

## Effects of the medial or basolateral amygdala upon social anxiety and social recognition in mice

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**Aim:** Though social anxiety and social recognition have been studied extensively, the roles of the medial or basolateral amygdala in the control of social anxiety and social recognition remain to be determined. This study investigated the effects of excitotoxic bilateral medial or basolateral amygdala lesions upon social anxiety and social recognition in mice.

**Materials and methods:** Animals at 9 weeks of age were given bilateral medial or basolateral amygdala lesions via infusion of N-methyl-D-aspartate and then were used for behavioral tests: anxiety-related tests (including open-field test, light-dark test, and elevated-plus maze test), social behavior test in a novel environment, social recognition test, and flavor recognition test.

**Results:** Medial or basolateral amygdala-lesioned mice showed lower levels of anxiety and increased social behaviors in a novel environment. Destruction of the medial or basolateral amygdala neurons impaired social recognition but not flavor recognition.

**Conclusion:** The medial or basolateral amygdala is involved in the control of anxiety-related behavior (social anxiety and social behaviors) in mice. Moreover, both the medial and the basolateral amygdala are essential for social recognition but not flavor recognition in mice.

**Key words:** Medial amygdala, basolateral amygdala, social anxiety, social behaviors, social recognition

### 1. Introduction

Anxiety disorders comprise a group of related mental illnesses characterized by pathologic worry and associated psychiatric and physical symptoms (1). Social anxiety is anxiety about social situations, interactions with others, and being evaluated or scrutinized by other people. The difference between social anxiety and normal apprehension of anxious situations is that social anxiety involves an intense feeling of fear in social situations, especially situations that are unfamiliar or in which one will be watched or evaluated by others. The feeling of fear is so great that for these types of situations one may be so worried that he or she feels anxious just thinking about them, and will go to great lengths to avoid them. One possible cause of social anxiety is the nervous system. Several studies have found that certain areas of the brain, such as a small, almond-shaped area called the amygdala, can be more active in individuals with anxiety (2). The medial or basolateral amygdala has been shown to be involved in the control of anxiety-related behavior. However, the effects of medial or basolateral amygdala lesions upon anxiety in a social situation remain unknown. In this study, we first investigated the effects of the excitotoxic bilateral medial

or basolateral amygdala lesions upon anxiety-related behavior, and then we examined the effects of the lesions upon social behavior in a novel environment in mice.

For proper social behavior, it is important to discriminate familiar animals from novel animals. This kind of memory is called social recognition. The ability to recognize a familiar conspecific is the foundation for all mammalian social relationships, including parent-offspring recognition, mate recognition, and dominant-subordinate hierarchies. All of these behavioral processes require social discrimination, which is a specific type of memory that differs from other types of learning and memory and may be subserved by distinct anatomical and neurochemical circuits in the brain. While humans and nonhuman primates rely primarily on visual and auditory cues for individual recognition, many other mammals rely on olfactory or pheromonal cues to differentiate individuals. In rodents the neural processing of these olfactory cues is critical to social memory (3). Both the medial and the basolateral amygdala have been shown to be involved in the processing of olfactory information in rodents. In this study, the effects of the excitotoxic bilateral medial or basolateral amygdala lesions upon social

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recognition, as well as whether the deficit is specific to social memory, were examined.

## 2. Materials and methods

### 2.1. Animals

Thirty-two healthy male C57BL/6 mice at 8 weeks of age were purchased from SLAC Laboratory Animal Co., Ltd (Shanghai, China). The animals had free access to food and water under a 12:12 h light/dark cycle (lights on 0730 hours) at  $22 \pm 2$  °C and approximately 40%–70% relative humidity. All animal experiments were approved by the Animal Care and Use Committee at the China Medical University with permit number CMU62043013, which complies with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

### 2.2. Surgical methods

For surgery for medial amygdala or basolateral amygdala lesions (as the lesion group, including 8 medial amygdala lesions as the medial-lesion group and 8 basolateral amygdala lesions as the basolateral-lesion group), 16 mice at 9 weeks of age were anesthetized with Avertin (tribromoethanol; 200 mg/kg intraperitoneally) and positioned in a stereotaxic frame. N-methyl-D-aspartate (NMDA) (Sigma) ( $3.2 \mu\text{g}$  in  $0.16 \mu\text{L}$ ) was infused via the cannula into 2 positions for each side of the brain. In sham-lesioned mice, 0.9% sodium chloride solutions were infused into the medial or basolateral amygdala of the other 16 mice as the sham group. The lesion sites were examined under a light microscope following Nissl staining.

