Vicarious Social Defeat Stress Induces Depression-related Outcomes in Female Mice

Supplemental Information

Supplemental Methods

Animals

Eight-week old female and male c57BL/6 mice were purchased from Charles River laboratories (Hollister, CA). Since the social defeat model of depression involves conflict stress (i.e., physical threat) from a more dominant resident counterpart (1), we also purchased retired CD1 male breeders to be used as aggressors for this investigation (2). Prior to vicarious defeat stress (VDS)-exposure, CD1 aggressors were single housed, while c57BL/6 mice were housed with littermates of the same sex and age, in groups of 3-4, in standard polypropylene cages containing wood shavings. Mice were maintained in a colony room with a 12-h light/dark cycle (lights on at 7:00 h), and with access to food and water *ad libitum*. Experiments were conducted in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and with approval of the Institutional Animal Care and Use Committee at the University of Texas at El Paso.

Control (CON) Housing Conditions

During the 10 days of VDS, ES females were housed adjacent to a novel aggressor after witnessing a defeat bout (Fig. 1B), whereas PS males were housed adjacent to its aggressor following the defeat episode (Fig. 1C). Thus, we conducted an additional experiment where a separate group of female ES mice was also housed overnight in the compartment next to the CD1 aggressor they had just witnessed aggress a conspecific (Table S1, Cohort 10). This approach was taken to match the male PS housing condition during VDS (as in Figure 1C).

Additional CON housing conditions (female paired with female; Fig. 1D) were included in order to evaluate baseline social interaction levels as a function of chemosensory stimuli (Table S1, Cohort 9). To do this, a separate group of female CONs were exposed to the bedding of PS male mice, across the 10 days of VDS. Similarly, a

separate group of CON female mice were housed as in Fig. 1D; however, in this case, they were house-paired with a male CD1 mouse that the female had not previously seen display aggressive behavior towards a conspecific.

Corticosterone Immunoassay

A separate set of female c57BL/6 mice was used to examine how VDS would influence the activation of the HPA axis, as inferred by levels of trunk blood serum corticosterone (3). Forty min after the tenth episode of VDS, female mice were decapitated, and trunk blood was collected into standard Heparin-coated collection tubes and placed on ice (4, 5). This time frame was selected because maximum activation of the HPA axis has a latency of 20-40 min post last defeat (6) – remaining elevated 1-24 hours after, in both the social- and witness-defeat paradigms, in male c57BL/6 mice (7, 8). As previously described (9), blood samples were centrifuged (3,000g for 10 min at 4°C) to obtain serum for corticosterone analysis. Corticosterone was analyzed by enzyme-linked immunosorbent assay kit (Neogen; Lexington, KY). Plates were read in a BioPlex Bead Array Reader (BioRad; Hercules, CA).

Sucrose Preference

The sucrose preference test has been widely implemented across the literature to examine the effects of stress-induced anhedonia (10). The test consisted of a 2-bottle procedure in which mice were given the choice between consuming water or a 1% sucrose solution (11, 12). Here, mice were habituated to drink water from two separate bottles during the first five days of the VDS procedure (i.e., PD70-74) – the two drinking bottles were located on the side that housed the experimental c57BL/6 mice (i.e., the left side of the cage; Fig 1B-C). On day six of VDS exposure (PD75), one of the bottles was replaced with a 1% sucrose solution, while the other bottle contained water. Water and sucrose consumption was measured every day prior to the beginning of the last five VDS bouts (PD75-79), as well as 24 h post stress regimen (PD80). The position of the sucrose bottle was balanced (left vs. right) across the different cages to control for potential side-preference bias. Preference for sucrose over water (sucrose/[sucrose + water]) was used

as a measure for sensitivity to reward (13). Total liquid intake was normalized to body weight ([water + sucrose]/body weight).

Tail Suspension Test

The tail suspension test is a behavioral procedure in which rodents are hung in an uncontrollable fashion by their tail for 6 min (14). Initially, mice engage in escape-directed behaviors but eventually adopt a posture of immobility – however, pharmacological antidepressant treatment can significantly increase their escape-directed behaviors, an effect that has been correlated with pharmacological antidepressant efficacy in humans (15). Conversely, an animal that spends more time immobile is considered to be more sensitive to the effects of inescapable stress (16). The total time (sec) spent immobile, during the last 5 min of the test, was the dependent variable.

