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Immediate post-defeat infusions of the noradrenergic receptor antagonist propranolol impair the consolidation of conditioned defeat in male Syrian hamsters

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HIGHLIGHTS

- Propranolol blocked the consolidation of conditioned defeat
- Peripheral and central administration significantly reduced submissive behavior.
- The effect of propranolol administration on consolidation was time-dependent.

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ABSTRACT

Social defeat occurs when an animal is attacked and subjugated by an aggressive conspecific. Following social defeat, male Syrian hamsters fail to display species-typical territorial aggression and instead exhibit submissive or defensive behaviors even when in the presence of a non-aggressive intruder. We have termed this phenomenon conditioned defeat (CD). The mechanisms underlying CD are not fully understood, but data from our lab suggest that at least some of the mechanisms are similar to those that mediate classical fear conditioning. The goal of the present experiment was to test the hypothesis that noradrenergic signaling promotes the consolidation of CD, as in classical fear conditioning, by determining whether CD is disrupted by post-training blockade of noradrenergic activity. In Experiment 1, we determined whether systemic infusions of the noradrenergic receptor antagonist propranolol (0, 1.0, 10, or 20 mg/kg) given immediately after a 15 min defeat by a resident aggressor would impair CD tested 48 h later. Hamsters that were given immediate post-training infusions of propranolol (1.0, but not 10 or 20 mg/kg) showed significantly less submissive behavior than did those given vehicle infusions supporting the hypothesis that there is noradrenergic modulation of the consolidation of a social defeat experience. In Experiment 2, we demonstrated that propranolol (1.0 mg/kg) given immediately, but not 4 or 24 h, after defeat impaired CD tested 48 h after defeat indicating that the window within which the memory for social defeat is susceptible to beta-adrenergic modulation is temporary. In Experiment 3, we examined whether central blockade of noradrenergic receptors could recapitulate the effect of systemic injections by giving an intracerebroventricular infusion of propranolol immediately after defeat and examining the effect on CD 24 h later. Centrally administered propranolol (20 μg/3 μl but not 2 μg/3 μl) was also effective in dose-dependently reducing consolidation of CD. Collectively, the present results indicate that noradrenergic activity promotes the consolidation of CD and suggest that CD is a valuable model to study the processes by which emotion and stress modulate memory in an ethologically relevant context. These data also suggest that the popular conception in the clinical literature that the anxiolytic effect of propranolol is primarily due to the drug's peripheral effects may need to be reconsidered.

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1. Introduction

Social defeat is a potent stressor that occurs when an animal is attacked and subjugated by an aggressive conspecific. Syrian hamsters are solitary animals that display territorial aggression against intruding conspecifics when singly housed under laboratory conditions [1,67,84]. Following social defeat, however, Syrian hamsters fail to display species-typical territorial aggression and instead exhibit submissive or defensive behaviors even when in the presence of a non-aggressive intruder [39,64,69]. This phenomenon is termed conditioned defeat (CD). Social defeat is considered a potent stressor because the effects of an initial defeat are profound and long lasting, and defeated hamsters exhibit activation of the hypothalamic-pituitary-adrenal (HPA) axis [39]. Specifically, exposure to agonistic encounters produces increases in plasma adrenocorticotropin (ACTH) and glucocorticoids in defeated but not in dominant hamsters [36–38]. Furthermore, defeated animals exhibit increased blood pressure and heart rate and compromised immune function in comparison to dominant animals [5,6,41]. CD is long-lasting; following social defeat, 100% of defeated hamsters exhibit a total absence of territorial aggression and increased submissive/defensive behavior in the presence of smaller, non-aggressive intruders. This response lasts for at least 10 days without further social defeat [39]. In fact, for a majority of the defeated animals, CD lasts at least 33 days even without a further social defeat experience [39].

Noradrenergic activity plays a role in anxiety-like processes and is important for stress-related changes in behavior [9,18,63]. Beta-adrenergic antagonists are widely prescribed, albeit “off-label”, in anxiety disorders such as social phobia [10,21], posttraumatic stress disorder [27,45,86], and panic disorder [35,83]. In addition, beta-blockers reduce acute stage fright [8,21], test anxiety [23], and contextual fear [32] in humans. In rodents, beta-adrenergic antagonists also decrease anxiety [2,3,30,82,89], reduce fear conditioning [19] and prevent behavioral changes caused by repeated stress [15].