### 2.3. Behavioral studies

#### 2.3.1. Anxiety-related test

This was an open-field test. Mice were placed in a corner of an open-field apparatus ( $60 \times 60 \times 40$  cm; 60 lx; Huaibei and Co., Anhui, China). The distance travelled and the time spent in the center area were measured over a 10-min period as indexes of anxiety-related behavior.

#### 2.3.2. Light-dark test

The apparatus consisted of a cage ( $40 \times 40 \times 30$  cm) divided into 2 equal chambers by a black partition containing a small opening (Huaibei and Co.). One chamber was made of white plastic and was illuminated (100 lx), and the other chamber was black and dark. Mice were placed in the dark chamber and allowed to move freely between the 2 chambers. The time spent in each chamber and the distance travelled were measured over a 5-min period.

#### 2.3.3. Elevated-plus maze test

The apparatus consisted of 2 open ( $25 \times 5$  cm) and 2 enclosed arms of the same size, with 15-cm-high transparent walls (60 lx, Huaibei and Co.). Locomotion activity, the time spent in open arms, and the frequency of entries into open arms were measured over a 5-min period as indexes of anxiety-related behavior.

### 2.4. Social behavior test in a novel environment

Two mice (11–12 weeks old) that had been housed separately were placed together in a novel cage ( $29 \times 18 \times 12$  cm) for 5 min and social behaviors between the 2 mice were observed. The system consisted of a cage and a filtered cage top containing an infrared video camera and infrared light emitting diodes. Animals that have high social anxiety show less social behavior in a novel environment.

### 2.5. Social recognition test

A resident mouse was exposed to an intact male mouse for 4 min. After a 30-min interval, both the previously exposed familiar mouse and a novel mouse were exposed to the resident mouse. Time spent for investigating the familiar or the novel mouse was measured. Mice tend to show more sniffing behavior towards a novel mouse as compared to a familiar mouse. The preference index, which equals time investigating a novel mouse divided by the sum of time investigating a familiar mouse, and time investigating a novel mouse were calculated.

### 2.6. Flavor recognition test

A resident mouse was exposed to a flavor stimulus for 4 min. After a 30-min interval, the resident mouse was exposed to both the previously exposed familiar flavor stimulus and a novel flavor stimulus. The time spent investigating the familiar or the novel flavor stimulus was measured. The preference index, which equals time investigating the novel flavor stimulus divided by the sum of time investigating the familiar flavor, and time investigating the novel flavor stimulus were calculated.

If the mouse cannot discriminate between a novel mouse (flavor) and a familiar mouse (flavor stimulus), the index becomes 50%.

### 2.7. Statistical analysis

Data are expressed as mean  $\pm$  SEM. The differences between 2 groups were analyzed using the 2-tailed Mann–Whitney U test.  $P < 0.05$  was considered statistically significant.

