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Research report

Lesions of the lateral habenula facilitate active avoidance learning and threat extinction

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HIGHLIGHTS

- LHb was damaged to study its role in avoidance and threat learning.
- LHb lesions facilitated two-way active avoidance learning.
- LHb lesions had no effects on the acquisition of conditioned threat responses.
- LHb lesions accelerated the extinction of conditioned threat memory.

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ABSTRACT

The lateral habenula (LHb) is an epithalamic brain structure that provides strong projections to midbrain monoaminergic systems that are involved in motivation, emotion, and reinforcement learning. LHb neurons are known to convey information about aversive outcomes and negative prediction errors, suggesting a role in learning from aversive events. To test this idea, we examined the effects of electrolytic lesions of the LHb on signaled two-way active avoidance learning in which rats were trained to avoid an unconditioned stimulus (US) by taking a proactive shuttling response to an auditory conditioned stimulus (CS). The lesioned animals learned the avoidance response significantly faster than the control groups. In a separate experiment, we also investigated whether the LHb contributes to Pavlovian threat (fear) conditioning and extinction. Following paired presentations of the CS and the US, LHb-lesioned animals showed normal acquisition of conditioned response (CR) measured with freezing. However, extinction of the CR in the subsequent CS-only session was significantly faster. The enhanced performance in avoidance learning and in threat extinction jointly suggests that the LHb normally plays an inhibitory role in learning driven by absence of aversive outcomes.

1. Introduction

According to the law of effect [1], two types of feedback signals are fundamental for regulating our everyday behavior and decision making: a punishment that would suppress the behavior and a reinforcement that would encourage the behavior. The two behavioral consequences must be encoded in the brain and used as instructive signals to adjust subsequent behavior. Several brain structures and related circuits have long been proposed to process the two types of signals [2–5]. In recent years, growing evidence has indicated that the lateral habenula (LHb) is one of the punishment-related brain regions, based on its encoding of negative prediction errors [6,7]. For instance, Hikosaka et al. reported that LHb neurons in primates are excited by aversive stimuli unexpected reward omission, and sensory stimuli that predict upcoming aversive events or omitted reward [8]. These neurons are inhibited in response to reward-predicting cues and unexpected reward delivery. Similar neuronal responses were also observed in the rodent LHb, even though the number of neurons with such response patterns were considerably low [9]. In addition, when LHb cells were optogenetically stimulated at a particular area, the animals developed behavioral avoidance of that area. These findings collectively suggest the LHb may play an important role in learning about punishment or aversive outcomes such as avoidance learning and threat (fear) learning.

Indeed, several previous studies examined the effects of electrolytic LHb lesions on instrumental avoidance conditioning procedures, in which animals acquire an avoidance response to a conditioned stimulus (CS) in order to prevent an aversive uncon-

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ditioned stimulus (US) [10–14]. However, their results were not in agreement. Some studies found an enhancement in the acquisition of avoidance learning [10,14], whereas others reported no effects [11] or even learning deficits [12,13]. The discrepancies might be due to differences in damaged areas and in learning paradigms that required preparative jumping in the current location or moving into an adjacent safe box. Especially, all of the previous studies did not selectively lesion the LHb, but rather made extensive lesions to other areas surrounding the LHb such as the medial habenula (MHb) and dorsomedial thalamus. Thus we revisited this issue with more discrete lesions confined within the LHb using a signaled two-way active avoidance task (2-AA), one of the most widely used instrumental conditioning paradigms [15]. The task required rats to shuttle back and forth between two compartments to avoid a footshock US signaled by an auditory CS.

In a separate experiment, we also investigated the role of the LHb in Pavlovian threat conditioning and extinction. In a typical acquisition of threat conditioning, a neutral CS is repeatedly paired with the footshock US regardless of the animal's response. During subsequent extinction, the CS is presented repeatedly without the US, which involves re-evaluation of learned negative value associated with the CS. A few studies have consistently shown that damage to the LHb does not alter the course of acquisition [16]. However, effect of LHb lesion on threat extinction has not been examined. Given the role of LHb neurons in negative prediction error signaling [6], we hypothesized that LHb lesion would alter the course of extinction, since extinction is a form of learning driven by the unexpected absence of the US.

2. Materials and methods

2.1. Subjects

Fifty eight and twenty five male Sprague-Dawley rats (270–320 g, Orient Bio, Kyunggi-do, Korea) were used in experiments 1 and 2, respectively. They were individually housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle (lights off at 9:00 a.m.). All subjects were given *ad libitum* access to food and water. All experimental procedures were conducted in compliance with the guideline of Care and Use of Laboratory Animals in Korea University.

