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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

高架式十字迷路テスト中のラット抗不安様行動に及ぼす下辺縁皮質および前辺縁皮質の電気刺激の影響とその行動に関連する神経活動のマルチユニット記録

清水朋子

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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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.

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

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

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 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 lux). 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 (21501x4E; 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.

2.5. Histology

After the elevated plus-maze test in both experiments, all rats were deeply anesthetized with an overdose of sodium pentobarbital (100 mg/kg, i.v.). Firstly, the location of the electrode tip was marked by applying a positive current (1 μA, 60 s) through the electrode to identify electrode position. Subsequently, rats were perfused transcardially with saline followed by 10% formalin in 0.1 M phosphate buffer. Brains were removed, cryoprotected in 30% sucrose, frozen, and sectioned through the mPFC (50 μm thickness) on a sliding microtome. The sections were mounted onto gelatinized slides and counterstained with 0.3% neutral red for examination.

2.6. Statistical analysis

Results were expressed as the mean ± standard error of the mean (SEM). Behavioral data were analyzed by two-way repeated measures analysis of variance (ANOVA) with the stimulation site (IL, PL, or control) as the between-subject factor and the period (PRE, ON1, OFF1, ON2, OFF2) as the within-subject factor. The Bonferroni test was used for subsequent post-hoc multiple comparisons. For the multiunit data, firing rates of IL and PL neurons (100-ms bin-size) were normalized in each animal so that the average bin values for the entire period (10 min) was 1.00 [28]. According to a previous related study, all significant changes in mPFC unit activity occur 1–3 s prior to the animal's transition from one arm to the other in the elevated plus-maze [29]. In the present study, to explore event-related activity of IL and PL neurons, multiunit activity was analyzed 1–3 s prior to open or closed arm entry. We examined the firing rates during four types of transitions: closed to open arm, closed to closed arm, open to open arm, and open to closed arm transitions (arm entry was defined when all four paws were placed in the arm). The normalized neural data were compared using either paired *t* test or one-way repeated measures ANOVA with the

Bonferroni post-hoc test. The criterion for statistical significance was $P < 0.05$ (SPSS Statistics 22.0; IBM, Tokyo, Japan).

3. Results

3.1. Histology

The tip positions of the stimulating electrodes in experiment 1 and the recording electrodes in experiment 2 were examined in coronal sections. The borders between the IL and PL were delimited based on the cytoarchitectonic features described by Krettek and Price [30]: the IL was characterized by relatively indistinct lamination and an uneven border between layer I and layer II, whereas the PL was characterized by a well-defined lamination, in which layer V contained large dark stained cells and layer VI contained horizontally elongated cells tightly packed into 2 thin layers. In experiment 1, the electrode tips in the stimulation group were located in the IL in 22 rats and in the PL in 19 rats (Fig. 1). In the control group, the electrode tips were located in the IL or PL in 26 rats. In experiment 2, the recording electrode tips were located in the IL in 24 rats and in the PL in 24 rats. In the PL, electrodes were implanted in the dorsal portion of the PL but not in the ventral portion of the PL in order to observe distinct differences between typical neural activities of PL and IL neurons.

3.2. Effect of stimulation of the infralimbic and prelimbic cortices on anxiolytic-like behavior of rats during the elevated plus-maze test

Data from 2 rats in the control group were excluded because the animals fell off of the open arm of the maze.

Fig. 2 depicts the percentage of time spent in the open arms. Most rats with IL stimulation showed an increase in the percentage of time spent in the open arms in ON1 and ON2 (Fig. 2A, B, Appendix B). In the case of ventral PL stimulation, a few rats also showed increased percentage of time spent in the open arms. Two-way repeated measures ANOVA revealed significant main effects of the stimulation site ($F_{(2, 62)} = 4.699$; $P = 0.013$) and period ($F_{(2.8, 175.9)} = 20.372$; $P < 0.001$). The interaction effect

between the stimulation site and the period was significant ($F_{(5.7, 175.9)}$ = 2.278; P = 0.041). Bonferroni post-hoc test revealed that rats in the IL stimulation group showed a significantly higher percentage of time spent in the open arms in ON1 and ON2 than rats in the PL stimulation group (ON1, P = 0.005; ON2, P = 0.014) or the control group (ON1, P = 0.005; ON2, P = 0.002) (Fig. 2C).

Fig. 3 depicts the percentage of open arm entries. Similar to the percent of time spent in the open arms, most rats with IL stimulation showed an increase in the percentage of open arm entries in ON1 and ON2 (Fig. 3A, B). Some rats in which the ventral PL was stimulated also showed increased percentage of open arm entries. Two-way repeated measures ANOVA revealed significant main effects of the stimulation site ($F_{(2, 62)}$ = 4.537; P = 0.014) and the period ($F_{(2.9, 181.4)}$ = 20.336; P < 0.001). The interaction effect between the stimulation site and the period was not significant ($F_{(5.9, 181.4)}$ = 1.428; P = 0.208). Bonferroni post-hoc test revealed that rats in the IL stimulation group showed a significantly higher percentage of open arm entries in ON1 and ON2 than those in the PL stimulation group (ON1, P = 0.020; ON2, P = 0.007) or the control group (ON1, P = 0.035; ON2, P = 0.003) (Fig. 3C).

Fig. 4 depicts the number of closed arm entries in each group. Two-way repeated measures ANOVA revealed significant main effects of the stimulation site ($F_{(2, 62)}$ = 7.160; P = 0.020) and the period ($F_{(2.8, 172.4)}$ = 15.298; P < 0.001). The interaction effect between the stimulation site and the period was not significant ($F_{(5.6, 172.4)}$ = 1.014; P = 0.415). Bonferroni post-hoc test revealed that the number of closed arm entries was significantly higher in the IL stimulation group than the control group in OFF1 (P = 0.006) and ON2 (P = 0.001), and also higher than in the PL stimulation in ON2 (P = 0.016). Rats in the IL stimulation group did not show a significant difference in the number of closed arm entries in ON1 compared to rats in the PL stimulation group (P = 1.000) or the control group (P = 0.739).

3.3. Multiunit recording of neural activity in the infralimbic and prelimbic cortices of rats during the elevated plus-maze test

The aim of Experiment 2 was to determine whether activation of the IL occurs when rats enter the open arms.

