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Research report

Rewarding brain stimulation reverses the disruptive effect of amygdala damage on emotional learning

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HIGHLIGHTS

- Lateral amygdala (LA) lesions in rats fully prevent active avoidance conditioning.
- SS treatment completely reverses the abolition of conditioning in damaged subjects.
- Changes in the cholinergic activity of LA nucleus caused by SS could be implicated.
- Results are consistent with clinical observations applying DBS in the hypothalamus.

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ABSTRACT

Intracranial self-stimulation (SS) in the lateral hypothalamus, a rewarding deep-brain stimulation, is able to improve acquisition and retention of implicit and explicit memory tasks in rats. SS treatment is also able to reverse cognitive deficits associated with aging or with experimental brain injuries and evaluated in a two-way active avoidance (2wAA) task. The main objective of the present study was to explore the potential of the SS treatment to reverse the complete learning and memory impairment caused by bilateral lesion in the lateral amygdala (LA). The effects of post-training SS, administered after each acquisition session, were evaluated on distributed 2wAA acquisition and 10-day retention in rats with electrolytic bilateral LA lesions. SS effect in acetylcholinestaresase (AchE) activity was evaluated by immunohistochemistry in LA-preserved and Central nuclei (Ce) of the amygdala of LA-damaged rats.

Results showed that LA lesion over 40% completely impeded 2wAA acquisition and retention. Post-training SS in the LA-lesioned rats improved conditioning and retention compared with both the lesioned but non-SS treated and the non-lesioned control rats. SS treatment also seemed to induce a decrease in AchE activity in the LA-preserved area of the lesioned rats, but no effects were observed in the Ce.

This empirical evidence supports the idea that self-administered rewarding stimulation is able to completely counteract the 2wAA acquisition and retention deficits induced by LA lesion. Cholinergic mechanisms in preserved LA and the contribution of other brain memory-related areas activated by SS could mediate the compensatory effect observed.

1. Introduction

Currently, deep-brain stimulation (DBS) therapies are used to reduce or alleviate symptoms of varying neurodegenerative diseases resistant to chemical therapies such as Parkinson's disease and major depression. Recently, it has been demonstrated that hypothalamic DBS might also be able to modulate memory [1]. Subsequently, the cognitive effects of DBS in different brain areas, such as the fornix/hypothalamus and the basal nucleus of Meynert, have been studied in patients with early- or middle-stage Alzheimer disease, showing that DBS may have beneficial effects on this disorder [2–4]. How these electrical brain-stimulation therapies can improve memory capacity and their potential ability to reverse cognitive deficits is still little understood, and basic research using animal models may provide insight and assistance

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Table 1
Lateral amygdala lesions coordinates in accordance with Paxinos and Watson [16], and current duration in experiments 1 and 2. Electrolytic lesion parameters are based on Nader et al. [27].

Location of LA lesion	Coordinates				Time Exp. 1	Time Exp. 2
	Posterior	Lateral	Ventral	Current		
Anterior	−2.28	±5.2	8.0	2.0 mA	9 s	10 s
Mid	−3.12	±5.3	8.0	2.0 mA	12 s	15 s
Posterior		±5.5	8.1	2.0 mA	15 s	18 s

in exploring their therapeutic potential for subjects with severe memory impairment.

Electrical stimulation in the lateral hypothalamus (LH) activates the medial forebrain bundle and many other regions forming part of the reward system; this is reinforcing, and experimental animals behave in a self-stimulating manner (intracranial self-stimulation behavior, SS). In rats, this kind of DBS constitutes an extremely effective treatment, improving the acquisition and retention of several tasks related to both implicit and explicit memory systems [5,6]. Behavioral data suggest that SS produces an acceleration of memory consolidation in healthy rats [7], but it has been shown to be especially effective in promoting learning and memory for subjects with a natural low capacity for learning [8] or with cognitive deficit caused by aging [9,10]. SS has also showed memory-deficit reversing properties in animals with brain damage. In this respect, in a previous study, we observed that SS was capable of reversing the amnesic effects of the thalamic parafascicular nucleus (PF) and the basolateral amygdala (BLA) lesions on two-way active avoidance (2wAA) conditioning [10,11].

