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Consequences of continuous social defeat stress on anxiety- and depressive-like behaviors and ethanol reward in mice

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ABSTRACT

This study employed the intruder-resident paradigm to evaluate the effects of continuous social defeat on depressive- and anxiety-like behaviors and the reinforcing and motivational actions of ethanol in male Swiss mice. Male Swiss mice were exposed to a 10-day social defeat protocol, while control mice cohabitated with a non-aggressive animal. Continuous defeat stress consisted of episodes of defeat, followed by 24 h or 48 h cohabitation with the aggressor until the following defeat. Mice were assessed for sucrose drinking (anhedonia), social investigation test, elevated plus-maze, conditioned place preference to ethanol, and locomotor response to ethanol. Plasma corticosterone was measured prior to, after the first and the final defeat, and 10 days after the end of defeat. Defeated mice exhibited a depressive-like phenotype as indicated by social inhibition and reduced sucrose preference, relative to non-defeated controls. Defeated mice also displayed anxiety-like behavior when tested in the elevated plus-maze. Stressed animals failed to present ethanol-induced locomotor stimulation, but showed increased sensitivity for ethanol-induced conditioned place preference. Corticosterone response to defeat was the highest after the first defeat, but was still elevated after the last defeat (day 10) when compared to non-stressed controls. Baseline corticosterone levels were unchanged 10 days after the final defeat. These data suggest that social defeat stress increased depressive- and anxiety-like behavior as well increased vulnerability to ethanol reward in mice.

1. Introduction

Social defeat stress has been widely used to model the effects of social stress in humans, since the main source for stressful stimuli in humans is of social nature (Björkqvist, 2001; Martinez et al., 1998; Ruis et al., 1999). In rodents, social defeat is accomplished by using the resident/intruder paradigm, in which naïve males (intruders) are introduced into the territory of a male aggressor (residents) to be defeated (Kudryavtseva et al., 1991; Martinez et al., 1998). The intruder is targeted with aggression from the resident, including attacks, bites, and threats. The "defeat" takes place when the intruder shows behavioral signs of submission towards the dominant aggressor (Miczek et al., 1982). Many variations in defeat protocols exist, including differences in species and lines of animals used; frequency and duration of aggressive confrontation; length of protocol; interval between defeats and testing, etc. This study will focus on repeated, continuous social defeat stress in outbred Swiss mice (see Hammels et al., 2015, for a review). In this case, right after the aggressive confrontation and subsequent defeat, the intruder is continuously exposed to the resident aggressor for a period of 24 h, separated only by a transparent perforated partition (Berton et al., 2006; Kudryavtseva et al., 1991; Martinez et al., 1998).

Previous studies have shown that this stressor induces a depressive-like phenotype, including social avoidance when presented to an unfamiliar conspecific in a social investigation test (Kudryavtseva et al., 1991). More recently, social avoidance was reported even after 4 weeks following a 10-day continuous defeat protocol, an effect that was reversed by treatment with antidepressants (Berton et al., 2006). Other depressive-like behaviors have also been observed, such as reduced sucrose preference (i.e., *anhedonia*) during the course of chronic defeat (Rygula et al., 2005) or within a few days after defeat stress (Covington et al., 2010; Krishnan et al., 2007), and decreased exploratory activity, indicating long-term effects of this social stressor (Rygula et al., 2005).

Social defeat stress stimulates the hypothalamic-pituitary-adrenal (HPA) axis, leading to increases in circulating corticosterone levels in defeated animals (Covington and Miczek, 2005; Keeney et al., 2006; Schuurman, 1980). There is, however, debate whether adaptation occurs in the HPA axis in response to social defeat stress. For example, Covington and Miczek (2005) reported similar corticosterone response

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after the first defeat, or the fourth defeat in rats. Conversely, sustained elevation in corticosterone levels after chronic defeat stress has been observed, indicating a deficiency in the regulation of the HPA axis (Keeney et al., 2006).

Several studies have shown that social stress also contributes to increased vulnerability to drug use (Miczek et al., 2008; Rhodes and Jason, 1990; Sarnyai et al., 2001; Sinha, 2008). Rats and mice undergoing repeated, brief episodes of social defeat stress over 10 days present enhanced locomotor response to psychostimulants and increased drug self-administration (Covington and Miczek, 2005; Miczek et al., 2008; Yap et al., 2005). On the other hand, when stressed rats are exposed to continuous defeat for 5 weeks, there is a blunted response to cocaine-induced hyperactivity and reduced cocaine intake (Miczek et al., 2011). Consequences of defeat stress on ethanol reward have been more inconsistent, with suppressive effects on drinking (Funk et al., 2004, 2005; Van Erp and Miczek, 2001) as well as increases in ethanol consumption (Caldwell and Riccio, 2010; Croft et al., 2005; Norman et al., 2015; Rodriguez-Arias et al., 2016). Recently, Norman et al. (2015) reported consistent increases in ethanol drinking in mice with a history of more severe episodes of defeat stress.

The present study aimed to characterize and further expand our understanding of chronic, continuous defeat stress model and its consequences on depressive- and anxiety-like behaviors, as well as corticosterone responses during and after the defeat protocol. We also assessed whether chronic exposure to continuous social defeat stress would increase ethanol conditioned reward and ethanol-induced locomotor stimulation.

