Effects of isolation after sexual experience on anxiety-like, depressive-like behaviors and affective states in male rats

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Received July 8, 2010; accepted October 15, 2010

Studies on social isolation have provided evidence for an enhanced anxiety-like phenotype, depressive-like symptoms and higher levels of 22-kHz ultrasonic vocalizations (USVs); whereas sexual experience could induce significant changes in affective states, decrease anxiety- and depressive-like responses. However, the behavioral and emotional alterations in male rats exposed to isolation after sexual experience remain poorly understood. Therefore, the present study examined anxiety- and depressive-like parameters, as well as USV emissions in male rats isolated for 1, 2, or 7 d following 1-week sexual (male-female paired housing) or social (male-male paired housing) interaction. Isolation for 1 d resulted in increased depressive-like symptoms, and 2-d isolation induced elevated anxiety-like behavior in sexually inexperienced rats, while rats with sexual experience exhibited comparable levels of emotionality at each isolation time. Moreover, isolated rats without sexual experience emitted less 22- and 50-kHz USVs, but sexually experienced rats exhibited similar affective states compared with control ones. These results suggested that prior sexual experience could have a modulatory effect on experimental rat emotional responses to isolation.

social isolation, sexual experience, anxiety-like, depressive-like, ultrasonic vocalization (USV)


Social and developmental factors are known to influence a wide array of affective behaviors [1,2]. Rats reared in social isolation exhibit distinct behaviors compared with socially reared ones [3–7], such as depressive-like behaviors [8–10], different levels of anxiety [11,12], increased negative states [13,14], enhanced aggressive response [15], and cognitive impairments [4].

Sexual encounter is a rewarding and reinforcing behavior [16–18] in adult male rats and may have effects on mood and affect [19]. Recent studies have reported that sexual experience can reduce anxiety- and depressive-like behaviors in male rats [19,20], facilitate positive states [21], and modulate contextual fear memory [22]. In addition, sexual behavior seems to reduce nervousness and anxiety following stressful events [22,23]. However, little is known about the emotional responses to environmental stressful stimuli, such as social isolation, after prior sexual experience.

The present study addressed the effects of sexual experience on anxiety- and depressive-like behaviors, as well as negative and positive states induced by isolation in male rats. Anxiety was measured in the elevated plus maze [24], and depressive-like behavior was examined using the forced swimming test [25]. Potential effects on affective states were assessed by ultrasonic vocalizations (USVs) [14,26]. Rats emit 22-kHz USVs when exposed to aversive stimuli, such as foot shock [27], social isolation [13], and predator exposure [28]. In contrast, 50-kHz USV emissions in adult rats have been associated with rewarding situations, including social exploration and increased sexual motivation [21].

Previous studies have shown that rats isolated for differ-
ent periods of time exhibit opposite emotional changes [11]. To explore this issue in more detail, the present study analyzed emotionality of male rats isolated for 1, 2, or 7 d and the potential effect of prior sexual experience. Results showed that sexual experience counterbalanced anxiety- and depressive-like behaviors, as well as reduced levels of 22- and 50-kHz USVs induced by isolation. These results provided further evidence that sexual experience might be an important intervention for preventing altered emotional responses to subsequent isolation.

1 Materials and methods

1.1 Animals and housing

Sprague Dawley rats 10 weeks old (Animal House Center, Kunming Medical College, Kunming) were used for the experiments. At postnatal week 11 (after 1 week habituation to the colony room), male rats were randomly assigned to one of 2 groups: social (2 male rats per cage) and sexual (1 male rat and 1 naïve female rat per cage). In the social group, both rats exhibited normal social behaviors, including social investigation, contact, and play fighting [29]; while classical sexual behavior consisted of mounting, intromission, and ejaculation [22,30] in the sexual group.

Animals were housed in a 12-h light/dark cycle (lights on at 8:00 and off at 20:00) and thermoregulated (22 ± 1°C) environment, with ad libitum access to water and food. All behavioral tests were performed between 13:00 and 17:00 to minimize circadian influences. Animal care and experimental protocols were approved by the Chinese Academy of Sciences, China.

1.2 Experimental procedures

A randomized design (Table S1) was utilized for the study (Figure 1). For the elevated plus maze and forced swimming tests, animals were housed either in social (male-male paired) or sexual (male-female paired) groups for 1 week. Subsequently, the socially-housed rats were randomly assigned to one of the following experimental groups: control (no isolation), 1, 2, or 7 d of male-male isolation rearing (a male rat was removed from the social group). The sexually housed rats were assigned to control (no isolation), 1, 2, or 7 d of male-female isolation rearing (the female one was withdrawn in the sexual group). Half rats in each group were exposed to the elevated plus maze test, and the remaining ones were submitted to the forced swimming test. To determine the affective states, USV measurements were performed on male rats immediately (control, no isolation), 1 d (1-d isolation), 2 d (2-d isolation), or 7 d (7-d isolation) after 1-week social or sexual interaction. Each rat underwent one behavioral assessment in the elevated plus maze and forced swimming test respectively to minimize any effects of previous stress, whereas vocalization measurements were performed on the same rat with no isolation and 1-, 2-, 7-d isolation.

1.3 Elevated plus maze

The elevated plus maze has two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm × 40 cm), with a central platform (10 cm × 10 cm) that joins the 4 arms to form a plus shape. The entire apparatus was elevated 60 cm off the floor. Rats were placed on the central platform facing an open arm and were allowed to freely explore the maze for 5 min. After each test, the maze was cleaned with 75% ethanol to eliminate odors. The number of entries into each arm (an entry was counted when the rat explored an arm with all 4 paws) and time spent on open arms were measured. The elevated plus maze test was used to examine anxiety-like behavior [24,31].

1.4 Forced swimming test

Depressive-like behavior was examined with the forced swimming test, which was performed as previously described [25,32]. Rats were individually placed in a vertical Plexiglas cylinder (65 cm high and 25 cm diameter) filled with water (25 ± 1°C) to a depth of 40 cm for 15 min training. Twenty-four hours later, the rats were replaced in the cylinder and total immobility time was measured during a 5-min test. A rat was judged to be immobile when it remained floating in the water almost motionless while making minimal activity necessary to maintain respiration.

Figure 1  Schematic diagram of experimental paradigm.