Extensive evidence from both human and animal studies indicate that catecholamines released peripherally and centrally during emotional arousal play a role in the consolidation of emotional experiences [13,62]. For example, post-training infusions of the stress hormone epinephrine, which is released by the adrenal medulla, enhance memory in a time- and dose-dependent manner in a variety of learning and memory tasks [24,29,61,74,75]. Interestingly, epinephrine-induced memory enhancement is reversed or impaired by removal of the adrenal medulla or by beta-adrenergic receptor antagonists in rodents [59,71,74]. Similarly, beta-adrenergic receptor antagonists prevent both the memory-enhancing effect of arousal in humans and rodents [12,46,66,87] as well as stress-induced impairments in extinction learning [25].

Despite the importance of catecholamines in stress responses and emotional memory consolidation, there is limited research examining the putative roles of noradrenergic transmission in conditioned responses to natural threats such as social defeat. The goal of the present set of experiments was to test the hypothesis that noradrenergic transmission is involved in the consolidation of CD by determining whether CD is susceptible to post-training manipulations of noradrenergic systems. Specifically, Experiment 1 determined whether immediate post-defeat, systemic infusions of the beta-adrenergic antagonist propranolol would dose-dependently impair CD tested 48 h after the defeat. If noradrenergic activity is involved in the consolidation of CD, then its effects should be restricted to the time period immediately following the social defeat. To test this, Experiment 2 examined the time-dependence of this post-training effect. Because propranolol effectively crosses the blood brain barrier [7,65], Experiment 3 was designed to determine whether the effect of propranolol observed in Experiment 1 could be due, at least in part, to an action of the drug in the central nervous system. In this experiment, we microinjected propranolol into the lateral ventricle immediately after defeat.

2. Materials and methods

2.1. Subjects

Adult male Syrian hamsters (*Mesocricetus auratus*; Charles River, Wilmington, MA) weighing 120–130 g (63–70 days) upon arrival were used in this study (Experiment 1 n = 80; Experiment 2 n = 50; Experiment 3 n = 32; individual group n's are indicated in the figures). Animals were housed in the animal facility for one week before the beginning of any manipulation (surgery and/or single housing, as indicated below). Thus, behavioral testing began a minimum of two weeks after arrival. Additional hamsters weighing 180 g on average were used as resident aggressors (RA) for CD training, and hamsters weighing 90–100 g on arrival were used as nonaggressive intruder stimulus animals during behavioral testing. All hamsters were housed in polycarbonate cages (20 × 40 × 20 cm) with wire mesh tops in a climate-controlled room (70–74 °F), and food and water was available ad libitum. Subjects and resident aggressors were housed individually, whereas nonaggressive intruders were group housed (five hamsters/cage) to minimize aggressiveness. The hamsters were maintained on a 14:10 h light:dark cycle with light off at 1100 h, and all training and testing occurred during the first 3 h of the dark phase of the daily light:dark cycle. All procedures and protocols involving hamsters were approved by the Georgia State University Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications Nos. 80–23, revised 1978).

2.2. Conditioned defeat (CD)

All subjects were single-housed for 7–10 days before the beginning of CD training during which time they were handled four to five times. Hamsters were matched by weight and randomly assigned to experimental or control groups. On the day of CD training, all hamsters were transported from the colony room to the behavioral testing room and were allowed to acclimate to the testing room for at least 30 min. CD training/acquisition consisted of a single resident/intruder pairing in which a subject was placed in a resident aggressor's home cage for 15 min. During the 15 min defeat session, experienced observers ensured that subjects were routinely attacked by the resident aggressor and that they displayed submissive and defensive behaviors towards this opponent. In the few cases wherein the resident aggressors did not attack within the first 2 min of the defeat session (n = 4, Experiment 1; n = 5, Experiment 2), the subject was immediately moved into the cage of another resident aggressor so that all animals experienced a social defeat. Resident aggressors were used a maximum of two times during any particular day to minimize variability in their behavior due to repeated testing.