2. Materials and methods

2.1. Animals

Adult Male Swiss mice (n = 294) aged 8–10 weeks, were purchased from CEDEME (Center for Experimental Models, Universidade Federal de São Paulo, Brazil). Animals were housed individually or in pairs, according to procedures described below. The vivarium was kept with controlled temperature (21 °C ± 2 °C) and humidity, and maintained on a 12 h light/dark cycle, with lights on at 7 a.m. Throughout the entire experimental procedure, food and water were available ad libitum. Experiments were conducted during the light phase (7h00–19h00) and were approved by the Ethics Committee on Animal Use at Universidade Federal de São Paulo (CEUA #308795/13, #8964170714, #0017/13).

2.2. Drugs

Drug treatments were delivered via intraperitoneal (i.p.) injections

- Saline (NaCl 0.9% w/v)
- Ethanol (Synth®, Diadema, Brazil) was prepared in saline (15% w/v in 0.9% NaCl).

2.3. Procedures and experimental design

2.3.1. Experimental design

Table 1 shows the different experiments conducted in this study and which behavioral tests or assays were carried out with each cohort of mice, with the respective number of animals/group in each test. Each experiment used a separate cohort of mice (control and stress groups). Most experiments were carried out with a 10-day protocol with daily defeats which were followed by continuous exposure to the aggressive resident for 24 h, with the exception of experiments 2 and 6, in which 5 defeats occurred during 10 days (Table 1). The timeline for the different tests and measurements is mentioned in footnotes to Table 1.

2.3.2. Continuous social defeat stress

Male resident mice (n = 32) were housed with a female for 3 weeks before being trained for aggressive behavior. During training for aggressive confrontations, younger and smaller male naïve mice (n = 40 stimulus animals) were introduced into the home cage of the residents, after removal of the female. Residents threatened, pursued and attacked the male stimulus animals, as previously described (Yap et al., 2005). Training for aggressive behavior was carried out for 5–9 sessions, in alternating days, until residents expressed stable levels of aggression towards stimulus animals (variation in attack bites < 15% over three consecutive sessions).

Experimental animals from both groups (control and stress) arrived at least 10 days before the beginning of the experiment, and were individually housed. Social stress occurred during 10 days, during which the female mouse was removed from the aggressive resident's cage. Each daily confrontation consisted of 5-min sessions of physical and social interactions, when, typically, the intruder was defeated by the aggressive resident. The confrontation was terminated earlier if the defeated mouse displayed submissive posture, including an upright posture exposing the abdomen, retracted ears and limp forepaws, during 4 consecutive seconds (see Miczek et al., 1982; Yap et al., 2005). After each defeat, the intruder remained in the home cage of the aggressive resident, physically separated by a perforated acrylic partition, as described in (Golden et al., 2011). The following day, the intruder was defeated by a new resident, in a rotation scheme, until completion of the 10-day protocol. During this 10-day period, control mice were pair-housed with another control animal, using the same acrylic partition that was used for the defeat sessions (allowing only sensorial contact). Housing partners were rotated every day, following the protocol described by Golden et al. (2011). At the end of the 10-day stress protocol, all subjects were returned to their individual home cages. Experimental animals were individually housed prior to, and after the social defeat protocol in order to prevent non-controlled aggressive interactions, which are commonly observed in group-housing conditions.

In experiments 2 and 6, the number of defeats was reduced to minimize stress exposure while still obtaining significant behavioral effects. These experiments occurred at a period when we were observing that mice undergoing defeats were becoming physically debilitated, which was not the purpose of our protocol. In these cases, 5 defeats occurred every other day, during 10 days. Defeated mice were housed in sensorial contact with the resident for 48 h, until the following defeat. Likewise, for these experiments, pairs of control animals were rotated every 48 h, during the 10-day protocol.

2.3.3. Sucrose preference test

Anhedonic-like behavior was evaluated by monitoring sucrose drinking. Mice had access to two bottles containing water or a 2.5% sucrose solution, during 2 h, in three different phases: prior to social defeat stress (10, 7 and 3 days prior to defeat protocol), during the defeat (D1, D4, D7, D10) and post-defeat (D12, D18, D22). Sucrose drinking was assessed in the home cage of mice, individually. During defeat period (D1–D10), mice were individually placed in their original home cages for sucrose drinking, which occurred at least 2 h after the defeat or rotation of control animals. After each test, stressed mice were then returned to the home cage of the aggressive resident, and controls were returned to pair housing condition, always in the presence of the divider. Bottles were weighed before and after the drinking session. Sucrose and water bottles were placed in randomly assigned sides of the cages, and bottle position was switched after each session to prevent side preference. Sucrose preference was calculated as percent intake of sucrose solution to total fluid intake.

2.3.4. Elevated plus maze

Five days after termination of the 10-day continuous defeat protocol, anxiety-like behavior was assessed in the elevated plus maze. The plus-maze consisted of two opposite open arms (30 cm length × 6 cm width) and two enclosed arms of the same size, with 15 cm high walls.