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Early deprivation increases high-leaning behavior, a novel anxiety-like behavior, in the open field test in rats

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ABSTRACT

The open field test is one of the most popular ethological tests to assess anxiety-like behavior in rodents. In the present study, we examined the effect of early deprivation (ED), a model of early life stress, on anxiety-like behavior in rats. In ED animals, we failed to find significant changes in the time spent in the center or thigmotaxis area of the open field, the common indexes of anxiety-like behavior. However, we found a significant increase in high-leaning behavior in which animals lean against the wall standing on their hindlimbs while touching the wall with their forepaws at a high position. The high-leaning behavior was decreased by treatment with an anxiolytic, diazepam, and it was increased under intense illumination as observed in the center activity. In addition, we compared the high-leaning behavior and center activity under various illumination intensities and found that the high-leaning behavior is more sensitive to illumination intensity than the center activity in the particular illumination range. These results suggest that the high-leaning behavior is a novel anxiety-like behavior in the open field test that can complement the center activity to assess the anxiety state of rats.

1. Introduction

Stressful events in early life can exert a persistent negative influence on cognitive performance and emotionality and increase the risk for psychiatric disorders such as anxiety disorder (Lupien et al., 2009; Heim et al., 2010). Rodent models of neonatal stress, maternal separation (MS) and early deprivation (ED) characterized by repeated separation of pups from their mother and littermates (Rees et al., 2006; Franklin et al., 2012) have been used to investigate the influences of early life experiences. However, previous studies using MS and ED reported inconsistent results. Some studies reported anxiogenic effect of MS and ED (Huot et al., 2001; Romeo et al., 2003; Daniels et al., 2004; Rees et al., 2006; Knuth and Etgen, 2007; Lee et al., 2007; Rentesi et al., 2010; Tsuda and Ogawa, 2012; Sachs et al., 2013) while other studies failed to find anxiogenic effects (Shalev and Kafkafi, 2002; Madruga et al., 2006; Diehl et al., 2007; Farkas et al., 2009; Hulshof et al., 2011; Takase et al., 2012; Suri et al., 2013; Nam et al., 2014; Xiong et al., 2014; Zhang et al., 2014).

The open field test is one of the most popular ethological tests to assess anxiety-like behavior in rodents. In this test, animals are allowed to freely explore an open arena surrounded by walls. In such a situation, rodents show thigmotaxis, a tendency to avoid the central region of the arena and stay at the periphery of the arena. Increases and decreases in center activity are indicative of anxiolysis and anxiogenesis, respectively (Prut and Belzung, 2003).

In the present study, we examined the effect of ED on anxiety-like behavior in the open field test in rats and failed to find a change in the center activity or periphery activity in the ED animals as shown in several previous studies. However, we found that the ED animals frequently leaned against the wall standing on their hindlimbs while touching the wall with their forepaws at a high position. We defined the behavior as high-leaning behavior and explored the relationship between high-leaning behavior and an animal's anxiety state. We found that the high-leaning behavior decreased with the anxiolytic drug, diazepam, and increased under intense illumination, which has an anxiogenic effect on nocturnal rodents (Valle, 1970; Bouwknecht et al., 2007; Hale et al., 2008). In addition, we compared the high-leaning behavior and center

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activity under various illumination intensities and found that the high-leaning behavior was more sensitive to the illumination intensity than the center activity in the dim illumination range. These results suggest that high-leaning behavior is a novel anxiety-like behavior in the open field test that can complement the center activity to assess the anxiety state of rats.

2. Materials and methods

2.1. Animals

Male and female Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan) were used. For the ED procedure, 4 pregnant female rats were used. For pharmacological and lighting condition experiments, 173 male rats (postnatal day (PD) 42–49) were used. All animals were housed in plastic cages (20 × 25 × 40 cm, 3–5 animals/cage, pregnant female rats were housed individually.) under controlled laboratory conditions (temperature: 21–24 °C) with free access to food and water under a 12 h light/dark cycle (light onset at 07:00 AM.). Nesting materials in each cage were changed once a week. The experimental procedures and the procedures for the care of the laboratory animals were approved by the animal care and use committee of Tottori University (approval numbers: ED experiments, 10-Y-30 and pharmacological and lighting condition experiments, 12-Y-27) in accordance with the National Institute of Health guide for the care and use of laboratory animals (NIH Publications No. 80-23, revised 1996).

