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Chronic mild stress in submissive mice: Marked polydipsia and social avoidance without hedonic deficit in the sucrose preference test

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HIGHLIGHTS

- Selectively bred Sub mice are highly sensitive to the anxiogenic effects of CMS.
- Stressed Sub mice, but not Wt, displayed impaired social exploration.
- Stressed mice displayed polydipsia, without altered sucrose consumption.
- The EPM and Three Chamber tests may be valuable complementary measures of CMS effects.

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ABSTRACT

In the Chronic Mild Stress (CMS) protocol, rodents are exposed to unpredictable stressors to induce anxiety-like behavior and hedonic deficit in the Sucrose Preference test (SPT). Since CMS-induced anxiety- and anhedonic-like behavior may depend upon individual vulnerability to stress, we hypothesized that selectively bred Submissive (Sub) mice would exhibit heightened anxiety- and anhedonic-like behavior, in response to CMS exposure. We anticipated that the testing of Sub mice alongside their Wt counterparts in a battery of behavioral assays would identify parameters most sensitive to CMS effects. To test these assumptions, Sub mice and their outbred Sabra (Wt) counterparts underwent a five-week CMS-SPT regimen.

CMS exposure led to reduced preference for sucrose (sucrose-sweetened water as percent of total intake) among both mouse strains ($p < 0.01$ Wt; $p < 0.05$ Sub). However, this effect was attributed to CMS-induced polydipsia, indicated by mice's increased water consumption, ($p < 0.01$ Wt and Sub), without changes in sucrose intake. Furthermore, CMS-exposed Sub mice, but not Wt, demonstrated impaired social exploration in the Three Chamber test ($p < 0.05$) and anxiety-like effects in the Elevated Plus Maze ($p < 0.05$). Moreover, in a separate experiment, social isolation alone was sufficient to induce polydipsia in Sub mice, without affecting Wt mice's drinking behavior. The present findings suggest that the EPM and Three Chamber tests may be valuable complementary measures of CMS effects, alongside the Sucrose Preference test, and introduce the Sub mouse strain for use in study of susceptibility to stress.

1. Introduction

Numerous variants of the Chronic Mild Stress (CMS) protocol are currently in common use for the research of stress-induced anxiety- and depressive-like behavior in rodents, in which unpredictable physical and psychological stressors induce behavioral changes [1,3] measurable by changes in body weight [4], locomotion [5,6], anxiety-like behavior [1], social exploration [7,8] and hedonic deficit [5,9,10]. Evaluation of the effects of chronic stress upon rodents has undergone standardization, with the Sucrose Preference test (SPT) widely accepted to measure the extent of anhedonic-like behavior [1,4,11]. However, the methodology of stress induction and behavioral assessment were originally developed using rats [12,13], and findings from CMS studies using mice indicate that they are not merely "miniature rats" [9]. Indeed, the adaptation of the CMS protocol for mice has proven not to be a trivial matter, with significant inconsistency of their behavioral reaction to stress, even within groups of inbred mice [9,14,15]. Conflicting findings of CMS-induced anhedonia in mice may depend upon the stability of anhedonic behavior over time and upon individual vulnerability to stress in mice [9,10,16]. Thus, mice exposed to CMS are frequently classified to 'anhedonic' and 'nonanhedo-

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nic' groups according to their CMS-induced changes in drinking behavior in the SPT [9,10].

Despite the high rates of comorbidity between depression and anxiety in the clinic, these two disorders are believed to be associated with differential pathogenic mechanisms [17–19]. Among CMS-exposed animals, anxiety-like behavior has been observed independently of anhedonia [9,10,20], while other groups reported no anxiogenesis [21,22], or even paradoxical, anxiolytic effects of CMS, alongside anhedonia [3]. In the current study, Elevated Plus Maze (EPM), Open Field (OF) and Three Chamber test (TCT), were used to measure anxiety-like behavior [1,5] and impairment of social exploration after stress exposure [7,8].

In order to further clarify the relationship between CMS-induced hedonic deficit and anxiety-like behavior, the present study made use of outbred Sabra mice (Wt) [23], as well as Submissive (Sub) mice derived by selective breeding from the Sabra strain [24,25], based upon their performance in the Dominant–Submissive Relationship (DSR) food competition test [26,27]. Sub mice demonstrate depressive-like behavior in a range of behavioral paradigms [24,25,28] and have been used in the preclinical testing of potential anxiolytics [28,29]. Based upon their previously reported sensitivity to stress [25,28], we hypothesized that Sub mice would exhibit heightened anxiety- and depressive-like behavior, relative to their Wt counterparts, in response to CMS exposure. Furthermore, we anticipated that the testing of Sub mice alongside their Wt counterparts in a battery of behavioral assays would indicate parameters most sensitive to CMS exposure, offering insight to the relationship between depressive- and anxiety-like behavior. Thus, we employed behavioral paradigms measuring different anxiety- and anhedonic-like behavioral parameters to identify CMS-induced changes in male Sub mice and their outbred Wt counterparts, in a five-week CMS-SPT setup.