The multiunit activity in the IL was analyzed in 10 of the 24 rats during the 10-min

elevated plus-maze test. This was because 5 of the 24 rats fell off of an open arm of the maze, 5 rats did not enter any open arm, and 4 did not show clear multiunit activity (signal-to-noise ratio > 2.0). The electrode tips in the 10 rats were shown in Fig.1. The mean number of open arm entries was 5.4 ± 1.1. The mean firing rate of IL neurons during testing was 3.5 ± 0.5 Hz (range, 1.3–5.7 Hz), and the mean firing rates during time spent in the open and closed arms were 4.2 ± 0.7 Hz (1.2–8.5 Hz) and 3.1 ± 0.5 Hz (1.2–6.4 Hz), respectively. The normalized firing rates in the open and closed arms were 1.23 ± 0.20 and 0.93 ± 0.87 respectively, and there was no significant difference between the firing rates in the open and closed arms (P = 0.253, paired t test, n = 10) (Fig. 5A). For event-related firing rates during transitions, we analyzed the firing rates 1–3 s prior to open or closed arm entry. The normalized firing rates 1–3 s prior to entries from one closed arm to the other, from a closed to an open arm, from one open arm to the other, and from an open arm to a closed arm were 0.83 ± 0.15, 1.49 ± 0.24, 0.98 ± 0.10 and 1.16 ± 0.14, respectively. One-way repeated measures ANOVA revealed significant effects of the transitions ($F_{(3, 27)}$ = 5.209; P = 0.006). Bonferroni post-hoc test revealed that the normalized firing rate 1–3 s prior to entries from a closed to an open arm was significantly higher than prior to entries from a closed to the other closed arm (P = 0.021, Figs 6A, 7).

The multiunit activity in the PL was analyzed in 7 of the 24 rats during the 10-min elevated plus-maze test; this was because 5 of the 24 rats fell off of an open arm of the maze, 10 did not enter any open arm, and 2 did not show clear multiunit activity (signal-to-noise ratio > 2.0). The tip positions of the recording electrodes in the 7 rats were shown in Fig. 1. The mean number of open arm entries was 6.7 ± 3.0. The mean firing rate of PL neurons during testing was 4.0 ± 1.0 Hz (range: 1.1–8.2 Hz), and the mean firing rates during time spent in the open and closed arms were 4.0 ± 1.0 Hz (1.2–8.5 Hz) and 4.1 ± 1.0 Hz (1.1–8.4 Hz), respectively. The normalized firing rates in the open and closed arms were 0.96 ± 0.30 and 1.06 ± 0.37, respectively, and there was no significant difference between the firing rates in the open and closed arms (P = 0.134, paired t test, n = 7) (Fig. 5B). The normalized firing rates 1–3 s prior to entries from the one closed arm to the other, from a closed to an open arm, from the one open arm to the other, and from an open to a closed arm were 0.84 ± 0.06, 0.96 ± 0.14, 0.96 ± 0.15 and 1.05 ± 0.11, respectively. One-way repeated measures ANOVA revealed no significant effects of the transitions ($F_{(3, 18)}$ = 0.575; P = 0.639) (Figs 6B).

4. Discussion

The present study demonstrated that IL stimulation induces a significant increase in the percentage of time spent and entries in the open arms of the elevated plus-maze compared to PL stimulation or no stimulation. In some cases, the stimulation applied to the ventral PL also increased the percentage of time spent and entries in the open arms. Several studies have reported that the dorsal and ventral portions of the PL show different connectivity [31–34], distribution of dopamine transporter [35] and extracellular dopamine levels [36]. These reports support the proposed division of the mPFC into a dorsal component (dorsal mPFC), which includes the dorsal PL, and a ventral component (ventral mPFC), which includes the ventral PL and IL [37]. Therefore, the present findings suggest that the IL, including the ventral PL, acts to mediate anxiolytic-like behavior. In contrast, we could not find any effect on anxiety-like behavior after PL electrical stimulation. Several studies have shown changes in anxiety-like behavior in the elevated plus-maze during PL modulation. For example, PL activation induced by N-methyl-D-aspartic acid or a pharmacogenetic agent injection in the PL reduces anxiety-like behavior [38,39], while PL inactivation induced by cobalt injection also reduces anxiety-like behavior [40]. Neuron-type specific stimulation in the PL may be needed to change anxiety-like behavior in rats in the elevated plus-maze. In addition, Hamani et al. [41] reported that PL stimulation at 130 Hz induces greater antidepressant-like responses in the forced swimming test compared to stimulation at 20 Hz, thus suggesting that PL stimulation with higher-frequency might provoke behavioral changes in the elevated plus-maze.

A higher percentage of time spent and entries in the open arms are usually interpreted as anxiolytic-like effects [14,24,25]. Accordingly, our findings suggest that IL stimulation mitigates anxiety-like behavior in rats. Consistent with our results, it was previously reported that rats with IL lesions spend less time in the open arms of the elevated plus-maze than non-lesioned rats [11]. IL stimulation has also been reported to improve fear and anxiety-like behavior in rats [42]. Moreover, stimulation of the mPFC, including the IL region, was shown to produce an antidepressant response in the forced swim test [15], and lesion of the IL abolishes behavioral resiliency to stress, induced by

an aggressor animal [43]. These reports on the function of the IL are in line with the present study. An increase in the number of closed arm entries in the elevated plus-maze is interpreted as an increase in locomotor activity [14,24,26,27]. Our finding that IL stimulation does not induce a significant increase in the number of closed arm entries in ON1, compared to PL stimulation or no stimulation, supports that the increase in the percentage of time spent and entries in the open arms in ON1 is induced by the anxiolytic-like effects of IL stimulation. In addition, we found that IL stimulation increases locomotor activity in OFF1 compared to no stimulation, as well as in ON2 compared to PL or no stimulation. This suggests that IL stimulation may exert not only anxiolytic-like effects but also some motivational effects, as it has been suggested in previous studies. Warden et al. [23] have shown that rat mPFC neurons are activated during a motivated active-escape state but not during a behavioral-despair state in the forced swimming test. Furthermore, Valdés et al. [44] and Riveros et al. [45] have reported that activation of the IL is related to an appetitive phase of motivated behaviors in rats. Further studies are required to clarify the effects produced by the cortical stimulation.