Results from different experimental approaches agree that the lateral nucleus of the amygdala (LA) is critical for the acquisition of fear memories and is an essential locus of fear-memory storage [12]. Moreover, it has been reported that the plasticity mechanism underlying fear conditioning involves acetylcholine receptors in the LA [13]. In fact, LA appears to be a key element in the neural circuitry involved in both of the 2wAA conditioning components, the Pavlovian fear-conditioning and the escape-from-fear response, in which the conditioned stimulus (CS) reinforces a locomotor response terminating the unconditioned stimulus (US). In particular, LA lesions blocked acquisition of both conditioned freezing responses and CS reinforcement of a new response, whereas BLA lesions blocked the escape-from-fear response but not conditioned freezing and central nucleus of the amygdala (Ce) is not required but may constrain the instrumental avoidance response [14,15].

The aim of this work is to explore the potential of post-training SS treatment to reverse a more severe impairment of a 2wAA conditioning caused by bilateral lesions of LA. An initial study established the experimental conditions in which LA lesion causes complete impairment of conditioning (experiment 1). We then assessed whether the treatment of SS was able to revert the impaired function in LA-damaged rats, and the degree to which this would occur. Additionally, in order to study in a preliminary manner the possible involvement of the cholinergic system in functional compensation caused by treatment, we analyzed acetylcholinesterase (AChE) activity in the LA-preserved area and the medial and lateral central amygdaloid nuclei (CeM and CeL) from SS-treated and non-treated lesioned animals (experiment 2).

2. Materials and methods

2.1. Subjects and experimental groups

A total of 93 naive male Wistar rats obtained from our laboratory breeding stock were used, with a mean age of 93.21 days (*SD* = 5.20) and mean weight of 389.45 g (*SD* = 34.31) at the beginning of the experiment. The animals were maintained on a 12 h light/dark cycle, and food and water were available ad libitum. All procedures were approved by the Ethics Committee at the Universitat Autònoma de Barcelona (protocol 2022).

In the experiment 1, rats were randomly distributed into three experimental groups: Lesion (bilateral lesion in the LA, $n = 20$); Sham-L (electrode introduction without lesion, $n = 10$); and Control (stereotaxic surgery without electrode introduction, $n = 6$). In the experiment 2, the groups were as follows: Lesion ($n = 10$), SS (SS treatment after each 2wAA acquisition session, $n = 15$), Lesion + SS (LA lesion and SS-treatment, $n = 10$), Sham-L ($n = 12$) and Sham-SS (SS electrode implantation, but without stimulation, $n = 10$). Some rats were not included in the main analyses because of problems with the 2wAA program or with histology (1 in the Sham-L; 3 in the Lesion + SS; 1 in the Lesion).

2.2. Stereotaxic surgery. Electrolytic lesions and/or SS electrodes implantation

Rats were anesthetized with a ketamine (110 mg/kg Ketolar®, i.p.) and xylazine (23 mg/ml; Rompun® i.p.) solution for surgery. Rats in the Lesion group were submitted to bilateral electrolytic lesions using a current of 2 mA for 9–15 s (Cibertec GL-2) with a bipolar insulated stainless-steel electrode (250 μm in diameter); LA lesions were produced in three locations along the rostrocaudal extent of the nucleus, as specified in Table 1. These were equivalent in the two experiments except for time of lesion, which was marginally extended in the second experiment as an attempt to increase the LA volume affected (see Table 1).

Rats in the Lesion + SS and SS groups were implanted with a monopolar stainless-steel electrode (150 μm in diameter) aimed at the LH, in accordance with coordinates from the Paxinos and Watson stereotaxic atlas [16]: AP = −2.28 mm from bregma, ML = 1.8 mm (right hemisphere) and DV = −8.8 mm. Behavioral procedures were initiated after a post-surgical recovery period of 12–14 days.

2.3. Behavioral apparatus

2.3.1. SS box

SS behavior was trained in a conventional Skinner operant chamber (24 cm long × 27 cm wide × 30 cm high; LE 850, Letica Scientific Instruments, Panlab, Barcelona, Spain) made of clear Plexiglas with a front door. One of the chamber walls—stainless steel—is fitted with a lever (4.5 cm above the floor) and lamp (22 cm above grid floor, 15w); this was lit to signal each reinforced response. The SS box was housed within a sound-attenuating box (LE 26, Letica Scientific Instruments, Panlab, Barcelona, Spain), with an observation hole in the front door and a constantly lit fluorescent bulb located on the back wall (22 cm above floor, 10w) of the sound-attenuating box, for general illumination.

Electrical brain stimulation, which animals self-administered whenever they pressed the lever, was delivered by a sine-wave stimulator (CS2-10 Cibertec, Madrid, Spain). Stimulation consisted of 0.3-s trains of 50 Hz sinusoidal waves at intensities ranging from 10 and 250 μA.