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Learning to actively cope with stress in female mice

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ABSTRACT

Repeated exposure to a same-sex resident stranger enhances subsequent indications of active coping that generalize across multiple contexts in intruder male mice. Here we investigate female mice for comparable learning to cope training effects. Stress coping research focused on females is important because stress related mood and anxiety disorders are more prevalent in women than men. Female mice were monitored for coping behavior in open-field, object-exploration, and tail-suspension tests conducted after repeated exposure to a same-sex resident stranger. During repeated exposure sessions of training staged in the resident's home cage, behavioral measures of aggression and risk assessment were collected and plasma measures of the stress hormone corticosterone were obtained from separate samples of mice. Repeated exposure to a same-sex resident stranger subsequently enhanced active coping behavior exemplified by diminished freezing and increased center entries in the open-field, shorter object-exploration latencies, and a tendency toward decreased immobility on tail-suspension tests. Open-field locomotion considered as an index of non-specific activity was not increased by repeated sessions of exposure and did not correlate significantly with any measure of active coping. During repeated sessions of exposure to a same-sex resident stranger, risk assessment behavior and consistent but limited aggression occurred and corticosterone responses increased over repeated sessions. Exposure to a same-sex resident stranger is mildly stressful and promotes learning to actively cope in mice assessed in three different contexts.

1. Introduction

Mood and anxiety disorders occur more often in women than men (Bale and Epperson, 2015). Stress triggers or exacerbates these disorders and various psychotherapies have been designed to enhance stress coping skills. Exposure psychotherapies train people to imagine a graded series of stressful situations and then encourage direct interaction with relevant stressors *in vivo* (McNally, 2007). Stress exposure is likewise a feature of stress inoculation training for people who work in conditions where performance in the face of adversity is required, *e.g.*, medical and military personnel, police, firefighters, and rescue workers (Meichenbaum and Novaco, 1985; Saunders et al., 1996; Stetz et al., 2007). Repeated stress exposure followed by recovery provides opportunities to learn and practice acquired stress coping skills (Craske et al., 2008; Lane et al., 2015).

Animal models of exposure psychotherapies often focus on learned extinction of conditioned fear (Milad and Quirk, 2012). Extinction occurs when a conditioned stimulus (CS) that was previously paired with an unconditioned stimulus (US) is repeatedly presented on its own. Repeated presentation of the CS alone results in new learning and subsequent inhibition of the previously conditioned response. Far less researched, but of equal importance, are indications that repeated presentation of the US alone also inhibits conditioned responses by devaluing or reducing the impact of the US through a process called US habituation (Rauhut et al., 2001; Storsve et al., 2010).

Inhibitory effects of learned extinction do not generalize to contexts that differ from those in which CS extinction learning occurs (Bouton, 2002; Rauhut et al., 2001). Context specificity limits the utility of animal models based on learned extinction (Craske et al., 2008; McNally, 2007) because the effects of exposure psychotherapies in humans tend to generalize across contexts (Preusser et al., 2017). Certain animal models of US habituation likewise generalize across contexts and appear to be minimally modulated by contextual cues (Chiandetti and Turatto, 2017; Nyhuis et al., 2010; Rauhut et al., 2001).

Recently we discovered that repeated exposure to a same-sex resident stranger enhances subsequent indications of active coping in multiple contexts for intruder male mice (Brockhurst et al., 2015). Here we investigate female mice for comparable learning to cope training effects. Same-sex intruders spontaneously elicit unconditioned attacks from resident male mice, but attacks are uncommon during same-sex interactions among female mice (Clipperton-Allen et al., 2011). Therefore we assessed aggression, risk assessment behavior, and the stress hormone corticosterone to determine whether repeated exposure to a same-sex resident stranger is stressful for intruder female mice.

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2. Methods and materials

Nulliparous female C57BL/6 mice weighing ~23 g (range 19–27 g) were purchased at 7 wk of age from Charles River (Gilroy, CA). These mice were housed for the duration of experimentation in groups of three per cage in climate controlled rooms with an ambient temperature of 26 °C and lights on from 07:00–19:00 h. Food and drinking water were provided *ad libitum*. After 2 wk of acclimation, group housed mice were randomized to repeated sessions of exposure to a same-sex stranger or a non-exposed control condition. All cage mates were exposed to the same conditions.

For the control condition, females remained undisturbed in groups. Females randomized to repeated sessions of exposure were treated as previously described for male mice (Brockhurst et al., 2015). Briefly, subject females (intruders) were transferred individually from their home cage into the cage of a gonadally intact, reproductively experienced, same-sex, resident stranger behind a mesh-screen barrier. The barrier confined residents to a small section of their cage while intruders had free access to ~80% of the resident's cage. The mesh-screen barrier also prevented direct physical contact, but allowed for non-contact social interaction. Repeated sessions of exposure to a same-sex resident stranger occurred 2–3 h after lights-on during the inactive phase for 15 min every other day over 21 days for 11 total sessions. Immediately after each exposure, subjects were returned to their own home cage to provide familiar conditions for learning and memory consolidation.

Residents were multiparous female Swiss Webster mice purchased as retired breeders from Charles River (Gilroy, CA) and subsequently acclimated to our facility for 2 wk. At Charles River, breeding starts as 8–10 wk of age and females are retired from breeding at 9–12 mon of age. In our facility, all residents were housed individually to enhance defensive behavior, and their prior reproductive experiences are known to promote same-sex aggression in mice (Miczek et al., 2001). Intruders engaged a new unfamiliar resident stranger during each exposure training session.