We also recorded multiunit activity from the IL and PL of rats during the elevated plus-maze test and found that IL but not PL neurons show significant increase in the firing rate prior to entry from a closed to an open arm compared with that prior to entry from one closed arm to the other. This finding supports the hypothesis that activation of the IL facilitates the entry into the open arms. Increased firing of IL neurons has been observed in neurophysiological studies of fear conditioning in rats; IL neurons were shown to exhibit increased firing during the retrieval of extinction learning and suppress fear- and anxiety-like behaviors after extinction [17,46]. Increased firing of IL neurons was also observed during leaving behavior in a social interaction test, in which one rat approaches another rat and then leaves to another direction, and was associated with stress relief [21]. Neuroimaging studies in humans have similarly reported decreased activation of the mPFC in individuals with posttraumatic stress disorder (PTSD) [47–51]. Exposure of patients with PTSD to traumatic material results in decreased activation in Brodmann's area 25 of the mPFC [48], which is considered to be the primate equivalent of the rat IL [37]. Taken together, these results may indicate that activation of the IL is associated with anxiolytic-like responses.

The firing rate of the IL during the time spent in the open arms of the elevated

plus-maze was not significantly different from that during the time spent in the closed arms. This may be due to the multivariate experiences of rats in the open arm. Rats in the open arm exhibit various behavioral aspects, including anxiety-related (immobility, head dipping, escape behavior) and non-anxiety-related ones (walking, exploratory sniffing, stretched posture, grooming) [52]. Therefore, it may not have been necessary to maintain IL activation in the open arms.

Several studies of fear conditioning have described opposing roles of the IL and PL in the expression of fear behavior [18,20,53,54]. The IL has been associated with the extinction of fear responses, whereas the PL has been associated with the expression of fear responses. Gourley and Taylor [55] reported that the IL supports habitual behaviors, whereas the PL regulates reward-related decision-making (the "PL-go/IL-stop" model). Minami et al. [21] reported an association between the IL and leaving behavior, as well as between the PL and approaching or contact behavior. In the present study, PL stimulation did not significantly reduce the percentage of the time spent in the open arms or the open arm entries, and there was no change in the firing rate of PL neurons preceding open or closed arm entry. These results suggest that the IL and PL have functionally dissociated but not definitively opposing roles in anxiolytic-like behavior, at least in the elevated plus-maze test.

The present study has certain limitations that could be addressed in the future. Firstly, we investigated the regulation of anxiety-like behavior by focusing only on the role of the IL and PL. However, many other brain regions are involved in the neural circuitry controlling anxiety-like behavior. For example, the hippocampal formation (HPC) has a robust projection to the mPFC, and the regulated interactions between the HPC and mPFC are critical for organizing defensive behaviors related to emotional learning and memory [56,57]. In addition, the ventromedial orbital prefrontal cortex (vmoPFC) plays a role in fear response [58,59]. Analysis of the role of the limbic system, including the HPC and amygdala, and of the prefrontal cortex, including the vmoPFC, in the regulation of anxiety-like behavior will provide further insight regarding the neural mechanisms for overcoming anxiety. Secondly, we investigated the multiunit activity of IL neurons but not the single neuron activity or field potentials. Multiunit activity is a result of the neural activities of various types of neurons (i.e., projection neurons and interneurons). Adhikari et al. [29] observed task-related firing of mPFC neurons 1–3 s prior to leaving or entering a closed arm by recording single

neuron activity in mice. Thus, it is possible that specific single IL neuron activity might correlate with many behavioral aspects exhibited in the elevated plus-maze. In addition, our wireless telemetry transmitter was provided with a band-pass filter (100–3000 Hz) circuit for selectively passing a frequency of spike potentials. Therefore, local field potentials, like theta oscillations, were not transmitted to the data acquisition system. Lu et al. [60] observed that the power of theta-band (4–12 Hz) oscillation of rat mPFC neurons decreases sharply prior to entry into the open arms. Further studies are needed to clarify the relationship between neural activity and behavioral aspects in this test.

5. Conclusions

Our study showed that IL stimulation exerts anxiolytic-like effects, evidenced by a higher percentage of time spent and entries in the open arms of the maze; this was not observed during PL stimulation or no stimulation. Multiunit activity recordings from the IL and PL indicated an increased firing rate of IL neurons 1–3 s prior to entries from a closed to an open arm, whereas no significant changes were observed for PL neurons. These findings suggest that the IL plays a key role in exerting active action to overcome anxiety-like behavior.

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Appendix A. Supplementary data

Appendix B. Supplemental video

References

[1] L. Weiskrantz, Behavioral changes associated with ablation of the amygdaloid complex in monkeys, J. Comp. Physiol. Psychol. 49 (1956) 381–391.

[2] J. LeDoux, The emotional brain, fear, and the amygdala, Cell Mol. Neurobiol. 23 (2003) 727–738.

[3] D. Paré, G.J. Quirk, J.E. Ledoux, New vistas on amygdala networks in conditioned fear, J. Neurophysiol. 92 (2004) 1–9.

[4] K.M. Tye, G.D. Stuber, B. de Ridder, A. Bonci, P.H. Janak, Rapid strengthening of thalamo-amygdala synapses mediates cue-reward learning, Nature 453 (2008) 1253–1257.

[5] N.H. Kalin, S.E. Shelton, R.J. Davidson, The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate, J. Neurosci. 24 (2004) 5506–5515.

[6] H.M.B. Lesscher, T. McMahon, A.W. Lasek, W.-H. Chou, J. Connolly, V. Kharazia, R.O. Messing, Amygdala protein kinase C epsilon regulates corticotropin-releasing factor and anxiety-like behavior, Genes. Brain Behav. 7 (2008) 323–333.

[7] A. Etkin, K.E. Prater, A.F. Schatzberg, V. Menon, M.D. Greicius, Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder, Arch. Gen. Psychiatry. 66 (2009) 1361–1372.

[8] B. Roozendaal, B.S. McEwen, S. Chattarji, Stress, memory and the amygdala, Nat. Rev. Neurosci. 10 (2009) 423–433.

[9] A.M. Lyons, T.E. Thiele, Neuropeptide Y conjugated to saporin alters anxiety-like behavior when injected into the central nucleus of the amygdala or basomedial

hypothalamus in BALB/cJ mice, Peptides 31 (2010) 2193–2199.