Testing for CD began 48 h (±1 h) after training for Experiments 1 and 2, and 24 h (±1 h) after training for Experiment 3. The extra time was allotted in the first two experiments to ensure that the peripherally administered drug would have ample chance to be metabolized fully before CD testing [52]. During testing, a non-aggressive intruder was placed into the home cage of the defeated subject for 5 min. All testing sessions were recorded and scored by observers blind to experimental condition using Noldus Observer (version 4; Noldus Information Technology, Wageningen, Netherlands). The following classes of behaviors were recorded as total duration in seconds during the 5 min testing session: (1) Non-social: locomotor/exploratory, self-groom, nesting, feeding, sleeping, (2) Social: attend, approach, investigate, sniff, touching nose, (3) Submissive/defensive: upright/side defense, tail lift, teeth chatter, flee, full submissive posture, and (4) Aggressive: upright/side offense, chase, bite, attack.

2.3. Surgery (Experiment 3)

One week after arrival, animals were initially anesthetized with a 5% concentration of isoflurane to oxygen. Maintenance of the surgical plane of anesthesia occurred at a 2.5–3% concentration of isoflurane, and this maintenance was verified by the lack of a withdrawal of the paw in response to toe-pinch. Animals were placed into the stereotaxic apparatus and the skull was exposed. Bregma and lambda were leveled and a unilateral cannula guide was placed into either the left or right side, aimed at the lateral ventricle with the following coordinates: 0.5 mm A/P, ±1.4 mm M/L, and 2.0 mm D/V (measuring from dura). A wound clip was attached to the skull posterior to the cannula guide to stabilize the mount and then dental cement was used to anchor the guide in place. During the surgery, animals received an s.c. injection of 1.0 ml of 0.9% saline and 5 mg/kg ketoprofen to restore hydration and to provide pain relief. An obturator was placed into the cannula guide after surgery to maintain patency. After at least 2 days of recovery, animals were then handled each day for 5 days by gently holding the animal in the experimenter's hand and unscrewing the obdurator, moving it up and down, and screwing the obdurator back onto the cannula guide. Animals were weighed on the last day of handling and assigned to one of the three weight-matched groups.

2.4. Drug injections

2.4.1. Experiment 1: effects of immediate post-defeat injections of propranolol on CD

Immediately after CD training, hamsters were given systemic infusions of propranolol (0.0, 1.0, 10, or 20 mg/kg IP in sterile 0.9% saline). The doses of propranolol were selected based on previous studies investigating the effects of systemic injections of propranolol in preventing stress-induced death in hamsters [57] and inhibiting fear learning and memory in rats [14,22,40,70,72,77,81,89]. In an effort to minimize the number of animals used in the present study, we did not include a group of animals given propranolol but not defeated. Given the dose and time specificity (see below) of the propranolol effect obtained, as well as the fact that it was given after defeat training, we felt that propranolol was highly unlikely to have an effect on submissive behavior that is independent of social defeat.

2.4.2. Experiment 2: time course of propranolol effect on CD

The same procedures were used as in Experiment 1 with the exception that after CD training, hamsters were given either an immediate or a delayed (4 or 24 h) systemic injection of vehicle (0.9% sterile saline, IP) or the effective dose of propranolol (1.0 mg/kg) as established in Experiment 1.

2.4.3. Experiment 3: effect of immediate post-defeat injection of propranolol given intracerebroventricularly on consolidation of CD