Elevated Plus Maze

The elevated plus maze is a classic test of anxiety-like behavior (17), that uses the natural reluctance of rodents to explore open spaces (18). The maze (Stoelting®, Wood Dale, IL) was made of gray plastic and consisted of two perpendicular intersecting runways (5 cm wide X 35 cm long). One runway had tall walls (closed arms; 15 cm in height), and the other one had no walls (open arms). The arms were connected together by a central area (5 X 5 cm), and the maze was elevated 40 cm from the floor. At the beginning of the test, under controlled light conditions (~90 lux), mice were placed in the central area, facing one of the open arms, and the cumulative time spent in the open-arms was recorded using a video tracking system (19).

Statistical Analysis

Data was analyzed using ANOVA techniques, with stress (CON, ES, PS), drug (saline, ketamine, chlordiazepoxide), as well as day of sucrose exposure and body weight (repeated measures), as sources of variance. Tukey post hoc tests were conducted to identify differences between the groups. Analyses implicating two-group comparisons were performed with Student's t-tests. Data are presented as mean + SEM. Statistical significance

Supplemental Figures

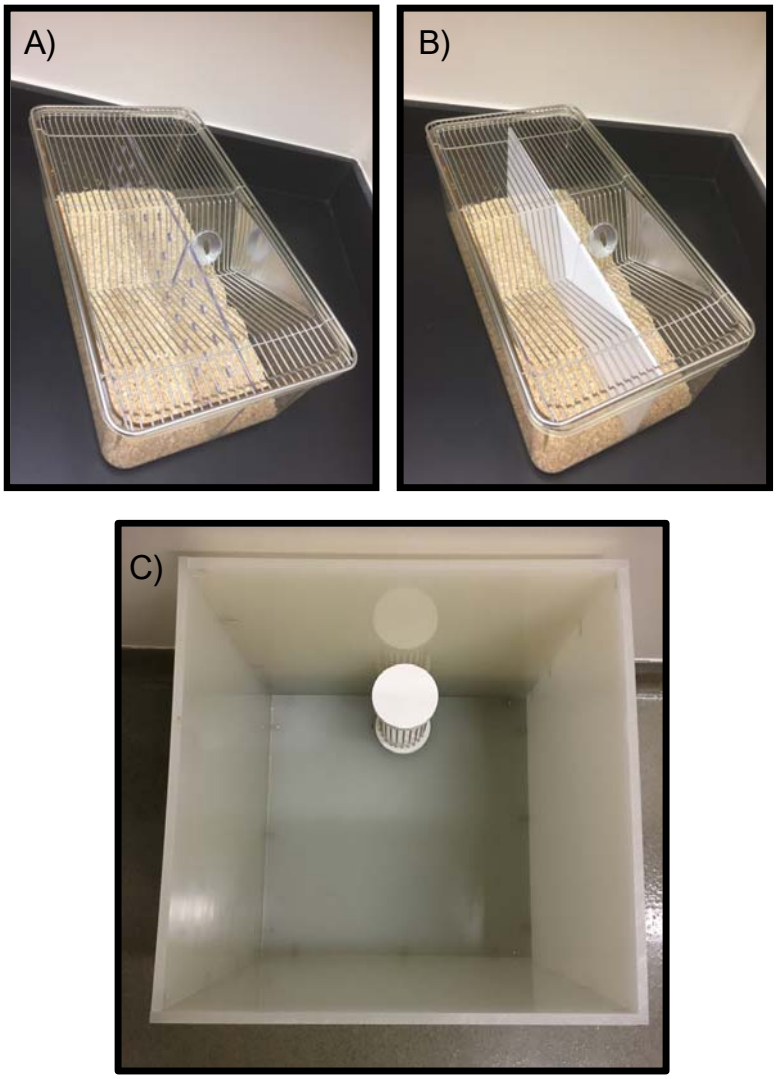

Figure S1. Photograph of defeat cages, with wood shavings bedding, used during the vicarious social defeat stress paradigm (Ancare®, N40 Large Mouse Cage and wire top). (**A**) Defeat mouse cage with fitted clear plexiglass divider with 1 cm diameter perforated holes. (**B**) Defeat mouse cage with fitted white opaque divider without holes. (**C**) Photograph of squared open field arena with a white circular wire cage (Stoelting Co.® Mouse Stranger Enclosure #60452), used during the social interaction test.