[10] R.P. Vertes, Differential projections of the infralimbic and prelimbic cortex in the rat, Synapse 51 (2004) 32–58.

[11] A.L. Jinks AL, I.S. McGregor, Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat, Brain Res. 772 (1997) 181–190.

[12] S.F. Lisboa, M.F. Stecchini, F.M.A. Corrêa, F.S. Guimarães, L.B.M. Resstel, Different role of the ventral medial prefrontal cortex on modulation of innate and associative learned fear, Neuroscience 171 (2010) 760–768.

[13] A.A. Shah, D. Treit, Excitotoxic lesions of the medial prefrontal cortex attenuate fear responses in the elevated-plus maze, social interaction and shock probe burying tests, Brain Res. 969 (2003) 183–194.

[14] A.A. Shah, D. Treit, Infusions of midazolam into the medial prefrontal cortex produce anxiolytic effects in the elevated plus-maze and shock-probe burying tests, Brain Res. 996 (2004) 31–40.

[15] C. Hamani, M. Diwan, C.E. Macedo, M.L. Brandão, J. Shumake, F. Gonzalez-Lima, R. Raymond, A.M. Lozano, P.J. Fletcher, J.N. Nobrega, Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats, Biol. Psychiatry 67 (2010) 117–124.

[16] A.A. Scopinho, M. Scopinho, S.F. Lisboa, F.M. Correa, F.S. Guimarães, S.R. Joca, Acute reversible inactivation of the ventral medial prefrontal cortex induces antidepressant-like effects in rats, Behav. Brain Res. 214 (2010) 437–442.

[17] M.R. Milad, G.J. Quirk, Neurons in medial prefrontal cortex signal memory for fear extinction, Nature 420 (2002) 70–74.

[18] M.R. Gilmartin, M.D. McEchron, Single neurons in the medial prefrontal cortex of the rat exhibit tonic and phasic coding during trace fear conditioning, Behav. Neurosci. 119 (2005) 1496–1510.

[19] P.J. Fitzgerald, N. Whittle, S.M. Flynn, C. Graybeal, C.R. Pinard, O. Gunduz-Cinar, A.V. Kravitz, N. Singewald, A. Holmes, Prefrontal single-unit firing associated with deficient extinction in mice. Neurobiol. Learn. Mem. 113 (2014) 69–81.

[20] I. Vidal-Gonzalez, B. Vidal-Gonzalez, S.L. Rauch, G.L. Quirk, Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear, Learn. Mem. 13 (2006) 728–733.

[21] C. Minami, T. Shimizu, A. Mitani, Neural activity in the prelimbic and infralimbic cortices of freely moving rats during social interaction: Effect of isolation rearing, PLoS One 12 (2017) e0176740. doi: 10.1371/journal.pone.0176740.

[22] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates. Academic Press; London, 2007.

[23] M.R. Warden, A. Selimbeyoglu, J.J. Mirzabekov, M. Lo, K.R. Thompson, S.Y. Kim, A. Adhikari, K.M. Tye, L.M. Frank, K. Deisseroth, A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge, Nature 492 (2012) 428–432.

[24] S. Pellow, S.E. File, Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat, Pharmacol. Biochem. Behav. 24 (1986) 525–529.

[25] A.A. Walf, C.A. Frye, The use of the elevated plus maze as an assay of anxiety-related behavior in rodents, Nat. Protoc. 2 (2007) 322–328.

[26] R.J. Rodgers, N.J. Johnson, Factor analysis of spatiotemporal and ethological

measures in the murine elevated plus-maze test of anxiety. Pharmacol. Biochem. Behav. 52 (1995) 297–303.

[27] A. Bahi, Sustained lentiviral-mediated overexpression of microRNA124a in the dentate gyrus exacerbates anxiety- and autism-like behaviors associated with neonatal isolation in rats, Behav. Brain Res. 311 (2016) 298–308.

[28] D.R. Collins, D. Paré, Reciprocal changes in the firing probability of lateral and central medial amygdala neurons, J. Neurosci. 19 (1999) 836–844.

[29] A. Adhikari, M.A. Topiwala, J.A. Gordon, Single units in the medial prefrontal cortex with anxiety-related firing patterns are preferentially influenced by ventral hippocampal activity, Neuron 71 (2011) 898–910.

[30] J.E. Krettek, J.L. Price, The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat, J. Comp. Neurol. 171 (1977) 157–191.

[31] H.W. Berendse, H.J. Groenewegwn, Restricted cortical termination fields of the midline and intralaminar thalamic nuclei in the rat, Neuroscience 42 (1991) 73–102.

[32] T.M. Jay, M.P. Witter, Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin, J. Comp. Neurol. 313 (1991) 574–586.

[33] R.C. Pierce, D.C. Reeder, J. Hicks, Z.R. Morgan, P.W. Kalivas, Ibotenic acid lesions of the dorsal prefrontal cortex disrupt the expression of behavioral sensitization to cocaine, Neuroscience, 82 (1998) 1103–1114.

[34] P.L. Gabbott, T.A. Warner, P.R. Jays, P. Salway, S.J. Busby, Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers, J. Comp. Neurol. 492 (2005) 145–177.

[35] S.R. Sesack, V.A. Hawrylak, C. Matus, M.A. Guido, A.I. Levey, Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter, J. Neurosci. 18 (1998) 2697–2708.

[36] G. Hedou, J. Homberg, J. Feldon, C.A. Heidbreder, Expression of sensitization to amphetamine and dynamics of dopamine neurotransmission in different laminae of the rat medial prefrontal cortex, Neuropharmacology 40 (2001) 366–382.

[37] C.A. Heibreder, H.J. Groenewegen, The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics, Neurosci. Biobehav. Res. 27 (2003) 555–579.

[38] H. Ahmadi, M. Nasehi, P. Rostami, M.R. Zarrindast, Involvement of the nucleus accumbens shell dopaminergic system in prelimbic NMDA-induced anxiolytic-like behaviors, Neuropharmacology 71 (2013) 112–123.

[39] S. Pati, A. Sood, S. Mukhopadhyay, V.A. Vaidya, Acute pharmacogenetic activation of medial prefrontal cortex excitatory neurons regulates anxiety-like behavior, J. Biosci. 43 (2018) 85–95.