Hamsters received microinjections of 0.0, 2.0, or 20 μg propranolol in 3 μl 0.9% sterile saline into the lateral ventricle immediately following defeat. These doses were selected based on previous studies where i.c.v. administration of 2 μg propranolol decreased LTP after high-frequency tetanization in rats [80] and 20 μg propranolol blocked the increase in corticosterone caused by i.c.v. norepinephrine [11]. To administer an intracerebroventricular dose after defeat, animals were gently restrained in the experimenter's hand, the obturator was removed, and a 1.2 mm projection needle was inserted into the cannula guide. This needle was attached to PE-50 tubing filled with water. A 0.2 μl air bubble separated the water and the drug or saline, and the tubing was attached to a 5-μl Hamilton syringe. The animal was then placed into a small cage where it could move freely during injection. A Harvard apparatus infusion pump was used to slowly administer 3 μl of solution over a 1-min period. The injection needle was left in place for an additional 1 min for diffusion of the drug, after which the needle was removed from the guide cannula, the obturator was replaced, and the animal was returned to its own cage. A successful injection was verified by movement of the air bubble down the tubing during infusion. Animals were tested for CD 24 h after defeat/injection as described above. Following the completion of the study, animals were euthanized with sodium pentobarbital and microinjected with 3 μl India ink into the cannula guide to verify successful cannula placement in the lateral ventricle.

2.5. Statistical analyses

The behavioral data were expressed as means and standard error of the means (S.E.M.). The aggression, submission, and some social data were not normally distributed; therefore, the non-parametric Kruskal–Wallis and Mann–Whitney U tests were used to detect differences between groups. The non-social behavioral data were normally distributed and were analyzed using One-Way Analysis of Variance (ANOVA). Significance was ascribed as $p < 0.05$.

3. Results

3.1. Experiment 1: immediate post-defeat injections of propranolol impair the consolidation of CD in a dose-dependent manner

Propranolol infusion given IP immediately following defeat training significantly decreased submissive behavior of defeated animals [$H(3) = 16.70$; $p < .05$] (see Fig. 1). The total duration of submissive behavior of hamsters given 1.0 mg/kg of propranolol was significantly lower than that of hamsters given systemic infusions of vehicle ($U = 50.50$, $p < .01$). There was no significant difference in the total duration of submissive behavior in hamsters given 10 or 20 mg/kg of propranolol and that of hamsters given infusions of vehicle ($U = 172.5$, $p > .05$ and $U = 105$, $p > .05$, respectively). Additionally, immediate post-defeat injections did not significantly effect aggressive [$H(3) = 4.64$; $p > .05$], social [$H(3) = 2.09$; $p > .05$], or non-social [$F(3,76) = .22$; $p > .05$] behaviors (see Fig. 1).

3.2. Experiment 2: post-defeat injections of propranolol impair the consolidation of CD in a time-dependent manner

Infusions of vehicle given 0, 4, or 24 h post-defeat did not significantly affect aggression [$H(2) = 1.71$, $p > .05$], submission [$H(2) = 1.37$, $p > .05$], social [$H(2) = 2.11$, $p > .05$], or non-social [$F(2,17) = .20$, $p > .05$] (data not shown). Therefore, these vehicle control groups

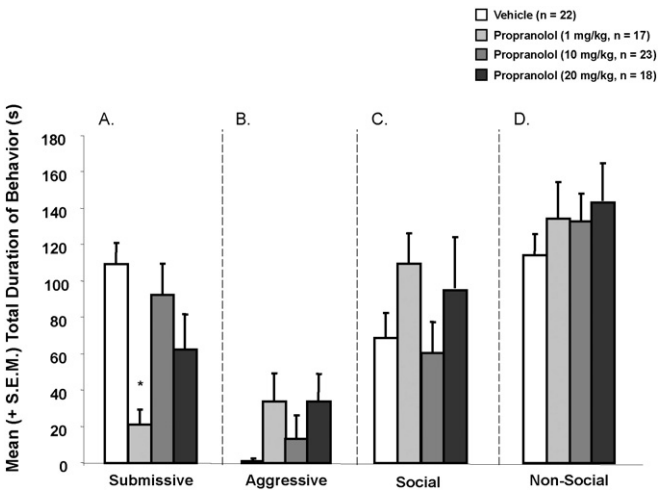

Fig. 1. Mean (±S.E.M.) total duration of (A) submissive, (B) aggressive, (C)social, and (D) non-social behaviors exhibited by defeated animals during the 5 min test with a non-aggressive intruder. Immediate post-defeat infusions of propranolol (1.0 mg/kg) significantly decreased the mean duration of submissive behavior (*$p < .05$, vs. saline controls) but did not affect aggressive, social, or non-social behavior.