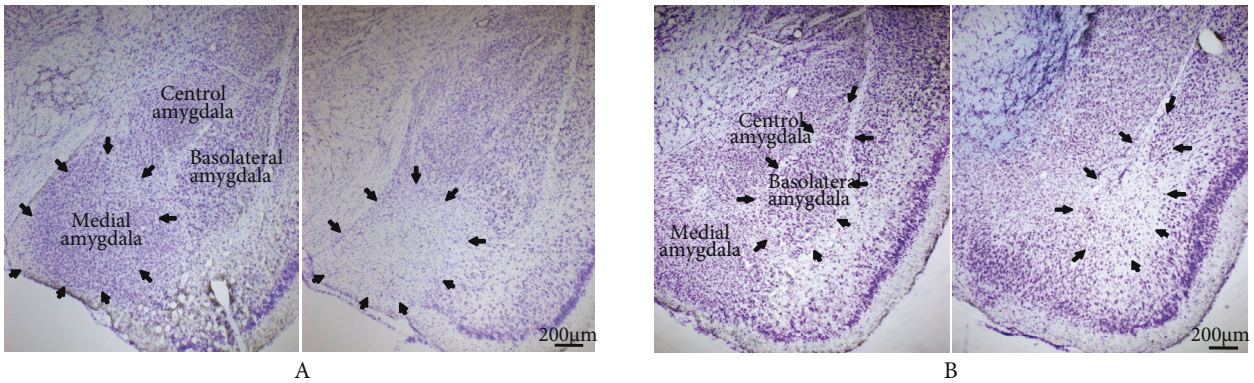
## 3. Results

### 3.1. Establishment of medial or basolateral amygdala lesions model

The medial or basolateral amygdala were destroyed by injections of excitotoxic NMDA solutions. Examination with Nissl staining showed that lesions were confined within the medial (Figure 1A) or basolateral (Figure 1B) amygdala. Medial or basolateral amygdala lesions were achieved in different mice.

### 3.2. Effects of the medial or basolateral amygdala lesions upon social anxiety

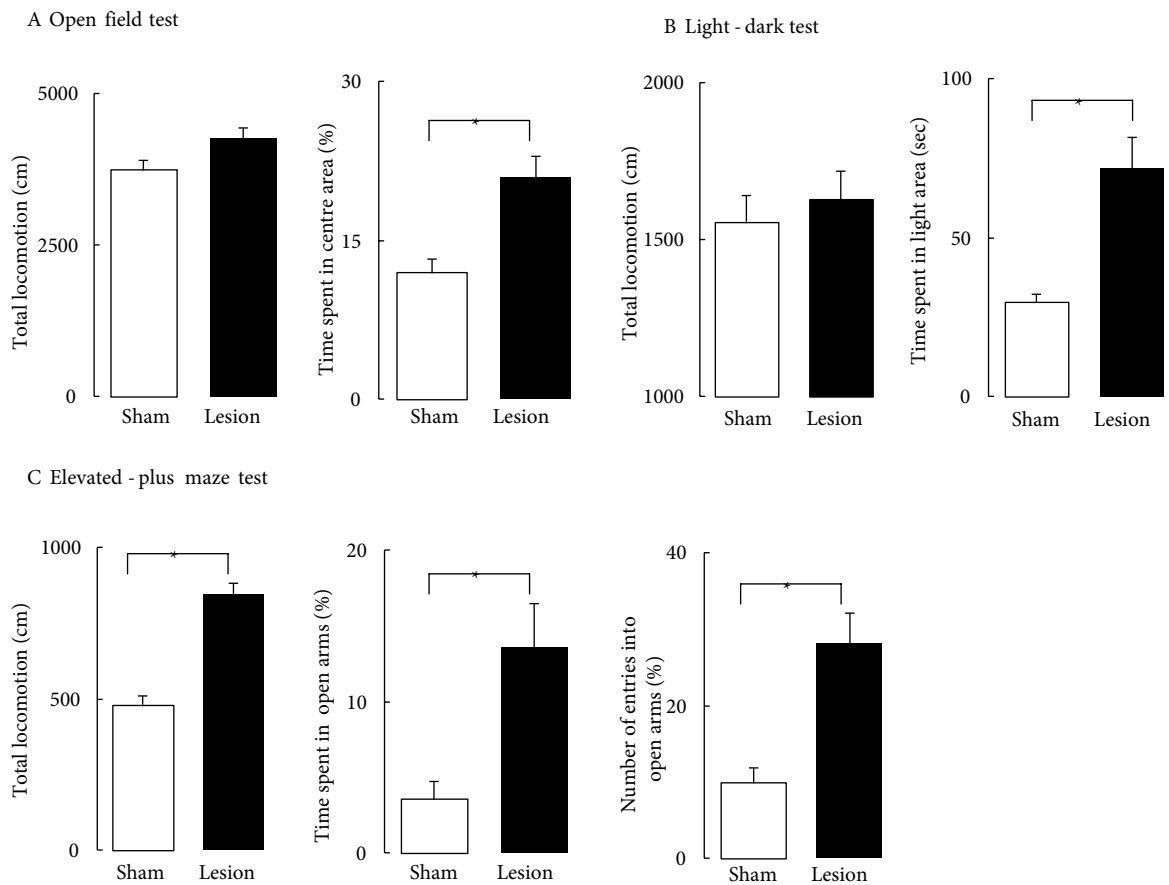
Mice with a high level of anxiety spent less time in the center area of the open field, in the light area of the light-dark box, and in the open arms of the elevated-plus