2.2. Early deprivation

The animals in the same litter were divided into the control and ED groups randomly (male: control, n = 7 and ED, n = 10, female: control, n = 10 and ED, n = 10) and they were housed in the same cage until weaning. All pups were painted with an animal marker on their abdomen and back. Their body weight was measured everyday between PD 2 and 35 and we found no significant difference between the control and ED groups. From PD 2–14, the ED pups were separated from their dam and kept individually in paper cups containing the bedding material from their home cage for 3 h between 10:00 and 13:00 every day. During the separation period, the ED pups were kept at 32 °C in an incubator and the dams and control pups were left in their home cages. All pups were weaned at PD 21 and housed with littermates of the same gender (2–5 animals/cage). Behavioral tests were performed at PD 40 or 41.

2.3. Open field test

2.3.1. Apparatus and setting

The open field apparatus consisted of a square arena (70 × 70 cm) with walls 40 cm high that was made of gray polyvinyl chloride plastic board (Muromachi Kikai Co., Tokyo, Japan) (Fig. 1A). The arena was lit by light-emitting diode lightings placed 145 cm above the arena. The illumination intensity was 75 lx in the ED test, 100 lx in the pharmacological test and 0 − 300 lx in the illumination test. The test sessions were recorded by a video camera placed 145 cm above the arena and analyzed using ANY-maze™ Video Tracking System (Stoelting Co. Wood Dale, IL, USA). The software detects the body shape of animals based on the contrast between the animal's body and the background. Then the position of animals is defined as the center point of the bounding rectangle of the body shape. The arena was divided into the center area (30 cm × 30 cm square) and the thigmotaxis area, which includes the peripheral region of the arena (less than 5 cm away from the walls) and the wall area on the captured image (Fig. 1B). When the animals lean against the wall at a high position, their position enters the wall area (See Results for details, Fig. 1A, C). We defined this behavior as "high-leaning behavior". During the test session, the total distance traveled, time in the center area, time in the thigmotaxis area and time in the wall area (high-leaning behavior) were measured automatically by ANY-maze. The frequency of rearing (defined as standing on the hindlimbs without touching the wall) and total leaning behavior (defined as standing on the hindlimbs and touching the wall at any height, thus including the high-leaning behavior) were counted manually. The angle between the animal and the floor during the leaning behavior was calculated from the body length of the animal and the distance between the animal's buttocks and the wall.

2.3.2. Experimental procedures

All animals were subjected to the open field test only once. The tests were conducted during the light phase of the illumination cycle. In the ED and pharmacological experiments, the animals in experimental and control groups were tested alternately so that both groups are tested in the same period of time. On the day of the test, rats were transported to the testing room and left in their home cages for 1 h before the test. To start each session, a rat was placed in a particular corner of the arena and allowed to explore for 5 min. The apparatus was cleaned with 70% ethanol before the test of each animal.

2.4. Drug treatment

In the pharmacological experiments, rats were pre-treated with diazepam (1.5 mg/kg, 1 mg/ml in the following vehicle, ip, Cercine, Takeda Pharmaceutical Company, Tokyo, Japan or Wako Pure Chemical Industries, Osaka, Japan) or vehicle (40% propylene glycol, 10% ethanol, 5% sodium chloride, 4.3% benzoic acid and 1.5% benzyl alcohol in water). The animals in the same cage were divided into the control and diazepam groups randomly. Drugs were administered to animals 60 min before the open field test.

2.5. Statistical analysis

All statistical analyses were performed using statistical software PASW Statistic Ver. 18 (SPSS Inc., Chicago, IL, USA) and EZR (Kanda, 2012). All data were analyzed using the Shapiro-Wilk test to examine the sample distribution and statistical comparisons between the two groups were carried out using an unpaired *t*-test or Mann-Whitney *U* test. Homoscedastic and heteroscedastic data were analyzed by Student's *t*-test and Welch's *t*-test, respectively. Multiple comparisons were carried out by one-way ANOVA and Tukey-Kramer test or Kruskal-Wallis test and Steel-Dwass test. Statistical significance was set at $P < 0.05$. Data are presented as the mean ± standard error (SE).

3. Results

3.1. The ED procedure increases high-leaning behavior in the open field test

To investigate the effect of ED on anxiety-like behavior, we performed the open field test. We found no significant difference between the ED and control animals in the time spent in the center or the thigmotaxis area, which are the common indexes of anxiety-like behavior (time in the center area: male, $F(1, 15) = 0.080$, $P = 0.781$, and female, $F(1, 18) = 1.125$, $P = 0.303$; time in the thigmotaxis area: male, $F(1, 15) = 0.387$, $P = 0.543$, and female, $F(1, 18) = 3.190$, $P = 0.091$, unpaired *t*-test) (Fig. 2D, E). The number of rearing and the total distance traveled, which are also reported to be implicated in anxiety-like behavior (Crawley et al., 1997), were similar in the two groups (number of rearing: male, $U(7, 10) = 31.500$,