[40] C.A.J. Stern, F.H.M. Do Monte, L. Gazarini, A.P. Carobrez, L.J. Bertoglio, Activity in prelimbic cortex is required for adjusting the anxiety response level during the elevated plus-maze retest, Neuroscience 170 (2010) 214–222.

[41] C. Hamani, M. Diwan, S. Isabella, A.M. Lozano, J.N. Nobrega, Effects of different stimulation parameters on the antidepressant-like response of medial prefrontal cortex deep brain stimulation in rats, J. Psychiatr. Res. 44 (2010) 683-687.

[42] R. Reznikov, F.R. Bambico, M. Diwan, R.J. Raymond, M.G. Nashed, J.N. Nobrega, C. Hamani, Prefrontal cortex deep brain stimulation improves fear and anxiety-like behavior and reduces basolateral amygdala activity in a preclinical model of posttraumatic stress disorder, Neuropsychopharmacology 43 (2018) 1099–1106.

[43] M.L. Lehmann, M. Herkenham, Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway, J. Neurosci. 31 (2011) 6159–6173.

[44] J.L. Valdés, P. Maldonado, M, Recabarren, R. Fuentes, F, Torralba, The infralimbic cortical area commands the behavioral and vegetative arousal during appetitive behavior in the rat, Eur. J. Neurosci. 23 (2006) 1352–1364.

[45] M. E. Riveros, M. I. Forray, F, Torrealba, Infralimbic cortex activation and motivated arousal induce histamine release. Behav. Pharmacol. 26 (2015) 338–344.

[46] A. A. Wilber, A. G. Walker, C. J. Southwood, M. R. Farrell, G. L. Lin, G. V. Rebec, C. L. Wellman, Chronic stress alters neural activity in medial prefrontal cortex during retrieval of extinction, Neuroscience 174 (2011) 115–131.

[47] J.D. Bremner, M. Narayan, L.H. Staib, S.M. Southwick, T. McGlashan, D.S. Charney, Neural correlates of memories of childhood sexual abuse in women with and without posttraumatic stress disorder, Am. J. Psychiatry 156 (1999) 1787–1795.

[48] J.D. Bremner, L.H. Staib, D. Kaloupek, S.M. Southwick, R. Soufer, D.S. Charney, Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study, Biol. Psychiatry 45 (1999) 806–816.

[49] L.M. Shin, S.P. Orr, M.A. Carson, S.L. Rauch, M.L. Macklin, N.B. Lasko, P.M. Peters, L.J. Metzger, D.D. Dougherty, P.A. Cannistraro, N.M. Alpert, A.J. Fischman, R.K. Pitman, Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD, Arch. Gen. Psychiatry 61 (2004) 168–176.

[50] L.M. Shin, C.I. Wright, P.A. Cannistraro, M.M. Wedig, K. McMullin, B. Martis,

M.L. Macklin, N.B. Lasko, S.R. Cavanagh, T.S. Krangel, S.P. Orr, R.K. Pitman, P.J. Whalen, S.L. Rauch, A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder, Arch. Gen. Psychiatry 62 (2005) 273–281.

[51] J.P. Hayes, S.M. Hayes, A.M. Mikedis, Quantitative meta-analysis of neural activity in posttraumatic stress disorder, Biol. Mood Anxiety Disord. 2 (2012) 9.

[52] M. Casarrubea, V. Roy, F. Sorbera, M.S. Magnusson, A. Santangelo, A. Arabo, G. Crescimanno, Temporal structure of the rat's behavior in elevated plus maze test, Behav. Brain Res. 237 (2013) 290–299.

[53] V. Laurent, R.F. Westbrook, Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction, Learn. Mem. 16 (2009) 520–529.

[54] T.F. Giustino, P.J. Fitzgerald, S. Maren, Fear expression suppresses medial prefrontal cortical firing in rats, PLoS One 11 (2016) e0165256.

[55] S.L. Gourley, J.R. Taylor, Going and stopping: dichotomies in behavioral control by the prefrontal cortex, Nat. Neurosci. 19 (2016) 656–664.

[56] J. Jin, S. Maren, Prefrontal-hippocampal interactions in memory and emotion, Front. Syst. Neurosci. 9 (2015) 00170.

[57] R. Marek, J. Jin, T.D. Goode, T.F. Giustino, Q. Wang, G.M. Acca, R. Holehonnur, J.E. Ploski, P.J. Fitzgenald, T. Lynagh, J.W. Lynch, S. Maren, P. Sah, Hippocampus-driven feed-forward inhibition of the prefrontal cortex mediates relapse of extinguished fear, Nat Neurosci. 21 (2018) 384–392.

[58] P.M. Wall, R.J. Blanchard, M, Yang, D.C. Blanchard, Differential effects of infralimbic vs. ventromedial orbital PFC lidocaine infusions in CD-1 mice on defensive responding in the mouse defense test battery and rat exposure test, Brain Res. 1020 (2004) 73–85.

[59] E.L. Zelinski, N.S. Hong, A.V. Tyndall, B, Halsall, R.J. McDonald, Prefrontal cortical contributions during discriminative fear conditioning, extinction, and spontaneous recovery in rats, Exp. Brain Res. 203 (2010) 285–297.

[60] J. Lu, H. Dong, X. Zheng, Strengthened functional connectivity among LFPs in rat medial prefrontal cortex during anxiety, Behav. Brain Res. doi: 10.1016/j.bbr.2018.04.013. [Epub ahead of print]

