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Modulation of the magnitude of conditioned taste aversion in rats with excitotoxic lesions of the basolateral amygdala

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ABSTRACT

The amygdala is one of the structures involved in the acquisition of conditioned taste aversion (CTA). Nevertheless, the specific roles that the nuclei of this structure play in CTA learning are controversial. Electrolytic lesions applied to the basolateral nucleus of the amygdala can eliminate or reduce the acquisition of this learning. This effect has been attributed to the involvement of fibers that pass through this nucleus and connect with other structures that are critical for CTA. Excitotoxic lesions may allow a clearer insight as to the potential involvement of this nucleus in the acquisition of CTA. The few studies to date that have used this paradigm have shown effects on taste aversion learning after applying extensive lesions to the amygdala. Thus, the aim of the present study was to determine the effect of selective excitotoxic lesions of the basolateral amygdala on the acquisition of CTA. The effects of these lesions on learning were compared with the effects observed in animals with sham lesions and animals with lesions of the hippocampus, which is a structure apparently not involved in CTA. The results revealed a decreased aversion in animals with basolateral lesions compared with both the sham and hippocampus-lesioned groups. Based on these findings, the role of this specific nucleus of the amygdala in the acquisition of taste aversion is briefly discussed.

1. Introduction

Conditioned taste aversion (CTA) learning depends on complex neural networks that include the brainstem and subcortical and cortical areas (Scott, 2011; Yamamoto & Ueji, 2011). Lesion studies provide relevant information regarding the specific brain structures involved in the acquisition of CTA (Bermúdez-Rattoni & Yamamoto, 1998). Specifically, lesion studies have demonstrated the involvement of the amygdala in associative processes related to CTA (Reilly & Bornovalova, 2005). However, the roles of the different nuclei of the amygdala in this conditioning remain unclear. For example, electrolytic lesions of the basolateral amygdala abolish the acquisition of CTA in some cases, but in most of the lesion studies, only a reduced aversion was found (Reilly & Bornovalova, 2005). Considering that electrolytic lesions induce damage to target cells and fibers of passage, it was argued that electrolytic lesions of the basolateral amygdala affect the acquisition of CTA only indirectly, through effects on critical connections for this learning (Dunn & Everitt, 1988; Fitzgerald & Burton, 1981; Schafe & Bernstein, 1996; Spray & Bernstein, 2004). But contrary to this assumption, in a subsequent behavioral and anatomical tract-tracing investigation, excitotoxic lesions of the basolateral amygdaloid complex severely compromised CTA without affecting the parabrachial-insular pathway (Morris, Frey, Kasambira, & Petrides, 1999).

It can be argued that excitotoxic lesions of the amygdala have not completely clarified the specific role of the basolateral amygdala as the studies that have analyzed effects on CTA have included extensive lesions of the amygdala that affected different nuclei (Schafe, Thiele, & Bernstein, 1998), and inconclusive results on taste aversion learning have been reported following lesions of the lateral and basolateral nuclei of the amygdala (Bermúdez-Rattoni & McGaugh, 1991; Dwyer, 2011; Ferry, Sandner, & Di Scala, 1995; Yamamoto, Fujimoto, Shimura, & Sakai, 1995). Since these studies have reported extensive lesions that have affected the basolateral amygdaloid complex, restricted lesions to the basolateral amygdala may shed light on the role of this particular nucleus on CTA.

The present study sought to explore the effects of selective bilateral excitotoxic lesions of the basolateral nucleus of the amygdala on CTA compared with sham and hippocampal lesions to determine the role of this amygdaloid nucleus in this particular conditioning. If the basolateral nucleus of the amygdala is necessary for the acquisition of CTA, animals with amygdaloid lesions

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will not acquire taste aversion when compared to animals with sham lesions. Because the hippocampus does not seem to be related to this type of conditioning (Yamamoto, 1993), hippocampal lesions should not affect CTA learning. In contrast, a simple reduction of taste aversion in animals with amygdaloid lesions could indicate a modulatory rather than an essential role of the basolateral amygdala in the acquisition of CTA.

2. Materials and methods

2.1. Animals

Thirty adult male Wistar rats, weighing between 280 and 320 g, were individually housed in boxes measuring 30 cm × 15 cm × 30 cm throughout the behavioral procedure, including periods of fluid intake. All animals were exposed to a 12-h light-dark daily cycle (lights on from 9:00 to 21:00), and the temperature was kept constant at 23 °C. Food was provided ad libitum, and the availability of fluid was restricted to 15 min daily throughout the behavioral procedure. The procedure was approved by the Ethics Committee for Animal Research of the University of Granada and was conducted in strict accordance with both the NIH Publications (N° 80-23) of the National Institutes of Health Guide (United States) for the Care and Use of Laboratory Animals (1996 revision) and the European Community Council Directive of 24 November 1986 (86/609/EEC). The National Legislation in agreement with this Directive is defined in the Royal Decree N° 1201/2005 of Spain.

