Behavioural Brain Research 353 (2018) 168–175

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Effect of electrical stimulation of the infralimbic and prelimbic cortices on anxiolytic-like behavior of rats during the elevated plus-maze test, with particular reference to multiunit recording of the behavior-associated neural activity

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ARTICLE INFO

Keywords:
Anxiolytic-like behavior
Brain stimulation
Elevated plus-maze
Infralimbic cortex
Medial prefrontal cortex
Multiunit recording

ABSTRACT

Fear and anxiety affect the activities of daily living and require concerted management, such as coping strategies, to preserve quality of life. The infralimbic (IL) and prelimbic (PL) medial prefrontal cortices have been implicated in the regulation of fear- and anxiety-like behavior, but their roles in overcoming fear- and anxiety-like behavior remain unknown. We investigated the anxiolytic-like effects of electrical stimulation of the IL and PL cortices in rats during the elevated plus-maze test. IL stimulation led to a significantly higher percentage of time spent and entries in the open arms, whereas PL stimulation did not have any significant behavioral effects. Subsequently, we recorded multiunit activity from the IL and PL cortices in rats using a wireless telemetry device, to determine whether activation of the IL occurs when rats enter the open arms in the elevated plus-maze test. The firing rate of IL neurons increased 1–3 s prior to entry from the closed arm to the open arm, whereas there were no corresponding changes in the firing rate of PL neurons. Taken together, the present findings suggest that the IL plays a key role in exerting active action to overcome anxiety-like behavior.

1. Introduction

Fear and anxiety often disturb the activities of daily living and sometimes require concerted coping strategies to preserve the quality of life. Although the amygdala has been identified as an important brain region in emotional processing [1–4], playing a role in the expression of fear and anxiety [5–9], the neural mechanisms underlying the process of overcoming fear and anxiety remain unclear. The medial prefrontal cortex (mPFC) has direct projections to the amygdala and is thought to regulate its activity [10]. Lesion or inactivation of the mPFC modify fear- and anxiety-like behavioral responses in rodents; however, the nature of this effect is somewhat controversial. For example, Jinks and McGregor [11] reported that lesion of the mPFC inhibits coping behavior in rats exposed to the threating or aversive environment in the elevated plus-maze test; similarly, Lisboa et al. [12] showed that pharmacological inactivation of the mPFC enhances anxiety-like behavior in rats submitted to the elevated plus-maze. In contrast, Shar and Treit [13,14] reported that lesion and pharmacological inactivation of the mPFC produces anxiolytic-like effects in the elevated plus-maze test. Hamani et al. [15] demonstrated that electrical activation of the mPFC induces antidepressant-like effects in the forced swimming test, while Scopinho et al. [16] found that pharmacological inactivation of the mPFC induces an antidepressant-like effect in this paradigm. Although these reports are not in agreement, they suggest that the mPFC functions as the neural basis for the control of fear and anxiety.

The mPFC is a heterogeneous structure. The ventral mPFC includes the infralimbic cortex (IL) and prelimbic cortex (PL), which have different projections to the amygdala. Whereas IL fibers project to widespread areas of the amygdala, PL fibers primarily project to the central and basolateral nuclei [10]. Electrophysiological studies of fear conditioning have shown that increased firing of IL neurons correlates with the recall of extinction learning, whereas increased firing of PL neurons correlates with sustained fear [17–19]. A study using intracortical stimulation similarly showed that IL stimulation decreases freezing behavior in response to a conditioned tone, whereas PL stimulation increases freezing behavior [20]. These studies of fear conditioning

Abbreviations: ANOVA, analysis of variance; HPC, hippocampal formation; IC, integrated circuit; IL, infralimbic cortex; mPFC, medial prefrontal cortex; PL, prelimbic cortex; SEM, standard error of the mean; vmoPFC, ventromedial orbital prefrontal cortex

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https://doi.org/10.1016/j.bbr.2018.07.005
Received 11 April 2018; Received in revised form 29 June 2018; Accepted 5 July 2018
Available online 06 July 2018

suggest that the IL and PL exert distinct functions with regard to fear and anxiety.

The purpose of the present study was to determine the roles of the IL and PL in anxiolytic-like behavioral effects. We investigated behavioral responses to electrical stimulation of the IL and PL and the neural activity of the respective neurons in rats during the elevated plus-maze test. We hypothesized that activation of the IL is associated with anxiolytic-like responses, whereas the activation of the PL is associated with anxiety-like responses.

2. Material and methods

2.1. Subjects

We used a total of 136 male Sprague-Dawley rats (Japan SLC, Inc., Shizuoka, Japan) that were 9–12 weeks of age at the time of testing: 77 rats were used to investigate behavioral responses to electrical stimulation of the IL and PL, and 59 rats were used to examine the neural activity of IL and PL neurons in the elevated plus-maze test. They were group-housed in a room with controlled temperature (24 °C) on a reverse 12-h light/dark cycle (lights on at 20:00 h) with food and water available *ad libitum*. The experiment protocol was approved by the Animal Research Committee of Kyoto University Graduate School of Medicine (permit number: Med Kyo 17508).

2.2. Elevated plus-maze test

The elevated plus-maze apparatus was located in a dark test-room illuminated by red lighting (5 lx). The maze was elevated 50 cm above the floor and consisted of 2 open arms (50 cm × 10 cm) and 2 closed arms (50 cm × 10 cm × 50 cm) joined by a central square (10 cm × 10 cm) (Fig. S1 in Appendix A). The floor and walls of the maze were made of opaque black Plexiglas. A video camera (CCD-2; Biotex Co., Kyoto, Japan) was fixed above the maze and relayed video to a computer monitor in an adjacent room.