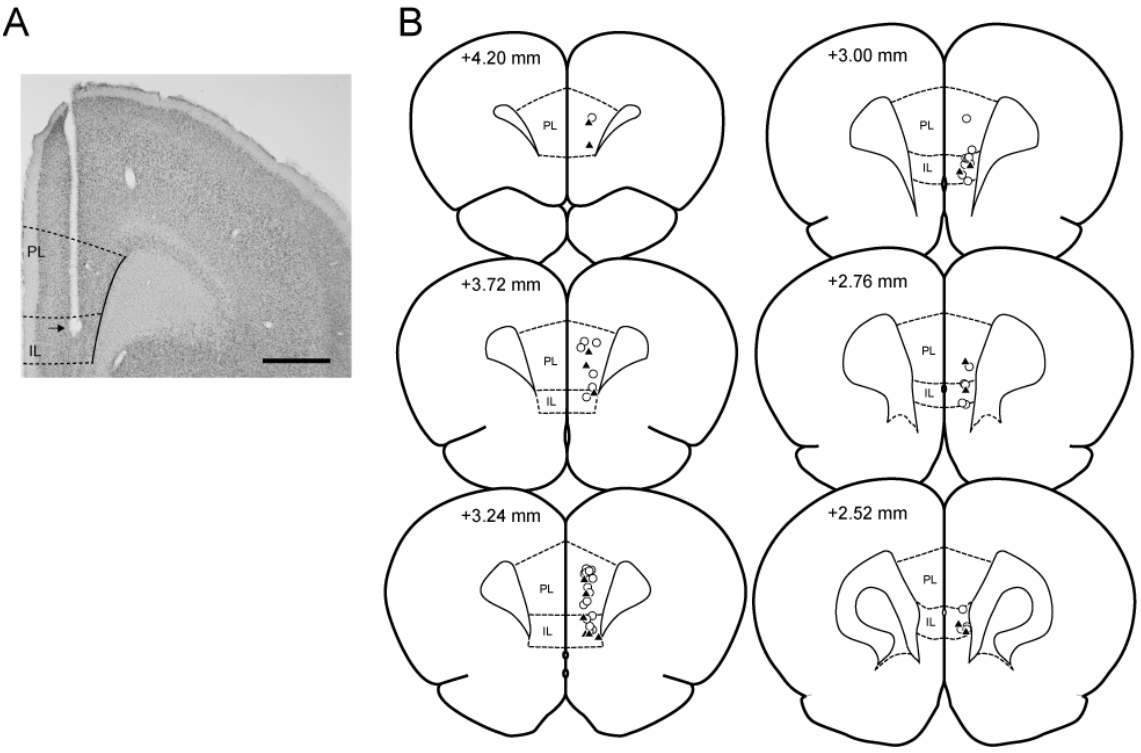

Fig. 1. Location of the tips of the stimulating and recording electrodes. (A) Representative photomicrograph showing the position of the stimulation site. The arrow indicates the mark of the tip of the stimulating electrode in the infralimbic cortex (IL). Scale bar = 1.0 mm. (B) Schematic representation of coronal brain sections illustrating the location of the tips of the electrodes in the prelimbic cortex (PL) and IL. The approximate positions of the tips of individual stimulating electrodes in experiment 1 (open circles) and recording electrodes in experiment 2 (solid triangles) are shown on 6 representative sections modified from a standard stereotaxic atlas [22]. Numbers on the left denote distances from bregma. The borders of the PL and IL are indicated with solid and dashed lines, respectively.

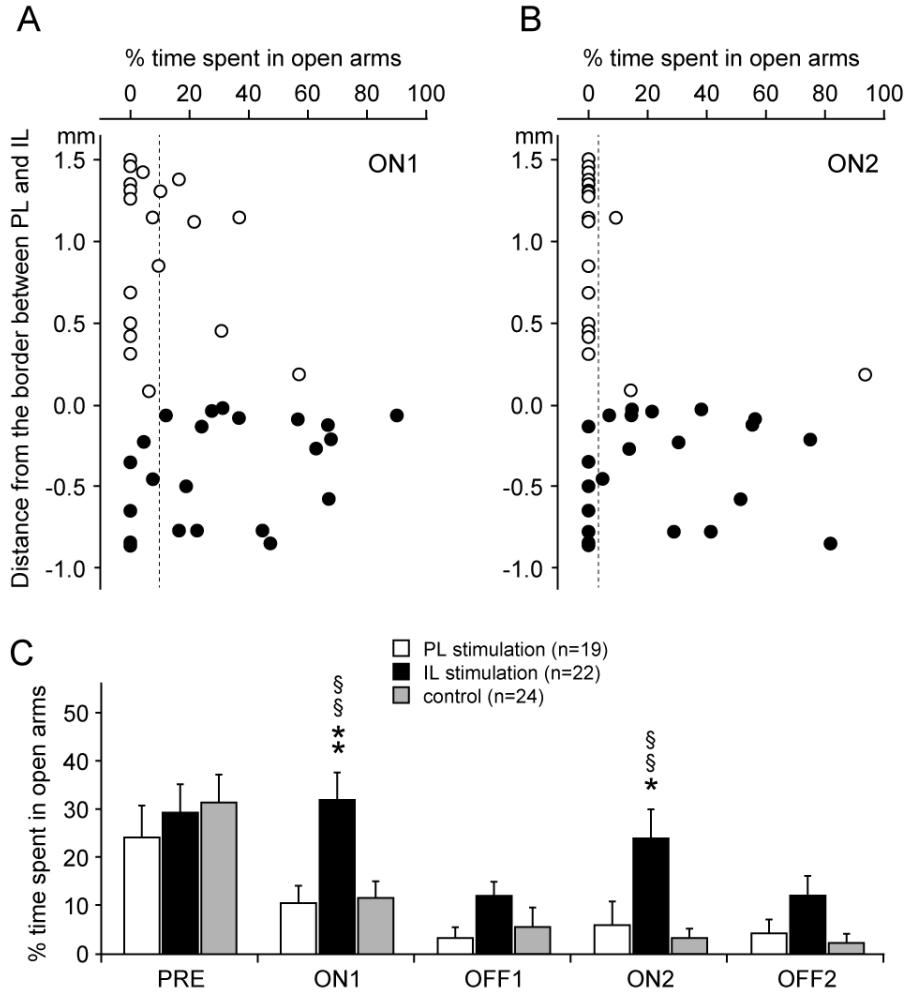

Fig. 2. Time spent in the open arms. (A, B) The percentage of time spent in the open arms in ON1 (A) and ON2 (B) is shown for each animal. The stimulation site, prelimbic cortex (PL) or infralimbic cortex (IL), is indicated with open and solid circles, respectively. The ordinate represents the distance from the border between the PL and IL (the negative distance indicates the position ventral to the border), and the abscissa represents the percentage of time spent in the open arms. The mean percentage of time spent in the open arms by control rats is shown by a vertical dashed line in (A) and (B). (C) Mean (± standard error of the mean) percentages of time spent in open arms by rats subjected to PL stimulation (n = 19, open columns), IL stimulation (n = 22, solid columns), and no stimulation (controls; n = 24, gray columns). The abscissa represents the experimental period (PRE, ON1, OFF1, ON2, and OFF2). $^{*}P < 0.05$ and $^{**}P < 0.01$, significantly different from the PL stimulation group; and $^{\S\S}P < 0.01$, significantly different from the control group; Two-way repeated measures analysis of variance with Bonferroni post-hoc tests.

