Published in final edited form as:

Brain Res. 2009 January 28; 1251: 112–120. doi:10.1016/j.brainres.2008.11.042.

Social defeat stress potentiates thermal sensitivity in operant models of pain processing

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Abstract

Higher-order processing of nociceptive input is distributed in corticolimbic regions of the brain, including the anterior cingulate, parieto-insular and prefrontal cortices, as well as subcortical structures such as the bed nucleus of stria terminalis and amygdala. In addition to their role in pain processing, these regions encode or modulate emotional, motivational and sensory responses to stress. Thus, pain and stress pathways in the brain intersect at cortical and subcortical forebrain structures. Accordingly, previous work has shown that acute restraint stress in female rats induces heat hyperalgesia in a forebrain-dependent operant test of thermal escape. In the present study, we investigated the effects of social defeat stress in male rats on the operant escape task, as well as in a test of nociceptive thermal preference. After establishing baseline behaviors in these tests, separate groups of rats were socially defeated by dominant “resident” male rats. They were tested for thermal preference after 5 successive social defeat sessions. Escape from cold, heat and a neutral warm temperature also was evaluated after social defeat. Defeated rats exhibited a significant increase in cold preference after social defeat compared to the baseline. In the escape task, the rats exhibited increased escape from warm and nociceptive cold and heat temperatures. Thus, chronic social stress produces hyperalgesia for both hot and cold stimuli in male rats, suggesting a mutually facilitatory cross-regulation between central pathways regulating stress and pain.

Keywords

Stress; Hyperalgesia; Pain; Social defeat; Operant behavior

1. Introduction

The sensation of pain signals hazards in the environment that pose an immediate threat to survival and well-being. However, damage to neural pathways that process pain can give rise to a variety of idiopathic pain syndromes that persist in the absence of any physical

stimulus and seem to have no somatic origin. Chronic pain detracts from quality of life by restricting daily activities, including exercise and sleep, and by activating stress pathways in the brain. Chronic pain is associated with heightened cortisol levels and an increased incidence of anxiety and depression (Ulrich-Lai et al., 2006). Pain-processing regions of the brain include corticolimbic regions such as the parieto-insular cortex (Craig, 2003) and the anterior cingulate cortex (Rainville et al., 1997; Johansen et al., 2001) which imbue incoming sensory information with emotional and motivational salience. The affective aspects of the pain experience are encoded in these and other limbic areas, such as the hypothalamus, amygdala, bed nucleus of stria terminalis (BNST) and hippocampus, that are associated with stress reactions.

Historically, research has shown that acute stress (e.g., foot shock, restraint) suppresses reflexive measures of nociception (such as tail flick and paw lick) (Madden et al., 1977; Amir and Amit, 1979; Lewis et al., 1983; Jørgensen et al., 1984), which are mediated by spinal and spinobulbar pathways. The conclusion from these studies was that the observed hyporeflexia represented stress-induced analgesia (SIA). However, acute restraint stress produces thermal hyperalgesia in an operant test (i.e., increased escape from nociceptive heat stimulation) in female rats (King et al., 2003, 2007). These results were obtained with temperatures that predominantly activate slow-conducting C nociceptors (Yeomans and Proudfit, 1996; Vierck et al., 2002; King et al., 2007), which in turn activate stress-sensitive limbic regions such as the anterior cingulate and insular cortices (Qiu et al., 2006). Thus, stress differentially modulates reflex and operant escape responses at different anatomical levels in the hierarchical processing of nociceptive stimuli. Stress-induced hyporeflexia manifests as a suppression of reflexive responses to thermal stimulation, whereas stress-induced hyperalgesia is demonstrated by increased escape from the same stimuli in an operant test of pain perception. (King et al., 2003, 2007).

Perception of pain includes higher level cerebral processing of nociceptive input (Coghill et al., 1999; Craggs et al., 2007), which cannot be measured using reflex-based behavioral tests (Vierck et al., 2002, 2008). The thermal preference test used in this study provides a choice between opposing heat and cold temperatures, both aversive. Likewise, the operant escape task offers a choice between a nociceptive temperature and an aversive bright light. The advantage of these operant tests of thermal sensitivity is that responses can be attributed to pain processing throughout the neuraxis, including the cerebrum, rather than the spinal or spinal-brainstem-spinal circuits that mediate reflexive responses.