**Figure 1.** Lesion sites of the medial and basolateral amygdala. Neurons in the medial (A) and basolateral (B) amygdala were destroyed by injection of excitotoxic NMDA solutions and the lesion was confined within the medial and basolateral amygdala. The black arrows indicated the lesion area. Bar: 200  $\mu$ m.

maze. In the open-field test, medial amygdala-lesioned mice spent increased percentages of time spent in the center area ( $P = 0.036$ ; Figure 2A). In the light-dark test, the medial amygdala-lesioned mice spent longer times in the light area ( $P = 0.028$ ; Figure 2B). In the elevated-

plus maze test, the total distance was longer in amygdala-lesioned mice. The medial group stayed longer in the open arms and entered into the open arms more often ( $P = 0.015$  and  $0.024$ , respectively; Figure 2C). Similar to the medial amygdala-lesioned mice, the basolateral amygdala-



**Figure 2.** Effects of medial amygdala lesions upon anxiety-related behavior in open-field test, light-dark test, and elevated-plus maze test. The total locomotion and percentages of time spent in the center area of an open field (A), time in the light area of a light-dark test (B), and time in the open arms and percentages of the number of entries into the open arms in an elevated-plus maze test (C) are shown. \*:  $P < 0.05$  when compared with sham mice. Sham group  $n = 16$ , lesion group  $n = 16$ .

lesioned mice also showed increased percentages of time spent in the center area ( $P = 0.041$ ; Figure 3A), spent longer times in the light area ( $P = 0.045$ ; Figure 3B), and stayed longer in the open arms and entered the open arms more often ( $P = 0.034$  and  $0.037$ , respectively; Figure 3C). All these data suggest that both the medial and basolateral amygdala-lesioned mice were less anxious.