were collapsed into one control group in order to increase statistical power. Post-defeat drug infusions significantly altered aggression [H (3) = 10.09; p < .05], submission [H (3) = 13.96; p < .05], and social behavior [H (3) = 9.89; p < .05] (see Fig. 2) but did not significantly affect non-social behavior [F (3,46) = 1.15; p > .05] (see Fig. 2). Immediate post-defeat injections of propranolol significantly decreased submissive behavior (U = 35, p < .05), but increased aggressive [U = 70, p < .05] and social [U = 57, p < .05] behavior in defeated hamsters. Injections of propranolol 4 h post-defeat did not affect aggression [U = 72; p > .05], submission [U = 80; p > .05], or social [U = 87.5; p > .05] behavior in defeated hamsters. Similarly, injections of propranolol 24 h post-defeat did not affect aggression [U = 80; p > .05], submission [U = 71; p > .05], or social [U = 69.5; p > .05] behaviors in defeated hamsters.

3.3. Experiment 3: immediate post-defeat, intracerebroventricular infusion propranolol impairs consolidation of CD in a dose-dependent manner

Post-defeat, intracerebroventricular infusion of 20 μg propranolol had a significant effect on submission [H (2) = 7.14; p < .05] but did not affect aggression [H (2) = 3.027; p > .05], social [F (2,29) = 0.488; p > .05] or non-social [F (2,29) = .036; p > .05] behavior. Immediate post-defeat injection of 20 μg of propranolol significantly reduced the duration of submission [U = 20.5; p < .01] exhibited by defeated hamsters during testing (see Fig. 3). There was no effect of the lower dose (2 μg) of propranolol on any behavior exhibited during testing as compared to vehicle control.

4. Discussion

These experiments demonstrate that systemic post-defeat infusions of the beta-blocker propranolol impair the consolidation of CD in Syrian hamsters in a dose- and time-dependent manner and that this impairment can be mimicked by delivery of propranolol directly into the central nervous system. Specifically, the present results show that a post-defeat infusion of propranolol impairs CD and that this impairment of CD is observed when propranolol is given immediately after the defeat but not when it is given 4 or 24 h post-defeat. This result indicates that the window within which the memory for social defeat is susceptible to beta-adrenergic modulation is less than 4 h under these conditions. In the final experiment, we determined that propranolol infused into the lateral ventricle after defeat also significantly reduced CD, demonstrating that the effect of systemically administered propranolol on stress- or fear-related memory may have been due, at least in part, to an effect of propranolol within the brain.

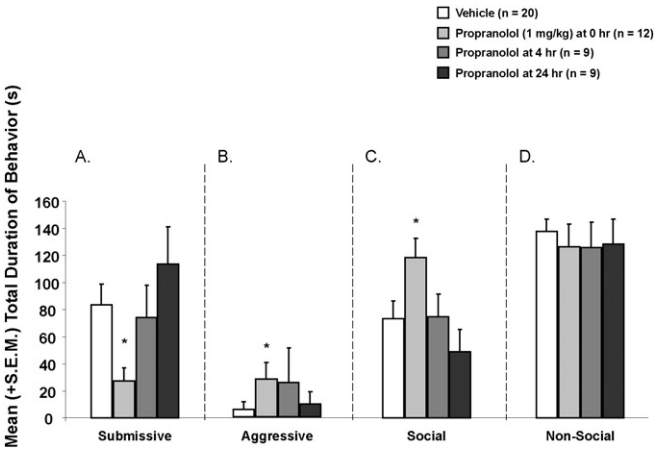

Fig. 2. Mean (±S.E.M.) total duration of (A) submissive, (B) aggressive, (C) social, and (D) non-social behaviors exhibited by defeated animals during the 5 min test with a non-aggressive intruder. Immediate post-defeat infusions of propranolol (1.0 mg/kg) significantly decreased the mean duration of submissive behavior (*p < .05, vs. saline controls) and significantly increased aggressive (*p < .05, vs. saline controls) and social (*p < .05, vs. saline controls) behavior. Infusions of propranolol 4 or 24 h after CD training did not significantly affect the mean total duration of submissive, aggressive, social, or non-social behaviors (p > .05, vs. saline controls).