Plasma levels of the stress hormone corticosterone were assessed in undisturbed home cage baseline conditions and immediately after the 1st, 3rd, 7th, and 11th exposure sessions. Tail vein blood samples were collected as described elsewhere (http://www.nc3rs.org.uk/mouse-tail-vessel-microsampling-non-surgical) between 09:00–10:30 h to control for circadian effects. Plasma extracted from blood was assayed in duplicate for corticosterone in a single radioimmunoassay from MP Biomedicals (Solon, OH) without knowledge of the experimental conditions. Assay sensitivity was 8 ng/ml and the intra-assay coefficient of variation was 10%.

Corticosterone was assessed in 9 mice and behavioral measures were collected from 24 different mice (n = 12 per condition) to control for potentially stressful blood sampling effects on behavioral measures that we used to assess active coping in mice (Harikrishnan et al., 2017). Sample sizes were powered to detect mean differences 80% greater than pooled variances with type I error risk of 5% and type II error probability equivalent to 80% power. Mean and variance estimates for statistical power calculations were taken from prior research with male mice (Brockhurst et al., 2015).

Repeated sessions of exposure were monitored for aggressive interactions scored when intruders or residents slapped, scratched, or bit at one another across the mesh-screen barrier using ethogram elements described elsewhere (Clipperton-Allen et al., 2011). Neutral interactions that consisted of sniffing or social exploration without aggression were also recorded. Determining the initiator of social interactions was hampered by the mesh-screen barrier and could not be reliably determined. All occurrences of aggressive and neutral interactions were therefore scored without reference to initiator on the 1st, 3rd, 5th, 7th, 9th, and 11th exposure sessions. On these same sessions, time spent in social proximity was scored when the intruder was located within one body length from the resident, and risk assessment behavior of intruders was scored as described elsewhere (Clipperton-Allen et al., 2011).

Three tests of coping were then administered sequentially in the same order 5–8 days after completion of the treatment conditions for intruders and controls. All tests were conducted 2–3 h after lights-on during the inactive phase to control for circadian effects. First, an open-field test was conducted by placing mice individually for 10 min in a novel white plastic open-field box (40 × 40 × 42 cm) illuminated at 225 lx. Time spent freezing in the open-field was scored as the absence of all movement except respiration and reflects anxiety-like behavior in mice (Ahn et al., 2013). Open-field center entries were scored whenever all 4 feet crossed into the open-field center area (16 × 16 cm), and total time spent engaged in locomotion in the open-field was scored as a measure of non-specific activity. Immediately after the open-field test, object-exploration was assessed in the same open-field apparatus with a familiar white plastic cap from the home cage and a novel black plastic pipe. Each mouse was removed temporarily from the open-field while objects were attached to the floor for each 10-min object-exploration test. Latencies to first explore the novel and familiar object were scored separately when an animal's head was located 1 cm from each object. Lastly, a standard 6-min tail-suspension test (Cryan et al., 2005) immediately followed the object-exploration test with total time spent immobile scored as the absence of movement except for respiration and nose or ear twitching. Tail climbing was prevented by use of a tail tube described elsewhere (Can et al., 2012). All tests and prior exposure training sessions were videotaped and scored offline by a trained observer without knowledge of the experimental conditions. Instead of using video-capture technology, a trained observer was used to reliably distinguish neutral from aggressive social interactions and identify risk assessment behavior. Observer reliabilities for behavioral measures expressed as intra-class correlation coefficients are presented in Supplementary material Table S1.

Data were analyzed with SYSTAT software. All measures were normally distributed according to Kolmogorov-Smirnov tests except for latencies to explore the novel object. Latencies to explore the novel and the familiar object were separately log transformed for parametric evaluation. Behavioral and hormonal measures acquired during exposure sessions were evaluated using repeated measures analysis of variance (ANOVA) and Bonferroni corrected t-tests for pairwise comparisons. Repeated sessions of exposure and type of social interaction (aggressive *vs.* neutral) were considered within-subjects factors. Five behavioral measures of coping behavior assessed in three contexts were simultaneously analyzed with multivariate analysis of variance (MANOVA) to provide a single statistical test of whether mice exposed to strangers differed from non-exposed controls. Exposure was considered a between-subjects factor in the MANOVA, as well as all follow-up univariate analyses used to separately evaluate each coping measure. Object type (novel *vs.* familiar) was considered a within-subjects factor in the univariate analysis of object-exploration latencies. Relationships between measures were assessed with Pearson correlation coefficients and descriptive statistics are presented as mean ± SEM. Test statistics were evaluated with two-tailed distributions at $P < 0.05$.

3. Results

Repeated exposure to a same-sex resident stranger consistently elicited corticosterone responses (Fig. 1) as discerned by repeated measures ANOVA ($F(4,32) = 19.8$, $P < 0.001$). Pairwise within-subjects comparisons indicated that corticosterone after each exposure session was greater than undisturbed baseline corticosterone levels measured in the home cage ($P < 0.001$). Corticosterone responses increased over repeated exposure sessions ($F(3,24) = 4.8$, $P = 0.009$). Pairwise within-subjects comparisons confirmed that corticosterone was greater after the 7th compared to the 1st exposure session ($P = 0.003$) and did not differ significantly between any of the other exposure sessions depicted in Fig. 1.