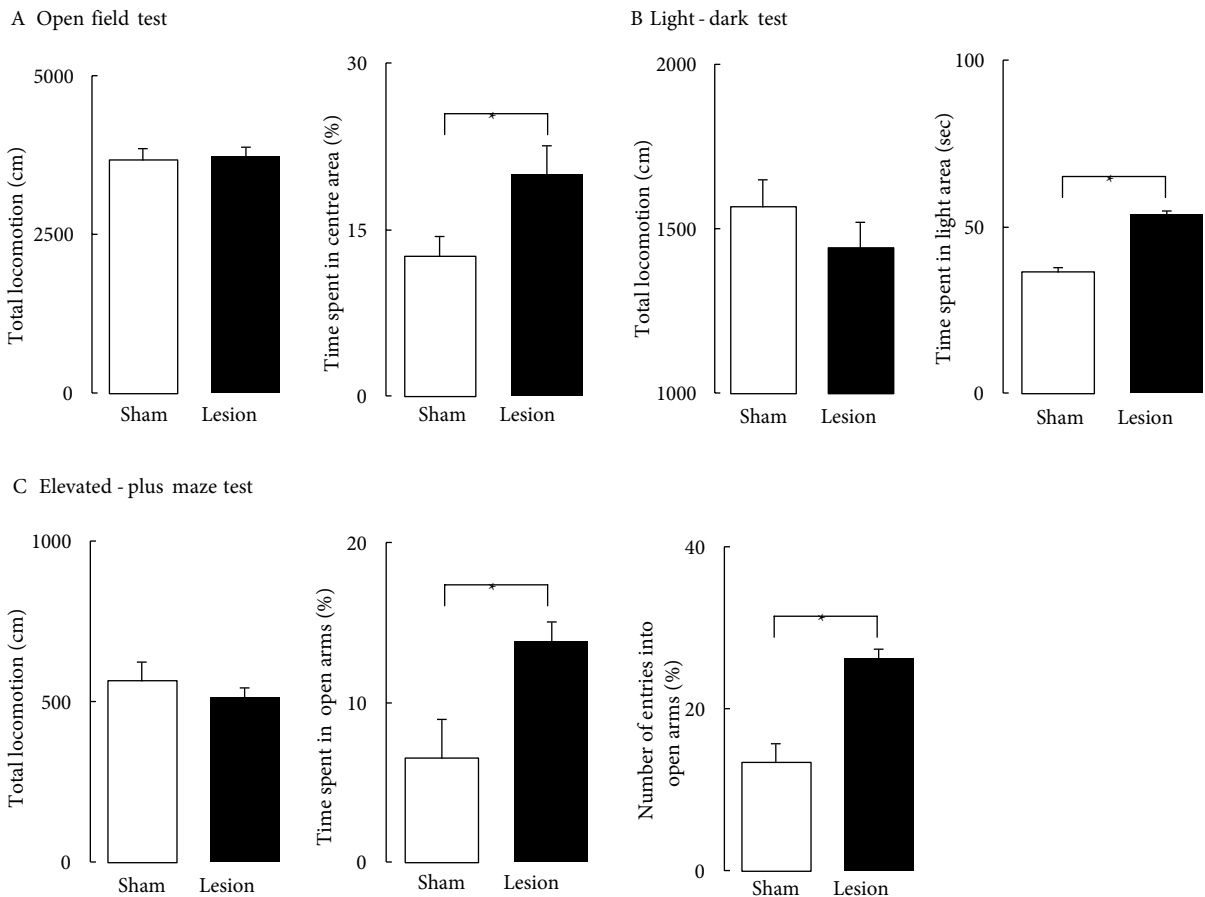
### 3.3. Effects of the medial or basolateral amygdala lesions upon social behaviors

Behaviors were recorded on digital video and the social behaviors of 2 mice (11–12 weeks old) were observed during the first 5 min following placement of the 2 mice into a novel test cage. Anxious animals exhibited less social behavior in a novel cage. Both medial and basolateral amygdala-lesioned mice spent more time in social behaviors compared with sham mice (For the medial-lesion group: approaching,  $P = 0.012$ ; following,  $P = 0.013$ ; sleeping together,  $P = 0.015$ ; anogenital sniffing,  $P = 0.021$ ; nose-to-nose sniffing,  $P = 0.029$ ; others,  $P > 0.05$ . For the basolateral-lesion group: grooming each other,  $P =$