Numerous issues must be resolved to determine the generality or specificity of stress-induced hyperalgesia. Acute restraint is considered a form of psychological stress, acting through limbic and hypothalamic circuits (Herman et al., 2005), but its effects habituate with repetition (Melia et al., 1994). Social defeat is a robust stressor, resulting in increases in circulating adrenocorticotrophic hormone (Heinrichs et al., 1992; Ebner et al., 2005) and corticosterone (Heinrichs et al., 1992; Covington and Miczek, 2001; Wommack and Delville, 2003), decreases in thymus and seminal vesicle masses (Buwalda et al., 2001), and increases in anxiety-related behaviors in tests of neophobia (Heinrichs et al., 1992; Calfa et al. 2006). In addition, social defeat is a form of emotional stress that takes advantage of the rats' natural social behaviors and does not readily habituate (Martinez et al., 2002); thus repeated exposures might be useful as a model of chronic stress effects on pain sensitivity. In our previous studies, the operant escape task was utilized to analyze the effects of acute (restraint) stress on pain sensitivity in female rats. The present study investigated the effects of repeated (social defeat) stress using thermal preference and operant escape as measures of thermal sensitivity in male rats.

2. Results

2.1. Experiment 1

2.1.1. Effect of social defeat on temperature preference—The effects of chronic social defeat on temperature preference behavior are summarized in Fig. 1A. A one-way repeated measures ANOVA revealed significant effects of social defeat on total time spent on the heated plate minus total time spent on the cold plate across 5 defeat sessions ($F_{(5,55)}=5.854$; $p=0.0002$). Bonferroni post-tests revealed increases in cold preference after social defeat on day 2 ($t_{11}=4.431$; $p<0.001$) and day 5 ($t_{11}=4.792$; $p<0.001$).

Preference behavior was then plotted as a function of pairs of responses within each test session. Since there was no apparent cumulative effect of social defeat (i.e., no progressive change in preference over the course of 5 social defeats), the behavior was averaged across the 5 days (Fig. 1B). The first pair of responses began at the start of the test session (on the heated plate) and ended when the rat crossed back from the cold plate to the heated plate. The cumulative time spent on the heated plate minus time on the cold plate for each successive pair was calculated. Prior to social defeat, the rats displayed a slight preference for the cold plate. After social defeat, this preference was significantly enhanced ($t_{11}=4.513$, $p=0.0009$).

2.1.2. Effects of social defeat on latency and the number of crosses between compartments—Rats were initially placed on the heated plate at the start of the test session. Latency to cross-over to the cold plate was significantly reduced by social defeat ($F_{(5,55)}=4.601$; $p=0.0014$), and Bonferroni tests revealed that the latencies were significantly reduced from baseline on days 2 to day 5 ($t_{11}=4.059$; $p<0.01$) (Fig. 2A). The number of crossings between the heated and cold plates remained relatively constant across days, indicating that there was no overall change in motor activity after stress (Fig. 2B). However, there was a significant effect of stress on the number of crosses ($F_{(5,55)}=2.560$; $p=0.0374$) on day 5 ($t_{11}=3.181$; $p<0.05$).

2.1.3. Variation in dominance/submission behavior over 5 days of social defeat—In an attempt to understand the variability in stress effects on days 1–5, relationships between measures of the dominance/submission interaction and the subsequent thermal preference of the intruders were statistically evaluated by the Pearson correlation test. Resident/intruder interactions were scored by the number of defeats, the latency to defeat, and the total duration of defeat (i.e. total time during which the resident rat exhibited dominance, standing over the intruder while the intruder lay in a supine posture) (Fig. 3). No significant correlation was found between these measures and the subsequent behavior of the intruders on the operant test. However, there was a significant increase in latency to defeat over time ($F_{(4,44)}=2.809$; $p<0.0367$). Student's *t*-tests were performed comparing the first day (day 1) of social defeat to each subsequent day (days 2–5), and a significant increase in latency to defeat was found on day 4 ($t_{11}=2.265$; $p=0.0447$). These differences in latency to defeat over the course of successive social defeat sessions could be the result of learned submissive behavior (e.g. freezing, etc.) on the part of the intruder.

