceivable that it might play distinct roles being a component of independent taste memory systems.

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Figures

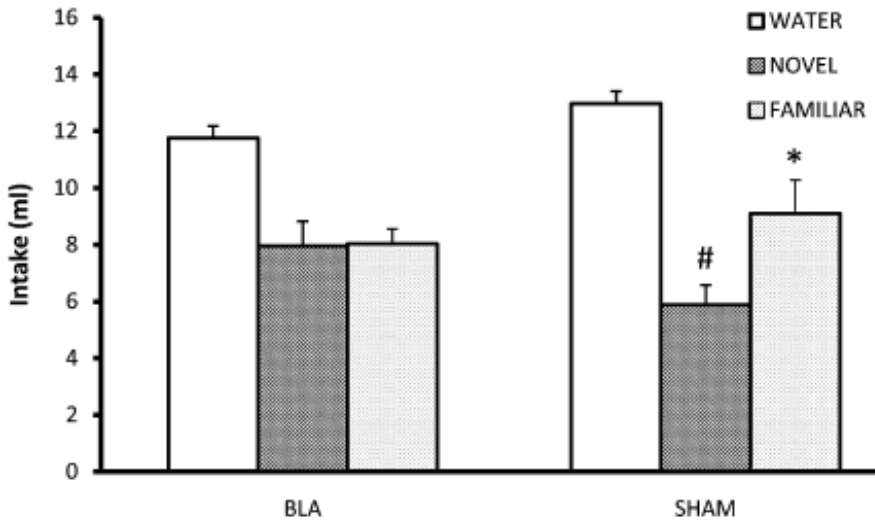

Fig. 1. Mean (±SEM) solution intake during the last day of water baseline (empty bars), the first (striped bars) and the second exposure (dotted bars) to the cider vinegar solution. * Novel versus familiar taste solution intake by the SHAM group ($p < 0.05$); # SHAM versus BLA groups' intake of the novel taste solution ($p < 0.05$).

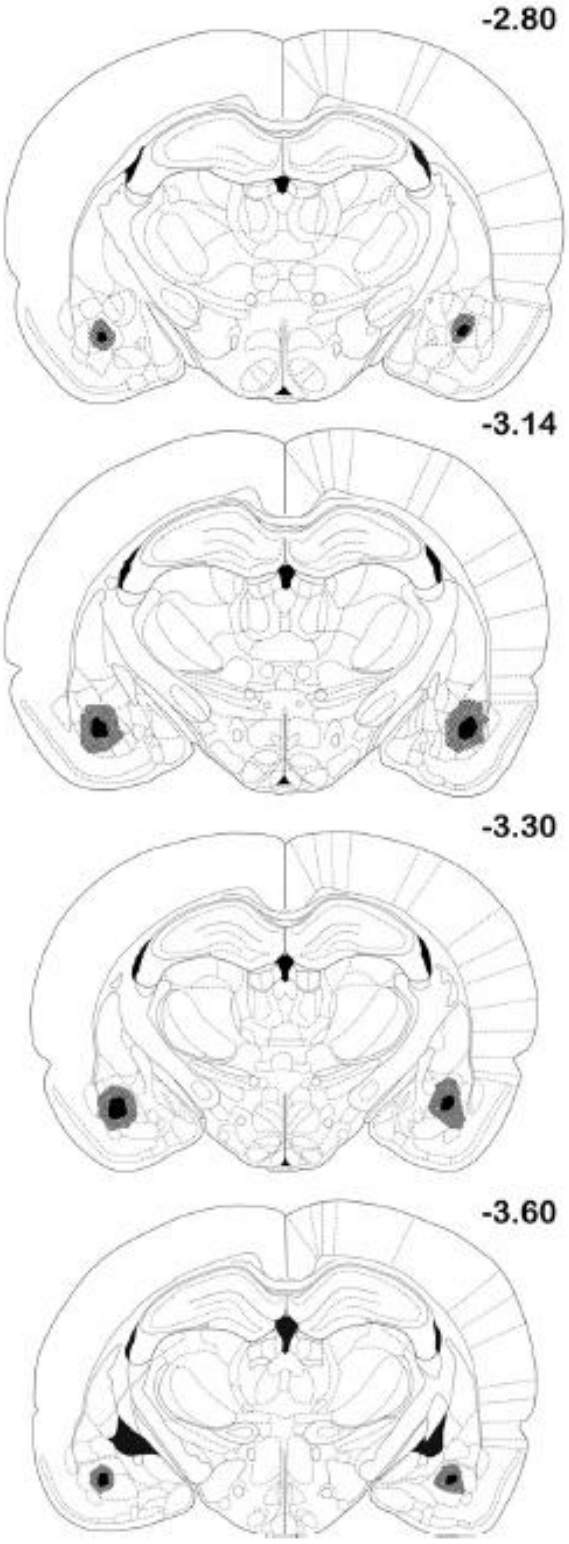

Fig. 2. Diagramatic reconstruction of the amygdala lesion, showing the máximum (gray shaded) and minimum (black) extent of the damage. The numbers indicate the A–P coordinates from bregma according to the Paxinos and Watson atlas [24].

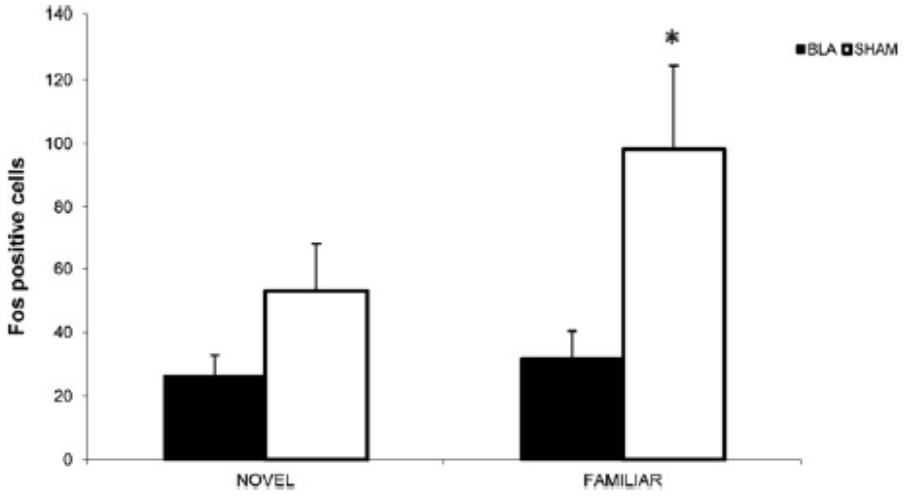

Fig. 3. Mean (±SEM) counts of Fos-positive cells in the perirhinal cortex after drinking the novel and the familiar solution in the BLA (black) and SHAM (white) groups.