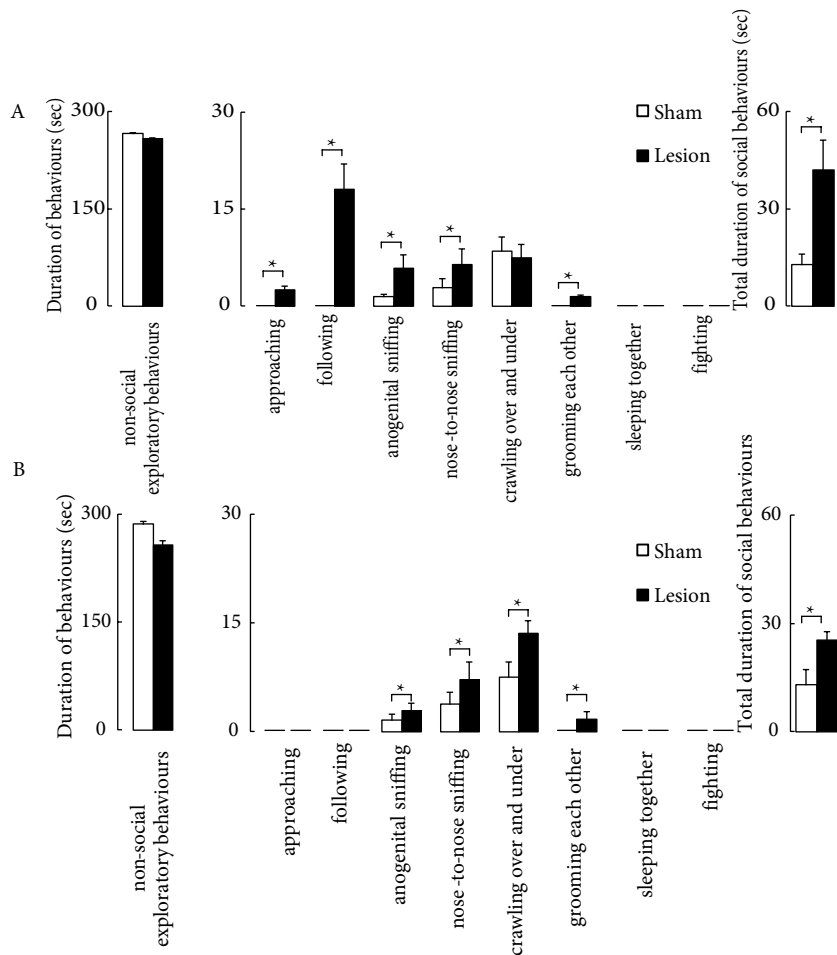
$0.014$ ; anogenital sniffing,  $0.042$ ; nose-to-nose sniffing,  $P = 0.038$ ; crawling over and under,  $P = 0.031$ ; Figures 4A and 4B). Medial or basolateral amygdala-lesioned mice showed lower levels of anxiety and increased social behaviors ( $P = 0.023$  in medial-lesion group;  $P = 0.035$  in the basolateral-lesion group) in a novel environment, suggesting that social anxiety was lower in medial or basolateral amygdala-lesioned mice (Figures 4A and 4B).

### 3.4. Effects of the medial or basolateral amygdala lesions upon social recognition

In both medial and basolateral amygdala-lesioned mice, the preference index in the social recognition test was significantly lower than that in sham mice and became approximately 50% (for the medial-lesion group,  $P < 0.001$ ; for the basolateral-lesion group,  $P = 0.007$ ; Figure 5A), suggesting that medial or basolateral amygdala-lesioned mice could not discriminate a novel mouse from a familiar mouse. Medial or basolateral amygdala-lesioned mice had difficulty in social recognition.



**Figure 3.** Effects of basolateral amygdala lesions upon anxiety-related behavior in open-field test, light-dark test, and elevated-plus maze test. The total locomotion and percentages of time spent in the center area of an open field (A), time in the light area of a light-dark test (B), and time in the open arms and percentages of the number of entries into the open arms in an elevated-plus maze test (C) are shown. \*:  $P < 0.05$  when compared with sham mice. Sham group  $n = 16$ , lesion group  $n = 16$ .



**Figure 4.** Effects of medial (A) or basolateral (B) amygdala lesions upon social behaviors in a novel cage. Total duration of social behaviors is also shown in the right panel. \*:  $P < 0.05$  when compared with sham mice. Sham group  $n = 8$ , lesion group  $n = 8$ .

In a flavor recognition test, the preference index was not significantly different between sham and medial or basolateral amygdala-lesioned mice (Figure 5B), suggesting that medial or basolateral amygdala lesions damaged the social memory specifically but not olfactory recognition.