2.2. Experiment 2

2.2.1. Effects of social defeat on thermal escape behavior—The average durations of escape from thermal stimulation for socially-defeated rats and unstressed control rats were calculated as cumulative totals (as in Experiment 1) and plotted across escape responses through response 7, when cumulative durations reached an asymptote (Figs. 4A–C). After social defeats, the rats displayed a progressive increase in escape behavior on the 10 °C, 44.5 °C, and 32 °C escape tests. The control rats, when tested at the same

temperatures and comparable time points as the socially-defeated rats, did not display any significant change in escape over time (Figs. 4D–F).

Social defeat produced a significant increase in the total escape duration after the 7th platform occupancy on the 10 °C test ($t_{11}=2.48$; $p=0.03$), the 44.5 °C test ($t_{11}=2.62$; $p=0.02$), and the 32 °C test ($t_{11}=4.43$; $p=0.01$) (Fig. 5A). After the 7th response, there were no further significant differences in escape duration (data not shown). Conversely, social defeat produced a significant decrease in time spent on the thermal plate during the 44.5 °C test ($t_{11}=4.12$; $p=0.002$) and the 32 °C test ($t_{11}=2.43$; $p=0.03$), but not the 10 °C test ($t_{11}=0.38$; $p>0.05$) (Fig. 5B).

3. Discussion

In agreement with our previous findings for the effects of restraint stress in female rats (King et al., 2003, 2007), social defeat potentiated heat sensitivity of males during thermal preference and escape testing. In addition, we found an increased aversion to cold during escape testing. Thus, there was an overall increase in sensitivity to nociceptive temperatures after social defeat. However, the preference for cold in the thermal preference test and the more robust effects seen for heat stimulation in the operant escape task suggest that increased sensitivity after stress is greater for heat than it is for cold.

Tornatzky and Miczek (1994) showed that repeated social defeat resulted in a 1.5 °C increase in core body temperature that was evident immediately and lasted for at least 3 h after termination of the stressor. Accordingly, an increased sensitivity to warmth (32 °C) and heat (44.5 °C) after social defeat (Figs. 4 and 5) could perhaps be partially attributed to stress-induced hyperthermia. However, there was also an increased aversion to cold in the operant escape task, and that aversion cannot be attributed to hyperthermia.

In the present study, the rats exhibited a slight preference for 5 °C over 45 °C under baseline conditions, which corroborates previous findings that male rats have an innate aversion to heat relative to females (Vierck et al., 2008). This may also contribute to the greater aversion to heat, versus cold, on the operant escape task. Thus, there appear to be multiple factors affecting relative preferences for and escape from nociceptive cold and heat, including sex, body temperature, and stress-induced thermal hyperalgesia.

Heat aversion in the thermal preference test was shown by lowered latencies to escape heat at the beginning of test sessions and by an increase in cold preference (heat aversion) over the course of testing sessions. The increased heat sensitivity after social defeat stress did not habituate over 5 successive exposures. The highest levels of heat aversion occurred on days 2 and 5 of the tests after social dominance. This variability in significant effects of stress could have resulted from day to day differences in the impact of social dominance on the intruders, but significant correlations with measures of dominance/submission were not obtained. It is not known if the number and duration of defeats are critical in determining the affective and physiological responses of the intruders or if the mere presence of the resident rats results in significant anxiety and stress.

To address whether the rats were responding to some cue in the environment other than a temperature differential, both thermal preference plates were set to 36 °C during thermal preference training, and the total time spent on either plate did not differ significantly (data not shown). Also, the average number of crossings on the hot (45 °C) vs. cold (5 °C) preference test remained high throughout the training period and after social defeat, indicating that the rats had not learned to avoid the hot plate by occupying the cold plate for extended periods and that motor/exploratory activity was not impaired by stress. In addition, in the operant escape task, the non-stressed control rats showed no change in escape over

time, controlling for changes in behavioral strategy (e.g., avoidance learning) with repeated testing.

