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Effect of chronic social defeat stress on behaviors and dopamine receptor in adult mice☆

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ABSTRACT

Victims of bullying often undergo depression, low self-esteem, high anxiety and post-traumatic stress disorder symptoms. The social defeat model has become widely accepted for studying experimental animal behavior changes associated with bullying; however, differences in the effects in susceptible and unsusceptible individuals have not been well studied. The present study investigated the effects of social defeat stress on behavior and the expression of dopamine receptors D1 and D2 in the brains of adult mice. Adult mice were divided into susceptible and unsusceptible groups after 10 days of social defeat stress. Behavioral tests were conducted, and protein levels in the brains were assessed by Western blotting. The results indicate that all mice undergo decreased locomotion and increased anxiety behavior. However, decreased social interaction and impaired memory performance were only observed in susceptible mice. A significantly decreased expression of D1 was observed in the prefrontal cortex and amygdala of susceptible mice only. No significant differences in D2 expression were shown between control and defeated mice in any area studied. These data indicate that depression-like behavior and cognition impairment caused by social defeat stress in susceptible mice may be related to changes in the dopamine receptor D1.

1. Introduction

Victims of bullying often undergo depression, low self-esteem (Björkqvist et al., 1982), high anxiety (Olweus, 1978) and typical post-traumatic stress disorder symptoms (Leymann, 1992). The social defeat model has become widely accepted for studying experimental animal behavior changes induced by chronic stress. This model is similar to human bullying in that the dominant animals bully the subordinate animals. Competition awareness for territory or food is used to induce animals to fight fiercely until one of them yields. As a result of the physical and psychological stress, the loser will have a significant mood disorder. Therefore, through the use of this model, the etiology of the emotional repercussions of bullying can be assessed.

It has been reported that in animal experiments, social defeat can induce anxiety (Denmark et al., 2010; Kinsey et al., 2007) and depression-like behaviors (Schloesser et al., 2010), which are similar to those experienced by the victims of bullying. Moreover, there is evidence (Razzoli et al., 2011; Adamcio et al., 2009) that social defeat can contribute to cognition impairment. According to the report of Yu et al. (2011), mice that undergo social defeat exhibit reduced memory in T maze tests, and reduced learning and memory in water maze tests. In object recognition tests, the learning ability of defeated mice is reduced when they are affected by social pressure caused by plundering (El Hage et al., 2004). Furthermore, social defeat can alter the signaling of dopamine, which participates in the adjustment of learning, memory and emotional activities through its receptors D1 and D2. Therefore, the social defeat model provides well-defined markers for assessing the effects of defeat for different populations of mice.

Despite the attributes of the social defeat model, few studies have been undertaken to investigate whether there is a difference in behavioral responses between susceptible and unsusceptible subpopulations of defeated mice. We hypothesized that the quality of depression,

☆ *Animal ethics statement*: This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of the Laboratory Animals of the National Health and Medical Research Council of China. The experimental protocol was approved by the Department of Psychiatry, The Second Affiliated Hospital of Xinxiang Medical University Animal Ethics Committee [SYXK(YU)2014-0005], and was endorsed by the Research and Ethics Committee of Henan Key Lab of Biological Psychiatry, China.

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anxiety-related behaviors and cognitive impairment in mice undergoing social defeat may differ between susceptible and unsusceptible individuals. To test this hypothesis, we assessed several different parameters of behavior. Furthermore, to explore the underlying molecular mechanisms for behavioral responses after social defeat stress, we assessed the effects of social defeat in susceptible and unsusceptible mice on levels of D1 and D2.

2. Material and method

2.1. Experimental animals

Seven-week-old (22–25 g) and 14-week-old (22–25 g) male C57BL/6J and CD1 mice (Beijing Vital River Laboratory Animal Technology Co., Ltd., China) were used in the present study. Dedicated efforts were made to minimize animal suffering and the number of animals used in accordance with the Guidelines for Animal Experiments of Xinxiang Medical University.

2.2. Administration of chronic social defeat stress

Induction of social defeat stress was carried out as previously reported (Berton et al., 2006; Tsankova et al., 2006). CD-1 mice, selected on the basis of their attack latencies (shorter than 30 s on three consecutive screening tests) were used as aggressive residents. Briefly, C57BL/6J mice were exposed to a different CD1 aggressor mouse each day for 10 min for 10 days. After the social defeat sessions, the resident CD1 mouse and the intruder mouse were housed in different halves of the cage separated by a perforated Plexiglas divider to allow visual, olfactory, and auditory contact for the remainder of the 24-h period. At 24 h after the last session, all mice were housed individually. Control mice were housed in similar cages but with members of the same strain, which changed daily. A scientifically and ethically refined chronic social defeat stress protocol was used (Azzinnari et al., 2014).