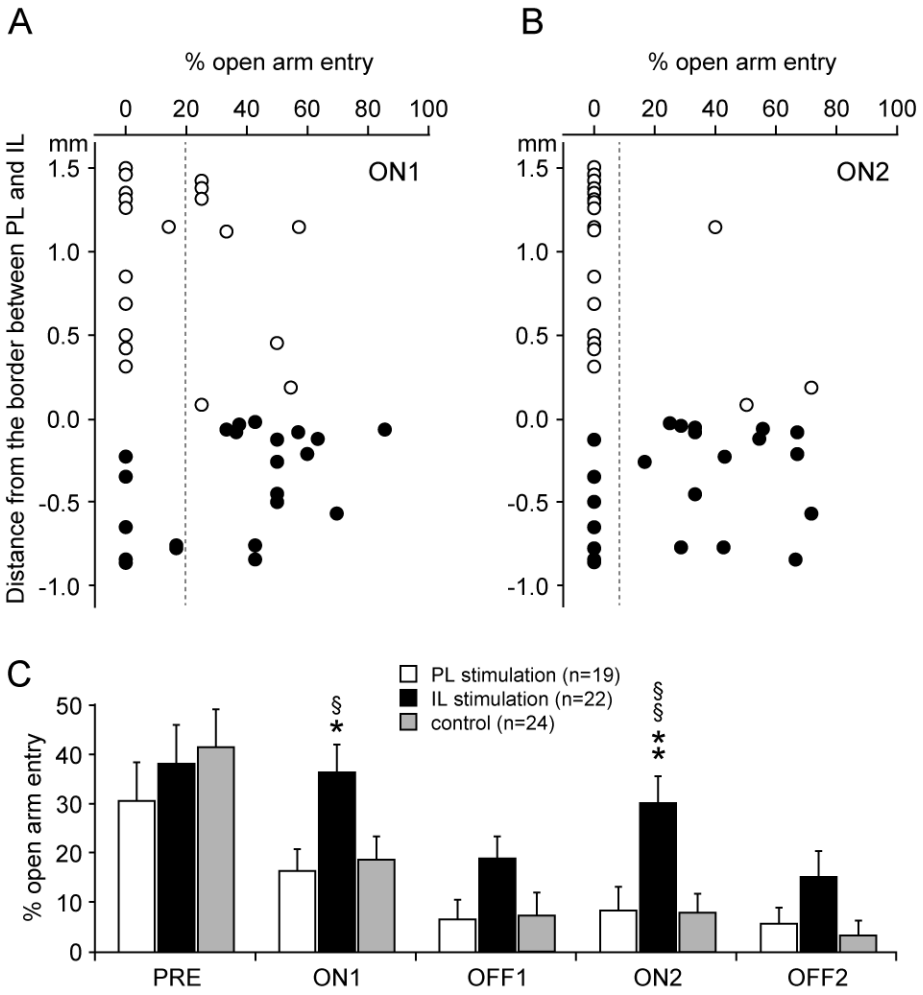

Fig. 3. Open arm entries. (A, B) The percentage of open arm entries in ON1 (A) and ON2 (B) is shown for each animal. The stimulation site, prelimbic cortex (PL) or infralimbic cortex (IL), is indicated with open and solid circles, respectively. The ordinate represents the distance from the border between the PL and IL (the negative distance indicates the position ventral to the border), and the abscissa represents the percentage of open arm entries. The mean percentage of open arm entries by control rats is shown by a vertical dashed line in (A) and (B). (C) Mean (± standard error of the mean) percentages of open arm entries in rats subjected to PL stimulation (n = 19, open columns), IL stimulation (n = 22, solid columns), and no stimulation (controls; n = 24, gray columns). The abscissa represents the experimental period (PRE, ON1, OFF1, ON2, and OFF2). $^{*}P < 0.05$ and $^{**}P < 0.01$, significantly different from the PL stimulation group; and $^{\S}P < 0.05$ and $^{\S\S}P < 0.01$, significantly different from the control group; Two-way repeated measures analysis of variance with Bonferroni post-hoc tests.

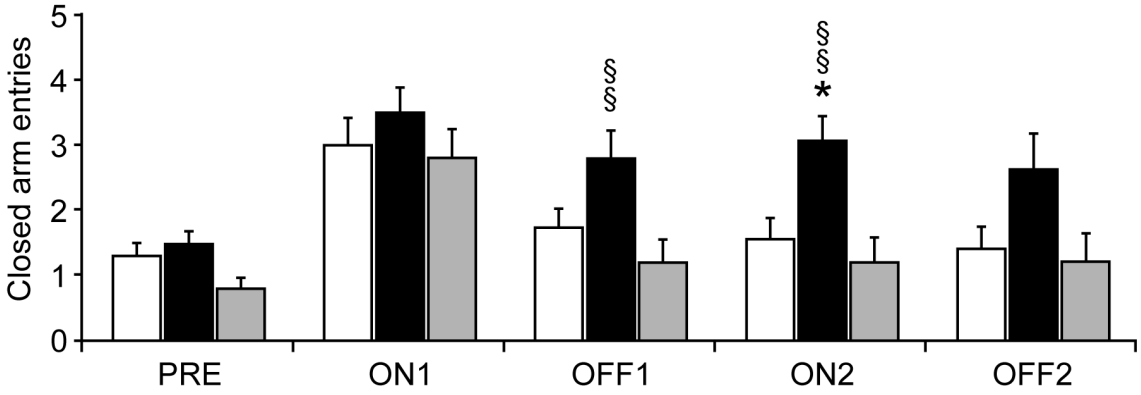

Fig. 4. Number of closed arm entries. Mean (± standard error of the mean) number of closed arm entries by rats subjected to prelimbic (PL; n = 19, open columns), infralimbic (IL; n = 22, solid columns), or no stimulation (controls; n = 24, gray coumns). The period of PRE was 1 min and other periods were 3 min. $^{*}P < 0.05$, significantly different from the PL stimulation group; and $^{\S\S}P < 0.01$, significantly different from the control group; Two-way repeated measures analysis of variance with Bonferroni post-hoc tests.