2.3. Surgery

Surgeries were performed with some modifications, as previously described [21]. Briefly, rats were anesthetized and maintained with a mixture of 2% isoflurane and nitrous oxide/oxygen (7:3) and placed in a stereotaxic apparatus (Model 1430; David Kopf Instruments, Tujunga, CA). The animal body temperature was maintained at 37 °C during surgery with the use of a heating lamp. The scalp was incised and retracted, a burr hole was drilled 10.0–11.5 mm anterior to the interaural line and 0.0–1.5 mm lateral to the midline, and the underlying dura was carefully retracted. Three additional holes were drilled in the skull for the placement of stainless-steel anchoring screws. A straight array of 2 urethane-insulated stainless-steel wires (50 μm in diameter, tip distance of 100 μm, impedance of approximately 140 kΩ; Unique-Medical Co., Tokyo, Japan) was connected to an integrated circuit (IC) pin (21,501 × 4E; Linkman Co., Fukui, Japan) and used as a bipolar stimulating electrode in experiment 1 and as a recording electrode in experiment 2. The electrode was lowered perpendicularly to the mPFC (10.5–11.0 mm anterior to the interaural line, 0.5–0.8 mm lateral to the midline, and 2.0–4.7 mm ventral to the cortical surface) into the PL and IL [22] by using a micromanipulator (Model 1760-61SB; David Kopf Instruments), and cemented to the skull and anchoring screws with dental acrylic. In experiment 2, an additional hole was also drilled in the nasal bone for the placement of a stainless-steel screw, which was soldered to a flexible electric wire and used as a signal reference. A U-shaped plastic plate was cemented to the skull and used to attach the wireless transmitter to the animal's head. All surgical incisions were carefully closed, and rats were treated with antibiotics (benzylpenicillin; Meiji, Tokyo, Japan) and placed in a comfortable position on a heating blanket. After waking, rats were housed in individual cages to avoid attacks of other animals on the wound and allowed to recover for at least 7 days prior to behavioral testing. Animals were monitored for potential signs of pain and infection or improper wound healing.

2.4. Experimental design

Experiments were conducted between 10:00 and 18:00 (during the dark phase of the light cycle).

In experiment 1, electrical stimulation was delivered through the bipolar stimulating electrode. The stimulation consisted of biphasic square-wave trains with pulses (0.2 ms pulse duration, 100 μA) delivered at 20 Hz for 3 min (total duration of stimulation) with a stimulator (MEN3302, Nihon Koden, Tokyo, Japan) and a biphasic stimulation isolator (MEN212, Nihon Koden). For choosing these stimulation parameters, two considerations were taken. First, a 20-Hz frequency was chosen to activate IL and PL neurons according to a related study [23]. Second, preliminary observations indicated that a current higher than 100 μA induced motor effects in some animals (i.e. head shaking). The current amplitude was monitored via the voltage amplitude across a 1-kΩ resistor, in series with the return lead of the current source. Individual rats were placed in the closed arm and behavior was recorded for 13 min [1 min to allow for recovery from handling (PRE) and a 12-min test session]. The 12-min session was divided into four 3-min periods: a first period of active stimulation (ON1), a second of no stimulation (OFF1), a third of active stimulation (ON2), and a fourth period of no stimulation (OFF2). The maze was cleaned with distilled water after each test-session and dried before introduction of the next animal into the maze. Behavioral data were scored after the sessions by a blinded experimenter. The experimenter quantified the time spent in the open and closed arms, as well as the number of entries into each arm. An entry was defined as the simultaneous placement of all 4 paws into the respective arm. The time spent in the open arms was quantified as the percentage of the total time spent in all of the arms, and the number of open arm entries was quantified as the percentage of the total number of arm entries [14,24,25]. A higher percentage of time spent in the open arms and higher percent of open arm entries were interpreted as measures of anxiolysis, and the number of closed arm entries was interpreted as an index of locomotor activity [14,24,26,27].

In experiment 2, the wireless telemetry transmitter used for recording neural activity in awake animals was described in our recent publication [21]. Briefly, the wireless telemetry transmitter was designed to attach to the head of a rat. The device was 2.6 cm in length, 1.0 cm in width, 1.9 cm in height, and weighed 6.0 g. The device was powered by a commercially available lithium coin cell battery (CR2032) and had a transmitter range of approximately 10 m. Due to its crest-like shape when attached to the head of a rat, we named it "Tosaka," which means "the crest of a rooster" in Japanese (Fig. S1 in Appendix A). The Tosaka transmitter was attached to the head of each rat prior to the animal's placement in the closed arm of the maze. Behavior and multiunit activity of the IL or PL were recorded for 10 min. The neural activity signal was received by 4 dipole antennas attached below the floor of the maze. The signal was band-pass filtered (100–3000 Hz) and was relayed to a data acquisition system. Neural activity was continuously acquired, sampled (10 kHz), and stored with a PowerLab/LabChart-7 system (ADInstruments, Nagoya, Japan). Firing data were analyzed off-line using custom-designed software with a template-based sorting program (Multiunit sorting, counting, and analyzing tools, ver. 8; Muscat-8) run on a personal computer. We discriminated and counted spikes with a signal-to-noise ratio > 2.0. The mean firing rates in the open and closed arms and event-related firing rates were analyzed for each rat. As the primary focus of this experiment was to investigate the population activity of IL and PL neurons (rather than to analyze differences in the responses of individual neurons), all recorded unit activity was analyzed as multiunit activity.