2. Methods

2.1. Mice

This study made use of two month-old male outbred Sabra (Wt) mice (Harlan Laboratories, Jerusalem, Israel), as well as Submissive (Sub) mice derived from the Sabra line by selective breeding on the basis of their behavior in the Dominant–Submissive Relationship (DSR) test [24,25]. Since the CMS protocol enabled each stress-exposed mouse to be housed individually, the SPT measurements of each mouse were taken seperately ($n = 8$ mice). However, the need to spare the naive mice from all stressors, including isolation, required their social housing, in groups of four, such that the cage was the experimental unit ($n = 5$ cages, 4 mice per cage, 20 mice). As depicted in Supplementary Table 1, 4 mouse groups were used in this study: (1) naive Wt ($n = 5$ (5 cages of 4 mice)); (2) CMS-exposed Wt ($n = 8$, individual housing); (3) naive Sub ($n = 5$ (5 cages of 4 mice); (4) CMS-exposed Sub ($n = 8$, individual housing). Among the naive mice ($n = 20$), a sub-cohort of mice ($n = 8$) underwent further behavioral assessment detailed below, in parallel to CMS-exposed mice ($n = 8$). In order to control for the potential effects of housing conditions, we conducted a parallel experiment, in which otherwise naïve Sub and Wt mice, housed either individually or 4 per cage were tested for five weeks in the SPT (Supplementary Figure 3). With exceptions detailed in the CMS protocol below, mice were given standard laboratory chow and water ad libitum, and were housed in groups of four per cage in a colony room maintained at 22 ± 1 °C, illuminated at ~200 lx on a 12 h L:12 h D cycle (lights on 07:00–19:00 h). The experiments were conducted in compliance with NIH/USDA guidelines, under the approval of the Ariel University Institutional Animal Care and Use Committee. Efforts were taken to minimize mice's discomfort during the course of the study, including continuous monitoring of mice's body weights. At the completion of experimentation, all mice were euthanized in a CO_2 chamber.

2.2. Chronic Mild Stress (CMS)

Mice underwent a CMS–Sucrose preference test (SPT) procedure based upon established protocols shown to induce anhedonia in mice [2,4,9,10,16]. As illustrated in Supplementary Fig. 1, at the age of two months, mice were split into naive and stressed groups. Naive mice remained in their original social housing groups (4 mice per cage), while stressed mice were placed in solitary housing, one per cage, albeit within the collective mouse colony room. Such social isolation of mice is considered to be a mild stressor when the mice can still hear and smell each other within the same room [2,30]. Stressed mice were exposed, in a room separate from the colony, to one randomly selected stressor daily, six days a week for five weeks. The daily stressors (Table 1) consisted of (1) food deprivation: mice were denied access to food for eight hours (23:00–07:00), with access to water ad libitum; (2) night illumination: mice were placed under illumination equivalent to that of the colony room (~200 lx) during the colony's 12 h cycle of darkness (19:00–07:00); (3) Cage tilt: mice's cages were placed at an angle of ~30°, from 09:00–17:00; (4) Tail pinch: to each mouse was attached a plastic clothespin to the base of the tail for 15 min [2]; (5) water immersion: mice were placed within 20 cm deep water (25 °C) for 5 min; (6) Cage crowding: eight stressed male mice were placed within a standard cage for eight hours (09:00–17:00); (7) Wet cage: 250 ml water was added to stressed mice's bedding. After eight hours (09:00–17:00), bedding was changed for dry bedding. (8) Empty cage: stressed mice spent eight hours (09:00–17:00) without cage bedding. Care was taken not to measure sucrose preference immediately following food deprivation.

2.3. Sucrose preference test (SPT)

During the course of the CMS protocol, preference of the mice for sucrose-sweetened versus tap water was measured once a week, in the mice's home cages [5,9,10]. Following 2 h pre-exposure to 2.5% sucrose to overcome neophobia [9], baseline preference of the mice for 1% sucrose or water was measured for 24 h. Bottles were weighed before and after use, by experimenters blind to the mice's group allocation. Sucrose and water bottles were placed in randomly assigned sides of the cage, and were switched after 12 h to eliminate side preference artifact. During the ensuing five weeks, mice's intake of water and 1% sucrose were measured once a week, on the day without exposure to stress, and not immediately following exposure to food deprivation. Mice's preference for sucrose was calculated as the percentage of total liquid intake attributed to 1% sucrose solution, according to the equation:

$$\frac{\text{Surcose solution(g)}}{(\text{Surcose solution(g)} + \text{water(g)}} \times 100$$

Water and sucrose solution bottles were prepared 24 h before their use, and stored on their side in the mouse colony room in order to avoid leakage. A random sampling of bottles placed in empty cages during SP testing demonstrated leakage to be negligible.

In order to control for the potential effects of housing conditions upon drinking behavior, we conducted a parallel experiment, in which otherwise naive Sub and Wt mice were housed either individually or 4 per cage and tested for five weeks in the SPT. Social isolation alone was found to be sufficient to induce polydipsia in Sub mice (Supplementary Fig. 3), precluding individual housing of the non-stressed control mice. Thus, in the current experiment, naive mice of both strains were spared from all stressors, including social isolation [31–36] and fluid intake was compared between