2.3. Social avoidance testing

On day 11 after chronic social defeat stress, social avoidance testing was performed to distinguish the "susceptible group" from the "unsusceptible group". Testing was performed in self-made chambers (42 cm × 42 cm × 42 cm). Before the tests, the mice to be tested were placed in the test room for 1 h. To delimit the testing area, a woven metal box (10 cm × 4.5 cm) was put in the interactive area. Mice inside and outside of the metal box could see, hear and smell each other, but could not touch each other. The 8 cm area around the box was regarded as the interaction area. The test had two phases of 2.5 min each. In the first phase, the mice to be tested were placed at the far-end of the interactive area. The box in the interactive area had no CD1 mice. The movements of the mice were recorded. In the second phase, CD1 mice were placed in the box within the interactive area and then the mice to be tested were placed at the same position. The interactions between the test mice and the CD1 mice were observed. A video tracking system was used throughout the test process to record and analyze the interaction, and the interaction rate was calculated (interaction rate = 100 × length of stay in interaction area in the second phase / length of stay in interaction area in the first phase). Mice with interaction rate ≥ 100 were determined to be the "unsusceptible group" and mice with interaction rate < 100 were determined to be the "susceptible group". Behavioral testing and Western blot analysis were performed consecutively after social avoidance testing (on day 12) as described below.

2.4. Open field testing

An automated recording of the locomotor activity was conducted in an open acrylic box (30 × 40 × 50 cm) using a video tracking system with SMART software (Panlab, Barcelona, Spain). Mice were allowed to habituate to the testing room for 30 min. Then, mice were placed into the testing apparatus, and their activity (distance moved) was measured for 30 min.

2.5. Light/dark preference determination

An apparatus was constructed that consists of a rectangular acrylic box (46× 27 × 30 cm), divided into one small (18 × 27 cm) and one large (27 × 27 cm) area, with a door-like opening (7.5 × 7.5 cm) in the center of the separation. Each animal was individually placed in the center of the bright compartment (facing away from the door), and the following parameters were measured for 5 min: latency of the initial movement from the light to dark area (latency of transition), total number of transitions between the light and dark areas, and total time spent in the light area.

2.6. Social interaction testing

The social interaction apparatus consisted of a transparent acrylic box (30 × 40 × 50 cm) without a top. Mice were habituated to the testing box for 10 min on the testing day. Then, each mouse was paired with an unfamiliar mouse of the same genetic background and similar weight. The following behaviors were recorded for 10 min under the dimly lit condition (40 lx): social sniffing, anogenital sniffing, social grooming, following, climbing or mounting, crawling under or over, aggressive or fighting behavior, and aggressive chasing.

2.7. New object recognition testing

The apparatus for this task consisted of a black open-field box (30 × 40 × 50 cm) located in a sound-attenuated room and illuminated with a 40-W bulb. Before the test, mice were habituated (10 min per day) in the box for 3 days. On day 4, two identical objects, such as a Duplo Lego toy or golf ball, were placed approximately 10 cm from each corner, and each mouse was allowed to explore the box for 10 min (acquisition trial). The time that the mice spent exploring each object was recorded. Retention trials were carried out at 1- and 24-h intervals following the acquisition trial. During the retention trials, each mouse was placed back in the same box with one of the familiar objects used during the acquisition and a novel object. The mouse was then allowed to explore freely for 10 min, and the time spent exploring each object was recorded. The recognition index (RI) and discrimination index (DI), defined in terms of the amount of time spent exploring a novel object (TN) and the total time spent exploring familiar (TF) and novel (TN) objects in the retention trial, i.e., TN / (TN + TF) and (TN − TF) / (TN + TF), respectively, were used to measure cognitive functioning.

2.8. Preparation of brain tissue

After the social avoidance test was completed, mice were terminated by decapitation under ether anesthesia. The prefrontal cortex, amygdala, and CA1 and dentate gyrus regions of the dorsal hippocampus were punched out bilaterally using a 1.0-mm Harris Uni-Core micropunch (Electron Microscopy Sciences, Hatfield, PA, USA).

2.9. Immune protein blotting

Tissue samples were homogenized in 20 mM ice-cold Tris–HCl (pH 7.4) containing 1% protease (P2714; Sigma-Aldrich Korea, Ltd., Yongin, Kyunggi-Do, Korea) and phosphatase inhibitor (P2850; Sigma-Aldrich Korea, Ltd., Yongin, Kyunggi-Do, Korea). The homogenates were centrifuged for 15 min at 14,000 rpm at 4 °C, and the resulting supernatant fractions were used for Western blot analyses. The protein samples (20 μg/lane) were separated and transferred to a hydrophobic polyvinylidene difluoride membrane. The membranes were blocked