2.2. Surgery

Each rat was fully anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and mounted on a stereotaxic frame (Stoelting Co., Wood Dale, IL). The skull was exposed and adjusted to place bregma and lambda in the same horizontal plane. The skull was drilled for electrode holes. Lesion electrodes (0.3 mm in diameter) which were insulated except for the 0.5 mm tip were bilaterally inserted into the LHb (3.6 mm posterior, 0.6 mm lateral, and 4.8 mm ventral to bregma). Once the electrodes were positioned, DC current (1.0 mA for 10 s) was passed [17,18]. Sham-lesioned animals received the identical surgical procedure except that no electrical current was delivered. After the scalp was sutured, the rat was placed back in the home cage and allowed to recover for at least 7 days.

2.3. Experiment 1: signaled two-way active avoidance

The 2-AA task was carried out in a shuttle box (28 × 63 × 26 cm) which was divided into two identical compartments by a Plexiglas divider with an opening in the center (8 cm wide). Each compartment contained a grid floor with 16 stainless steel bars through which a footshock unconditioned stimulus (US; 0.5 mA) was delivered using a scrambled shocker (Coulbourn instruments, Whitehall, PA). An auditory tone as a conditioned stimulus (CS; 2 kHz, 80 dB) was delivered by two speakers located on opposing walls of the box. The box was placed in a sound-attenuating chamber (120 × 150 × 210 cm). Rats' movement was monitored by a video camera mounted on the ceiling of the box. The CS and US presentation and detection of animals' position were fully automated by custom-written LabView software (National Instrument, Austin, TX).

Forty four and fourteen rats in the lesion and sham groups, respectively, were individually acclimated to the shuttle box by allowing them to freely explore for 3 min. On the next 5 consecutive days, they were trained to shuttle between the two compartments in order to avoid a footshock US that were signaled by a preceding CS [15]. In each day, rats received 30 avoidance trials with an averaged inter-trial interval (ITI) of 60 s. The ITI was set to be shorter than those used in other studies [15,19] in order to make the task more demanding, based on pilot experiments in the laboratory. A trial began with a CS that lasted a maximum of 15 s. A shuttle response during the CS led to immediate CS termination and no US delivery. Failure to make the avoidance response resulted in the delivery of the US which lasted until the animal moved to the other compartment or 15 s, whichever comes first.

2.4. Experiment 2: threat conditioning and extinction

A rectangular Plexiglas box (30 × 34 × 42 cm) was located in a red-lit, sound-attenuating chamber (58 × 58 × 68 cm). The box had a grid floor wired for scrambled footshock [18,20]. An auditory CS (2 kHz, 80 dB) was delivered via a speaker attached to one side wall. A video camera was fixed on the side wall of the chamber to record and monitor the animal's behavior. The presentation of CS and US was automated by custom-made LabView software.

For threat conditioning, fifteen and ten rats in the lesion and sham groups, respectively, were individually exposed to the conditioning box for 3 min. On the next day, they were returned to the box and given five CS-US pairings with an averaged ITI of 120 s. Each CS (15 s) was co-terminated with a footshock US (0.5 s, 1.0 mA). On the following day, the animals received extinction training during which the CS was presented 20 times without the US in a novel context. The box for extinction contained a flat Plexiglas floor instead of the grid floor and it was located in a blue-lit chamber. The rats were again returned to the extinction context 24 h later and presented with four CSs for extinction retention. To measure rats' threat responses during the CS presentation, freezing behavior, defined as the absence of movement except for respiration, was analyzed offline using a digital stopwatch.

2.5. Open field test

A square arena (77 × 77 × 25 cm) was located in a room surrounded by a black curtain. The open field was virtually subdivided into central area (45 × 45 cm) and peripheral areas. One week after completing the threat conditioning and extinction task, rats were individually placed in the center of the arena and allowed to explore freely for 10 min. Their movement trajectories were recorded by a video camera and analyzed using a tracking software (ANY-Maze, Stoelting, Wood Dale, IL).

2.6. Histology

After completion of all experiments, all rats were deeply anesthetized with overdose of pentobarbital sodium (120 mg/kg, i.p.) and transcardially perfused with 0.9% saline, followed by a 10% formaldehyde solution. After extraction, the brains were stored in a 10% formalin/30% sucrose solution and cut in coronal sections (50 μm) on a sliding microtome (SM 2000R, Leica, Nussloch, Germany). Every third section containing the LHb (from −3.1 to