Neural mechanisms that process emotional stress and pain converge at multiple sites in the CNS. Nociceptive input has been shown to activate the paraventricular nucleus (PVN) of the hypothalamus (Ziegler and Herman, 2002; Ulrich-Lai et al., 2006), from which stress-related neuroendocrine activities of the hypothalamic–pituitary–adrenal (HPA) axis are triggered. In the acute condition, these HPA axis actions constitute highly adaptive and important physiological responses to environmental hazards. On the other hand, prolonged exposure to pain or chronic stress can lead to maladaptive changes in the limbic pathways that regulate HPA output. In one study, an animal model of chronic neuropathic pain was found to increase corticotropin releasing hormone (CRH) and glucocorticoid receptor (GR) expression in the central amygdala, and to downregulate GR expression in the hippocampus (Ulrich-Lai et al., 2006), consistent with a chronic stress profile. The central amygdala (CeA) has neurons that respond strongly to noxious stimuli, and administration of low levels of CRH results in increased responsivity to noxious stimulation (although very high levels of CRH produce the reverse) (Ji and Neugebauer, 2008). In addition, reduced GR expression in the hippocampus could contribute to impaired negative feedback of the HPA axis. Thus chronic pain could contribute to changes that result in sensitization of stress response systems, which in turn may potentiate pain perception by acting on descending pathways that facilitate nociceptive transmission (Millan, 2002; Ren and Dubner, 2002).

Widely distributed transmitter systems appear to be altered by both stress and pain. These include substance P, brain-derived neurotrophic factor (BDNF), norepinephrine, and GABA. Each of these systems can be altered at multiple levels of the nervous system, including the spinal cord, the brainstem, and the cerebrum. For example, Bradesi et al. (2006) found that forced swim stress resulted in colorectal hyperreflexia, which was blocked by antagonism of the neurokinin 1 receptor (NK1R, the substance P receptor). In this study, the stressful stimulation also increased NK1R mRNA and protein in the lumbar spinal cord and decreased expression in the colon. Acute and repeated restraint stress and acute and chronic inflammation (formalin and Complete Freund's adjuvant or CFA, respectively) reduced NK1R and BDNF mRNA expression (Duric and McCaaron, 2006, 2007) in the hippocampus, while CFA decreased expression in the dorsal horn of the spinal cord. Deyama et al. (2008) found that formalin injections into the hindpaw increased norepinephrine release from the BNST, a structure known to be important in evaluating the emotional salience of potentially threatening stimuli. In this study, antagonism of beta-adrenoceptors attenuated conditioned place aversion related to the formalin injections. Finally, Suarez-Roca et al. (2008) found decreased GABA release in the spinal cord and increased sensitivity to formalin after forced swim stress. The formalin response was attenuated by diazepam and restored by a GABA antagonist. Collectively, these studies provide evidence for a strong interplay between the stress and pain response systems, not only at levels involved in reflexive responding, but also at higher levels of cognitive and emotional processing.

The anterior cingulate cortex, which has been implicated in the affective aspects of pain, exhibits distinct morphological and biochemical changes in chronically stressed rats (Figueiredo et al., 2003; Radley et al., 2004; Perez-Cruz et al., 2007). There is also evidence of substantial gray matter loss in the anterior cingulate of patients who suffer from post-traumatic stress disorder (PTSD) after prolonged exposure to intense stress (Kitayama et al., 2006; Kasai et al., 2008). Functional imaging studies have implicated the anterior cingulate cortex in emotional processing of pain (Derbyshire and Jones, 1998; Price, 2002), and lesions of the anterior cingulate (Craig, 2003) and parietoinsular cortex (Greenspan et al.,

1999) drastically reduce pain and temperature sensitivity. Thus, stress may act at the level of the anterior cingulate cortex to facilitate thermal nociception.

Overall, it appears that chronic emotional stress produces alterations in CNS structures that mediate convergent processing of emotional stress and nociceptive stimulation. Therefore, it would be beneficial to have a laboratory animal model that reveals progressive changes in pain sensitivity during exposure to acute and chronic emotional stress. In the present study, an expected increase in hyperalgesia over the series of 5 social encounters was not observed. Therefore, longer or more frequent periods of stress may be required to establish alterations in neurobiological mechanisms and behavioral responses that are consistent with the effects of severe and chronic emotional stress that are observed in humans.

In conclusion, psychosocial stress activates the HPA axis and the limbic system which could affect pain sensitivity. Accordingly, social defeat stress augmented thermal sensitivity in two operant tests of pain perception. These findings in male rats are consistent with our previous results in females using restraint stress and provide a model for investigating the neural substrates underlying social stress-induced hyperalgesia.

