Neurobiology of Learning and Memory 93 (2010) 117–126

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Intracranial self-stimulation recovers learning and memory capacity in basolateral amygdala-damaged rats

Pilar Segura-Torres *, Laura Aldavert-Vera, Anna Gatell-Segura, Diego Redolar-Ripoll [1], Ignacio Morgado-Bernal

Departament de Psicobiologia i de Metodologia de les Ciències de la Salut, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

ARTICLE INFO

Article history:
Received 15 April 2009
Revised 3 September 2009
Accepted 9 September 2009
Available online 15 September 2009

Keywords:
Rewarding brain stimulation
Intracranial self-stimulation
Basolateral amygdala lesion
Active avoidance
Memory modulation
Memory recovery

ABSTRACT

We studied the capacity of post-training intracranial self-stimulation (SS) to reverse or ameliorate learning and memory impairments caused by amygdala damage in rats. A first experiment showed that lesions of the basolateral amygdala (BLA) slow down acquisition of two-way active avoidance conditioning (2wAA). In a second experiment we observed that a post-training SS treatment administered immediately after each 2wAA conditioning session is able to completely reverse the disruptive effects of the BLA lesions, and the facilitative effect lasts for 10 days. A third experiment allowed us to differentiate the strong recuperative effects of the SS treatment from the slight effect caused by overtraining the same conditioning response. We concluded that SS is able to counteract the behavioral deficit induced by BLA damage, probably by activating alternative undamaged brain structures related to learning and memory, such as the hippocampus.

1. Introduction

Post-training intracranial self-stimulation (SS) facilitates the acquisition and retention of several tasks related to both implicit and explicit memory systems in rats (Coulombe & White, 1980, 1982; Huston & Mueller, 1978; Huston, Mueller, & Mondadori, 1977; Redolar-Ripoll, Aldavert-Vera, Soriano-Mas, Segura-Torres, & Morgado-Bernal, 2002; Ruiz-Medina, Morgado-Bernal, Redolar-Ripoll, Aldavert-Vera, & Segura-Torres, 2008a; Segura-Torres, Capdevila-Ortis, Marti-Nicolovius, & Morgado-Bernal, 1988; Soriano-Mas, Redolar-Ripoll, Aldavert-Vera, Morgado-Bernal, & Segura-Torres, 2005). Beside being especially effective in subjects with a naturally low learning capacity (Aldavert-Vera, Segura-Torres, Costa-Miserachs, & Morgado-Bernal, 1996) or a cognitive deficit caused by aging (Aldavert-Vera et al., 1997; Redolar-Ripoll et al., 2003), experiments from our laboratory have shown that SS is also able to ameliorate two-way active avoidance (2wAA) conditioning deficits caused by extensive bilateral lesions of the parafascicular nucleus of the thalamus (PF) (Redolar-Ripoll et al., 2003). In the present study, we aim to investigate if SS can be also able to functionally compensate the impairment caused by the damage of other brain structures belonging to neuroanatomic systems more critically involved in 2wAA conditioning, such as the amygdala.

The amygdala has been identified as a central structure in the emotional memory system. In general terms, it is well established that amygdala damage in humans and animals may impair emotional learning and memory (Bechara, 2005; Cahill, 2000; LeDoux, 2000, 2003; Rodrigues, Schafe, & LeDoux, 2004). There is also evidence that post-training treatments stimulating amygdala functioning can positively influence learning and retention in appetitively or aversively motivated tasks in rats (Lalumiere & McGaugh, 2005). The basolateral area of the amygdala (lateral, basal, and accessory basal nuclei) (Pitkanen, Savander, & LeDoux, 1997), is especially important for associative learning related to emotional stimuli (Dwyer & Killcross, 2006; Sah & Westbrook, 2008), and for 2wAA conditioning in particular (Savonenko, Werka, Nikolaev, Zielinski, & Kaczmarek, 2003). With reference to the acquisition of 2wAA, it has been proposed that the reactive response that responds to stimuli that predict danger by eliciting hard-wired defense responses (the classical component of 2wAA) would be mediated by direct pathways from the lateral nucleus (LA) to the central (Ce) nucleus of the amygdala, and that the ability of a conditioned stimulus to reinforce the acquisition of new or "active" responses (instrumental component of 2wAA) would be mediated by projections from the LA to the BLA (basolateral nucleus composed by the basal and basal accessory nuclei) (Amorapanth, LeDoux, & Nader, 2000; Savonenko, Filipkowski, Werka, Zielinski, & Kaczmarek, 1999).

* Corresponding author. Fax: +34 93 581 20 01.
E-mail address: pilar.segura@uab.cat (P. Segura-Torres).
[1] Universitat Oberta de Catalunya, Rambla de Poble Nou, 156, Barcelona 08018, Spain.

1074-7427/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2009.09.001

Another major function of the amygdala is the enhancement of memory consolidation for emotionally arousing experiences (McGaugh, 2004). Considerable evidence suggests that the basolateral complex of the amygdala (LA and BLA nuclei) is a key structure in a memory-modulatory system that regulates, in concert with other brain regions, stress and glucocorticoid effects on different memory functions (Nathan, Griffith, McReynolds, Hahn, & Roozendaal, 2004; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006).

In this context, we have carried out three experiments. The first evaluates the degree of impairment of 2wAA conditioning that can be caused by BLA lesions. A second experiment determines whether the same kind of post-training SS treatment that in our previous research has been able to reverse 2wAA impairments in rats with PF lesions, is also able to reverse the observed impairments in rats with BLA damage. To find out more about the nature of the observed effects, a third experiment compared the facilitative effect of the SS treatment with the improved performance produced by supplementary 2wAA training in rats with BLA lesions.