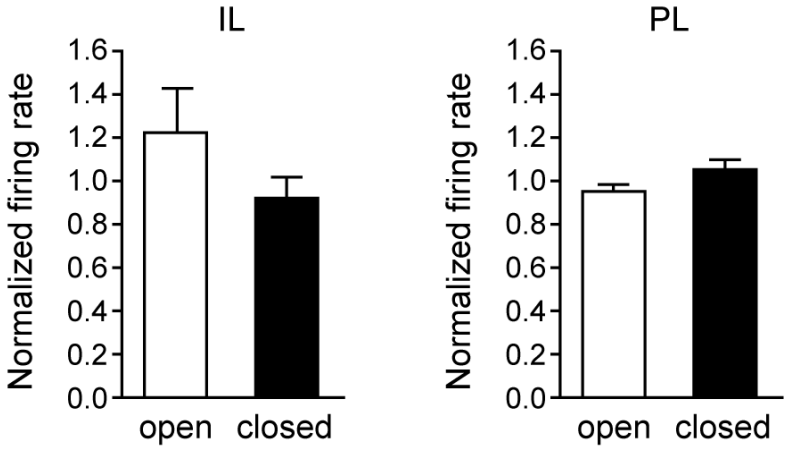

Fig. 5. Firing rates of infralimbic (IL) and prelimbic (PL) neurons in open and closed arms. Firing rates were normalized so that the average bin value for the entire period (10 min) is 1.00. (A, B) Mean normalized firing rates of IL (A) and PL (B) neurons when rats were in open arms (open columns) and closed arms (solid columns). Data (IL; n = 10, PL; n = 7) are presented as the mean ± standard error of the mean (bars).

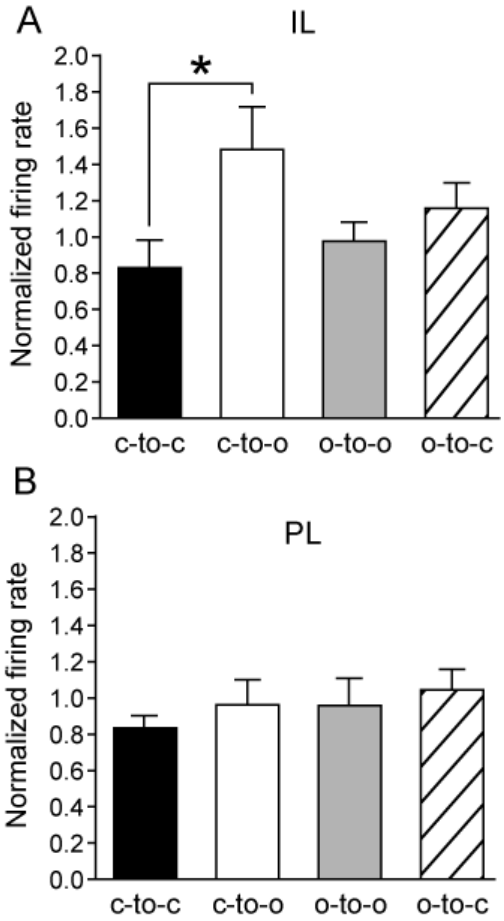

Fig. 6. Event-related firing rates of infralimbic (IL) and prelimbic (PL) neurons. (A, B) Mean normalized firing rates of IL (A) and PL (B) neurons 1–3 s prior to entries from one closed arm to the other (c-to-c), from a closed to an open arm (c-to-o), from one open arm to the other (o-to-o), and from an open arm to a closed arm (o-to-c) are shown with solid, open, gray, and hatched columns, respectively. Data (IL; n = 10, PL; n = 7) are presented as the mean ± standard error of the mean (bars). $^{*}P < 0.05$; One-way repeated measures analysis of variance with Bonferroni post-hoc tests.

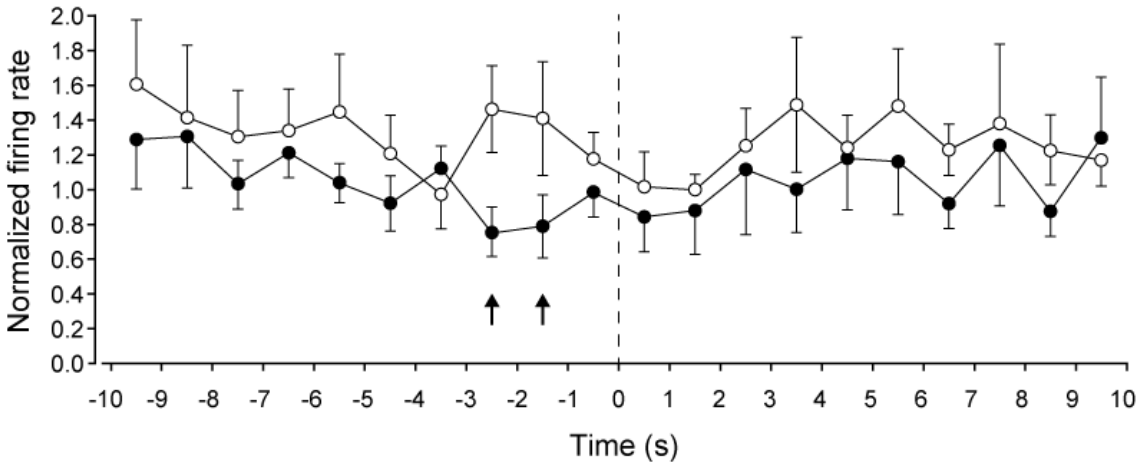

Fig. 7. Changes in the firing rate of infralimbic (IL) neurons during transitions from a closed to an open arm or to the other closed arm. Firing rates were normalized in 1-s bins. Changes in the normalized firing rate of IL neurons during 10 s before and after entry from the closed to the open arms (open circles) and from one closed arm to the other (solid circles) are shown. Arrows indicate the time points at which significant differences in firing rate are shown in Fig. 6A. Ordinate, normalized firing rate per second (Hz); abscissa, time (s) before and after the entry into the arm (arm entry was defined when all four paws were found in the arm). Data (n = 10) are presented as the mean ± standard error of the mean (bars).