2.2. Surgery

The rats were randomly distributed into the following three groups: BlAm, animals with lesions in the basolateral nucleus of the amygdala ($n = 10$); Hippocampus, animals with lesions in the dorsal hippocampus ($n = 10$); Sham, animals with sham lesions in the basolateral nucleus of the amygdala ($n = 10$). Each animal was anaesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and then placed in a stereotaxic apparatus for surgery (Stoelting Co. Instruments, Wood Dale, IL, USA). The incisor bar was set at 3.3 mm below the interaural line. After a longitudinal scalp incision was made to expose the skull, the bregma and lambda points were leveled in the horizontal plane, and two trepanations were made in each hemisphere for each animal using bregma as a reference point to create the lesions in the basolateral nucleus of the amygdala or the dorsal region of the hippocampus. The stereotaxic coordinates used to locate the areas of the lesions were taken from the atlas of Paxinos and Watson (2005) (Table 1).

All the animals, with the exception of the sham animals, received two successive injections of N-methyl-D-aspartate (NMDA) (0.6 μL; 0.077 M), i.e., one in each trepanation of each hemisphere, through an injection cannula (0.3 mm external diameter × 0.15 mm internal diameter) connected to a micro-syringe (Hamilton, 10 μL). The neurotoxin was injected at a rate of 1 μL/min using an injection pump (Harvard, USA). The cannula remained at the lesion site for 2 min after the injections to allow for the complete diffusion of the neurotoxin. Immediately thereafter, the cannula was removed and the incision was sutured. This procedure was identical for the sham group with the exception that no fluid with neurotoxin was infused through the cannula. The surgical procedure was the same for all three groups and the bilateral placements of the injection cannula were the same for the sham and BlAm groups. The rats were allowed a postoperative recovery period of seven days in which water and food were available ad libitum.

2.3. Behavioral procedure

At the end of the postoperative period, all of the animals were deprived of fluid for a daily period of 23 h and 45 min throughout the experiment. The drinking sessions (15 min) and subsequent recordings were performed in their home boxes at 11.00 am. The mean consumption of water by each group on the baseline day was recorded. Subsequently, on the conditioning day, all groups were exposed to a sodium saccharin solution (0.1%) for 15 min and their consumptions were recorded. Twenty minutes later, the animals received an injection of lithium chloride (LiCl; 0.15 M, 2% of body weight, ip) and were then returned to their home boxes to remain until the completion of behavioral testing. After one day of recovery with water (available for 15 min), all the animals were exposed again to saccharin for 15 min, and their consumptions were recorded to analyze the acquired aversion.

2.4. Histology

When the experiment was completed, the animals received a lethal overdose of sodium pentobarbital and were transcardially perfused. Then, the brains were extracted and stored in a 10% formalin solution and subsequently cryo-sectioned (Erma-422 cryostat, Tokyo) at approximately 45 μm. The slides were stained with cresyl violet and examined using an optical microscope (CH-30, Olympus). Images of the slides were captured with an Olympus TV camera (U-PMTVC, Japan).

2.5. Statistical analysis

The effects of the lesions on the acquisition of CTA were analyzed using a 3 × 3 factorial design, with one between-group factor (lesion) with three levels (*amygdaloid, hippocampal,* and *sham lesions*) and one inter-group factor (day) with three levels (*baseline, conditioning,* and *test days*). The data from the baseline, conditioning, and test sessions were analyzed with a factorial analysis of variance (ANOVA), and the significant factors were analyzed using one-way ANOVAs. For the significant factors and interactions, Newman-Keuls post hoc tests were applied to analyze the differences. The consumption on the test day was analyzed with a between-group (lesion) factorial covariance analysis (ANCOVA). The strength of taste aversion was calculated based on the consumption on the test day relative to the consumption on the conditioning day, and the resulting percentage was analyzed with a between-group (lesion) factorial ANOVA. In all tests, the critical level of significance for differences was set to $P < 0.05$.

Table 1
Stereotaxic coordinates.

Group	A-P 1	A-P 2	M-L 1	M-L 2	D-V 1	D-V 2
BlAm	−2.8	−3.3	±4.6	±4.6	+8.2	+8.8
Hippocampus	−2.8	−3.6	±1.6	±2.2	+3.4	+3.4
Sham	−2.8	−3.3	±4.6	±4.6	+8.2	+8.8

BlAm, basolateral nucleus of the amygdala; Sham, sham lesion in the basolateral nucleus of the amygdala; A-P, anterior-posterior axis; M-L, medial-lateral axis; D-V, dorsal-ventral axis.