4. Experimental procedures

4.1. Animals

All the rats were of the Long–Evans strain, and were housed in polycarbonate cages (43×21.5×25.5 cm) on a 12–12 h light–dark cycle in an AAALAC-approved, climate-controlled vivarium. Standard laboratory chow and tap water were available *ad libitum* throughout the experiment. All procedures were pre-approved by the University of Florida's Institutional Animal Care and Use Committee, and the experiments were conducted in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

4.1.1. Intruder rats—The male intruder rats in experiment 1 ($n=12$; 250–300 g at the beginning of the experiment) were pair-housed throughout the duration of the experiment. The rats were adapted and trained and then tested for 4 weeks on the thermal preference test to establish baseline behavior before social defeat. The rats were exposed to 10 min of social defeat once per day for 5 days and tested for thermal preference at the completion of each social interaction.

In experiment 2, the male intruder rats ($n=12$; 170–210 g at the beginning of the experiment) were pair-housed until 3 days prior to the first social defeat; then they were individually housed. The unstressed control rats in this experiment ($n=5$; 140–200 g) were group housed (2 in one cage, 3 in another) throughout the duration of the experiment. These rats were trained and baseline tested for 4 weeks on the operant escape test. Like in experiment 1, the rats were then exposed to 10 min of social defeat once per day for 5 days and tested on the escape task at the completion of social interaction.

4.1.2. Resident rats—The male resident rats were vasectomized, and allowed to recover for 7–10 days ($n=6$). These rats (weighing between 600–700 g) were then pair-housed with female rats (300–400 g). The residents used in this study consistently exhibited dominant behaviors toward smaller males in behavioral pre-tests of social dominance.

4.2. Social defeat stress

Prior to each social defeat session, the resident female rat was removed from the home cage 10 min before the resident–intruder encounter. The subsequent social defeat procedures

consisted of two stages. In the first stage, an intruder rat was placed into the cage of a dominant resident. The encounter continued for 5 min or until the intruder displayed submissive behavior defined as entering into a supine posture 3 times for at least 2 s each. Immediately following this defeat encounter, the second stage of the social defeat stress commenced. The intruder was removed and placed into a 10 cm×10 cm×15 cm (inner dimensions) double-walled wire mesh protective cage. The rat was then returned, inside the protective cage, to the cage of the resident rat until 10 min had passed from the start of the defeat encounter. The wire cage served to separate the rats, avoiding physical contact but maintaining stressful sensory stimuli. After the 10-minute session, each intruder was individually placed into the thermal preference test (experiment 1) or the operant escape task (experiment 2).

4.3. Experiment 1: temperature preference test

Temperature preference testing was conducted in an operant apparatus that permits rats to choose between two compartments. A shuttle-box was used, in which an enclosure was divided into two 6×11.5 in. compartments, each with a distinct thermally regulated floor plate. The compartments were separated by a partition with an opening that allowed free passage between the compartments.

During training, the temperature of both plates was initially set to 36 °C so that the rats could acclimate to the testing apparatus, and also to determine if there was a preference for one compartment over another, independent of temperature. Then, during the first week of the training period, the temperature of both plates was gradually changed to produce an equal preference for the two compartments. The final temperatures used, and those selected for evaluation of stress, were 5 °C for the cold plate and 45 °C for the heated plate. The training period lasted for 3 weeks, until the rats had reached a stable level of responding to the target temperatures (less than 10% variation of the mean on each of 5 consecutive days). The averages for the last 5 days of thermal preference training were then taken as the baseline.

A 15 min pre-test exposure to 36.0 °C in a single compartment was used to standardize foot temperatures and to acclimate the rats to the testing apparatus. Subsequent preference trials with 5 °C vs. 45 °C stimulation were 15 min long, with the rats being placed initially on the hot side. Temperature preference responses were assessed by frequency (number of crossings between compartments during a trial), duration (sequential times spent occupying each compartment during a trial), and latency to the first cross-over response.

4.4. Experiment 2: thermal escape test

Tests of operant escape from thermal stimulation were conducted in a shuttle-box, in which a 6×14 in. enclosure was divided into two compartments by a hanging septum with an open doorway. One compartment (6×9 in.) contained a temperature-regulated floor plate, allowing presentation of a nociceptive stimulus to the rats' paws. This compartment was only dimly illuminated. The adjacent compartment contained a thermally neutral 5×6 in. platform tilted at 12°. This compartment was brightly lit, producing a conflict between the nociceptive temperature of the thermally regulated plate and the bright light in the platform compartment. The occupancy of each platform was detected by electronic position detectors and tabulated by proprietary software. In this test, rats reliably partition their time between the two compartments based upon the intensity of cold and hot temperatures (Vierck et al., 2002).