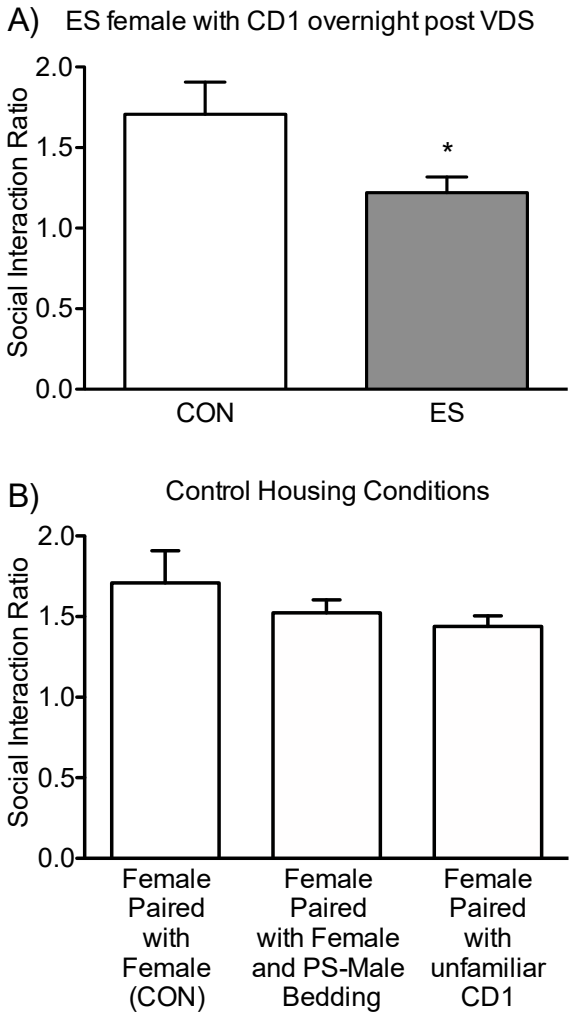

Figure S2. (**A**) Social interaction ratio of female mice exposed to emotional/psychological stress (ES) that spent 24 h with CD1-male aggressor (overnight), which physically defeated a c57BL/6-male conspecific (PS). After 10 days of vicarious defeat stress (VDS), the ES-female group spent significantly less time in the interaction zone, when compared to controls (CON; n=11; female house-paired with another female), 24 hours post last stress exposure. (**B**) Housing and chemosensory conditions do not influence baseline levels of social interaction in CON female mice (i.e., females that do not experience VDS). Specifically, no differences in time spent in the social interaction zone were evident between females CONs (house-paired with separate female) and females housed in similar conditions but also exposed to the bedding of physically stressed (PS) male mice (n=12), or pair-housed with an unfamiliar CD1 male mouse (n=10). Note: CON group in sections A and B are the same group (Table S1, Cohort 1). *p>0.05.

Table S1. Experimental groups.

Cohort	Stress	n	Sex	Procedure I	Interval	Procedure II	Data
1	CON	11	Female	VDS	24 hr	Social Interaction	Figure 2
	ES	11	Female				
	CON	11	Male				
	ES	11	Male				
	PS	11	Male				
2	CON	11	Female	VDS	24 hr	Social Interaction with Same Strain/Sex Target	Figure 3A Figure 4
	ES	11	Female				
3	CON	10	Female	VDS with Opaque Divider	24 hr	Social Interaction	Figure 3B
	ES	10	Female				
4	CON	8	Female	VDS	40 min	Blood Collection Corticosterone Assay	Figure 3C
	ES	6	Female				
5	CON	10	Female	VDS	24 hr	Sucrose Preference	Figure 5
	ES	10	Female				
6	CON	9	Female	VDS	24 hr	Tail Suspension	Figure 6A
	ES	11	Female				
7	CON	12	Female	VDS	24 hr	Elevated Plus Maze	Figure 6B
	ES	11	Female				
8	CON	18	Female	VDS	24 hr	Pharmacological Study	Figure 7
	ES/SAL	8	Female				
	ES/KET	8	Female				
	ES/CHLR	10	Female				
9	CON*	11	Female	CON Housing	24 hr	Social Interaction	Figure S2A
	CON/BEDD	12	Female				
	CON/CD1	10	Female				
10	CON*	11	Female	VDS Overnight with CD1	24 hr	Social Interaction	Figure S2B
	ES	11	Female				

BEDD, bedding from physically stressed male mice; CHLR, chlordiazepoxide; CON, non-stressed control; ES, emotional/psychological stress; KET, ketamine; PS, physical stress; SAL, saline; VDS, vicarious defeat stress; *Same group in cohort 1.

Supplemental References

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