2. Experiment 1: effects of basolateral amygdala (BLA) lesions on 2wAA conditioning

This experiment was intended to test the effects of bilateral electrolytic lesion of BLA on subsequent 2wAA distributed conditioning. Rats were trained daily until they reached an established learning criterion.

2.1. Material and methods

2.1.1. Subjects

We used 32 naive male Wistar rats, obtained from our laboratory breeding stock, with a mean age of 92.19 days (*SD* = 4.20) at the beginning of the experiment, and mean weight of 446.47 g (*SD* = 37.34) at the time of surgery. All rats were housed singly, always kept under conditions of controlled temperature (20–24 °C) and humidity (40–70%), and subjected to an artificial light/darkness cycle of 12/12 h (lights on at 08:00 h). Food and water were available ad libitum. The rats were tested during the first half of the light cycle. The experiments were carried out in compliance with the European Community Council Directive for care and use of laboratory animals (CEE 86/609) and the Generalitat de Catalunya Decret (DOGC 2073 10/7/ 1995, DARP protocol number 2181).

2.1.2. Stereotaxic surgery

Before surgery rats were randomly distributed into three experimental groups: BLA-lesion (rats that would receive a bilateral lesion in the BLA nucleus, $n = 16$), BLA-sham (rats that would receive a bilateral sham implantation without lesion in the BLA nucleus, $n = 8$), and Control (rats that would receive a sham stereotaxic surgery without lesion or sham implantation, $n = 8$). Stereotaxic surgery (Model 1504, David Kopf Instruments) was performed under general anesthesia (150 mg/kg Ketolar® *Ketamine chlorhydrate* and 0.08 mg/kg Rompun® *Xylazin*; i.p.). Rats in the BLA-lesion group were submitted to bilateral electrolytic lesions using a direct current of 2 mA (Cibertec GL-2 electrical stimulator) for 9–15 s depending on the antero-posterior coordinate, with a bipolar insulated stainless steel electrode (250 μm in diameter). Although electrolytic lesions are considered as non-specific, they can be restricted to simple regions with minimal infringement onto neighboring structures (Nader, Majidishad, Amorapanth, & LeDoux, 2001). Moreover, it has been shown that this kind of lesions is more effective than other kind of lesions, such as ibotenic acid, to affect conditioning when applied to the amygdala (Lanuza, Nader, & Ledoux, 2004). The incisor bar was set at −2.7 mm below the interaural line, and the following stereotaxic coordinates were used: *AP* = −2.12, −2.8, −3.3, −4.16 mm from bregma; *L* = ±4.9, ±4.9, ±5.1, ±5.3; *P* = −9.0, −9.2, −9.0, and 9.0 with the cranium surface as dorsal reference (Paxinos & Watson, 1998).

2.1.3. Procedure

Once the rats had recovered from surgery (15 days), they were trained in 2wAA conditioning conducted in an automated shuttle-box (50 cm long × 24 cm wide × 25 cm high, Letica Li-916, PANLAB, Barcelona, Spain) enclosed in a sound-attenuating box ventilated by an extractor fan. The conditioning box was illuminated by a fluorescent bulb located on the sound-attenuating box. The two compartment floors (without any physical separation between them) were independently electrifiable and constructed of stainless steel bars (3.9 mm in diameter, 8.8 mm apart) that formed a shock grid. The conditioned stimulus was a 60-dB, 1-kHz tone of 3 s duration. The grid delivered a scrambled footshock unconditioned stimulus (0.5 mA intensity, maximum duration of 30 s) provided by a shock generator. The current supplied by the shocker was a positive semiwave of 100 Hz. The shuttle-box was connected to a computer that controlled the training schedule of 1 min (±10 s). Rats received one daily 10-trial 2wAA session until they obtained 80% or more correct responses in two consecutive sessions. Rats that did not achieve the established learning criterion were allowed to perform a maximum of 15 sessions (distributed in 3 blocks of 5 sessions separated by 2 days of rest). Just before the first acquisition session, the rats were allowed to ambulate freely in the shuttle-box for 10 min to become familiarised with the learning environment. During the learning training, animals avoided the shock by crossing to the adjacent compartment when the conditioned stimulus was on. Apart from the number of avoidance responses made in each training session (considered as the level of performance), inter-trial crossings and crossings during the free ambulation period were also scored and considered measures of locomotor activity.

2.1.4. Histology

At the end of the experiment, histological analyses were performed to verify the extent and location of the lesions. The animals were given an overdose of sodium pentobarbital (150 mg/kg, i.p.) and transcardially perfused with 0.9% physiological saline followed by 10% formalin (water and 37–40% formaldehyde). The animals' brains were removed and placed in a 30% sucrose solution before being cut into 40 μm sections on a freezing stage microtome (Cryocut 1800 with microtome 2020, JUNG). The tissue sections were stained following the acetylcholinesterase method and examined under a microscope (Olympus BX41, Olympus Optical CO., LTD Japan) connected to a photographic camera (Olympus DP70). They were examined separately by three people who were not aware of either the group or the performance of the subjects. Lesions were assessed neuroanatomically by examining sections for areas of neuronal loss. The damaged areas were represented by drawing them onto standardised sections of the brain from the atlas of Paxinos and Watson (1998).

2.1.5. Data analyses

The statistical computer package program SPSS 14.0 was used to process the data. The main analyses were carried out considering the independent variable as qualitative (three experimental groups), and the dependent variables as quantitative (performance in 2wAA conditioning sessions and locomotor activity). Multivariate analyses of variance (MANOVA) were performed with their corresponding contrast analyses. Survival analyses were also carried out to analyse and compare the mean number of sessions required by each experimental group to reach the established learning criterion (80% avoidance responses during two consecutive 2wAA