The rats were trained to escape the thermally regulated floor at 10 °C, 44.5 °C or 32 °C. Training and baseline measures were taken for 5 weeks prior to social defeat. After baseline

testing, the rats were subjected to 3 days of social defeat (or no defeat for unstressed controls) followed by escape testing on 10 °C, 44.5 °C, or 32 °C floors. Thermal sensitivity was measured by successive durations of platform and plate occupancies up to the 7th escape response (when escape behavior reached a plateau). The last week of baseline testing was used for the expressed baseline measures.

4.5. Statistical analyses

In experiment 1, thermal preference scores were analyzed with one-way repeated measures ANOVAs (total time), for baseline vs. social defeat on each of the 5 days using Graphpad Prism software. All significant effects ($p < 0.05$) were analyzed by Bonferroni post-tests. The thermal preference scores for social defeat days 1–5 were averaged and plotted against response numbers. Differences between baseline and post-social defeat preference scores were analyzed by t -test for the 15th response, a point on the behavioral plateau. The effects of social defeat on the number of entries to the cold plate and the initial latency to cross-over to the cold plate were analyzed using a one-way repeated measures ANOVA. All significant effects ($p < 0.05$) were analyzed by Bonferroni post-tests. Between-session differences in the number of defeats, latency to the first defeat, and duration of defeats were analyzed with t -tests, comparing the data for each session with the data for the initial defeat session. Pearson correlation tests were performed to evaluate any possible interaction between number of defeats, latency to defeat, and defeat duration on subsequent thermal preference behavior.

In experiment 2, differences between baseline and post-social defeat measures of total platform and plate durations after the 7th escape were analyzed with t -tests for each temperature condition.

Acknowledgments

This work was supported by the University of Florida Office of Research and the Comprehensive Center for Pain Research.

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Fig. 1.

Effects of chronic social defeat on thermal preference behavior. (A) After social defeat, the rats displayed increased preferences for the cold plate as evidenced by decreases in total time spent on the heated plate minus time on the cold plate (Hot minus Cold). The data are expressed in seconds over a 15 min test session at baseline (average of 5 days) and during 5 consecutive days of social defeat. (B) The increase in cold preference after social defeat was evident early in a trial and reached maximum after the 8th response pair. The data are expressed as time (seconds) spent on the heated plate minus the cold plate over 15 consecutive response pairs during a 15 min test session at baseline (average of 5 days) and across 5 days of social defeat. All values are expressed as mean $\pm$ SEM ($n=12$). *** $p<0.001$.

Fig. 2.

Effects of social defeat on first latency to cross-over and number of crossings. (A) After social defeat, the rats displayed a decrease in initial latency to cross-over from the heated plate to the cold plate, while (B) the number of crossings from the heated plate to the cold plate (number of entries) was significantly reduced only on the 5th day. All values are expressed as mean $\pm$ SEM ($n=12$). *($p<0.05$) and **($p<0.01$).

Fig. 3.

Variation in dominance behavior over 5 days of social defeat. (A) Number of defeats, (B) latency to defeat (in seconds), and (C) defeat duration (in seconds) over 5 consecutive days of social defeat was variable, but only latency to defeat differed significantly on the 4th day, compared to the 1st day. Defeat duration is defined as the total time over a single defeat session that the intruder spends in a submissive posture with the resident. All values are expressed as mean±SEM ($n=12$).

Fig. 4.

Effects of social defeat on cumulative escape duration. After social defeat, the rats displayed greater escape behavior during (A) 10 °C, (B) 44.5 °C, and (C) 32 °C trials, in comparison with baseline responses. In contrast, the unstressed control rats displayed a slight (statistically insignificant) decrease in escape responses when tested at (D) 10 °C and (E) 44.5 °C, and no change in escape behavior when tested at (F) 32 °C. Behavior is expressed as the cumulative time spent on the escape platform for sequential escape responses up to response # 7. Values expressed are group means. The values for the unstressed control rats in D, E, and F were taken at time points that are comparable to the measures collected for the stressed rats in A, B, and C.

Fig. 5.

Effects of social defeat on total (accumulated) escape duration on the 7th response. After social defeat, the rats spent (A) more total time on the escape platform and (B) less total time on the thermally-regulated plate relative to baseline for all temperature conditions except plate duration at 10 °C. Values expressed are mean±SEM ($n=12$). * $p<0.05$, ** $p<0.01$.