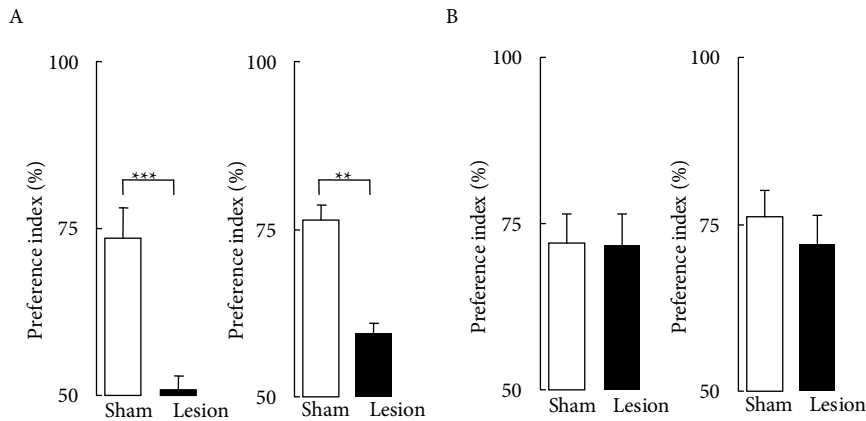
#### 4. Discussion

The amygdala is critical for the processing of emotions including fear and anxiety (4,5). In the present study, anxiety was lower in both the medial and basolateral amygdala-lesioned mice, which is consistent with previous studies that demonstrated anxiogenic-like effects after electrical stimulation (6) and anxiolytic-like effects after ablation of the medial amygdala (7,8).

Anxiety is a normal reaction to stress. Social anxiety disorder indicates a persistent irrational fear of situations. It is clear that certain domains, which have been well studied in the literature, provide the basis for a specific

neuroanatomical structure for anxiety disorder. In the present study, both medial and basolateral amygdala-lesioned mice showed lower levels of anxiety but increased social behaviors in a novel environment, including approaching, following, anogenital sniffing, nose-to-nose sniffing, crawling over and under, and grooming each other. Our findings demonstrated for the first time that the medial or basolateral amygdala is involved in the control of anxiety-related behavior, and both of them are involved in the control of social anxiety disorder in mice.

In the present study, destruction of the medial amygdala neurons impaired social recognition but not flavor recognition. Social recognition has been shown to be facilitated by application of oxytocin (9,10) and suppressed by an oxytocin receptor antagonist (11). Oxytocin-deficient or oxytocin receptor-deficient mice show deficits in social recognition. Anatomical studies have shown that pheromone information is conveyed to the hypothalamus, where oxytocin is mainly synthesized, via the medial



**Figure 5.** Effects of medial or basolateral amygdala lesions upon social recognition and flavor recognition. (A) Social recognition test. \*\*\*:  $P < 0.001$ , \*\*:  $P < 0.01$  when compared with sham mice. (B) Flavor recognition test. Sham group  $n = 16$ , lesion group  $n = 16$ .

amygdala (12,13). It is possible that the medial amygdala mediates activation of oxytocin neurons during social behavior. In the present study, mice showed difficulty with social recognition following medial amygdala lesions. It is thus supposed that impairment of social recognition following medial amygdala lesions is due to a blockade of oxytocin neuron activation following exposure to a novel mouse. However, the sites of oxytocin actions that recover social recognition remain to be determined. All these data suggest that the medial amygdala facilitates social recognition via oxytocin.

Destruction of the basolateral amygdala neurons also impaired social recognition. The basolateral amygdala is an integration center for sensory information that is sent from the cortex, thalamus, and hippocampus (14,15). Research has investigated recognition cues in social memory and found that the recognition cue is olfactory in nature (16). In contrast to the medial amygdala, the nuclei of the basolateral amygdala do not (or only sparsely) receive

direct projections from the olfactory bulb (17). Research has shown that basolateral lesions do not influence odor discrimination in a place preference task. However, animals with basolateral amygdala lesions were able to learn to approach a food magazine during the presentation of a light-conditioned stimulus that signaled food delivery. The authors hypothesized that the basolateral amygdala uses multimodal sensory cues as a basis for associations. This may be true for olfactory and gustatory stimuli, but not for visual and auditory ones (18). However, the neural mechanism that underlies the role of basolateral amygdala in mediating social recognition remains unknown.

In conclusion, the present study suggests that medial or basolateral amygdala-lesioned mice showed a lower level of anxiety but increased social behavior in a novel environment. Destruction of the medial or basolateral amygdala neurons impaired social recognition but not flavor recognition.

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