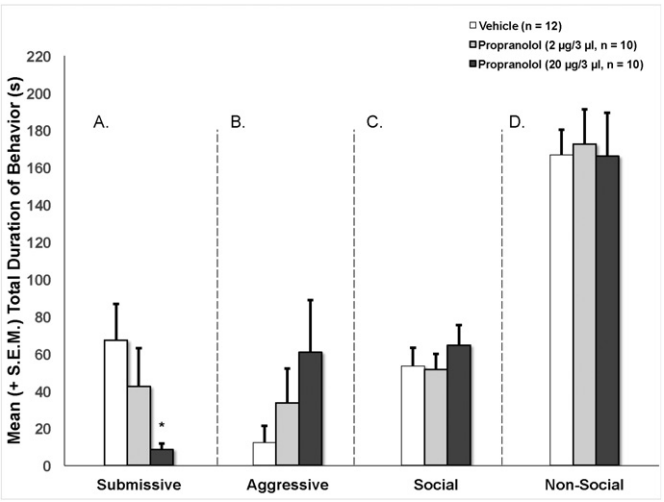

Fig. 3. Mean (±S.E.M.) total duration of (A) submissive, (B) aggressive, (C) social, and (D) non-social behaviors exhibited by defeated animals during the 5 min test with a non-aggressive intruder. Immediate post-defeat infusions of 20 μg, but not 2 μg, propranolol significantly reduced submissive behavior (*p < .05, vs. saline controls).

Several aspects of the propranolol effect are potentially interesting. First, each time we repeated the systemic treatment, it appeared that there would be an inverted u-shaped dose–response curve for the effect of propranolol on submissive/defensive behavior. This apparent effect never reached significance, however, despite the fact that the manipulation was repeated several times, the same pattern emerged each time, and the group n's, particularly in Experiment 1, were very high. It is possible that we would have obtained a significant effect if an additional, higher dose of propranolol were included, but given the fact that the receptor specificity of the drug at high doses would have been in question, it is not clear what would have been gained by this addition. Another interesting observation is that doses of propranolol that appeared to increase aggression also stimulated social behavior. This is not surprising in that we have consistently observed across many years of work that social behavior increases as social avoidance decreases and that a return to species typical territorial aggression closely follows an increase in social behavior.

CD is a profound behavioral change in defeated hamsters that is characterized by a total absence of species typical territorial aggression accompanied by a pronounced increase in submissive and defensive behavior [69]. In our past work, we have often found that pharmacological manipulations that are effective in altering the amount of submission do not concomitantly alter aggression, indicating that separate circuits may regulate particular aspects of the behavioral profile that we call CD. It also suggests and that we know less about how defeat inhibits aggression than we do about how it stimulates submission. Interestingly, propranolol treatment appeared to reliably increase aggressive behavior similarly in all three experiments (although only significantly in Experiment 2) suggesting that this treatment stimulates a more complete reinstatement of species-typical territorial behavior. It would be interesting to determine if the aggression-stimulating effect of systemic propranolol is recapitulated following systemic administration a beta-adrenergic drug that does not cross the blood–brain barrier. Such a manipulation would be needed to establish definitively that the change in behavior is dependent on central nervous system blockade of beta-adrenergic receptors. Noradrenergic receptor antagonists have previously been shown to induce maternal aggression when given in the lateral septum [79] as well as to block the aggression-reducing effect of chronic variable stress [92]. In the latter study,

peripherally administered noradrenergic drugs that crossed the blood–brain barrier (e.g., propranolol) were shown to be more effective than were drugs that did not cross the blood–brain barrier (e.g., acebutolol). Together, these findings strongly suggest that the effect of noradrenergic manipulations on aggression is mediated centrally, although more research is needed to determine where centrally the effects of social defeat on aggression are mediated. Previous research from our lab demonstrated that infusion of a $GABA_A$ receptor agonist [53] or a dopamine receptor antagonist [31] into the nucleus accumbens before testing also restored aggression in previously defeated hamsters, so it is possible that the nucleus accumbens in a component of this circuit. Finally, we have demonstrated that the pharmacological inactivation of the lateral septum using muscimol also increases aggression in previously defeated hamsters, but it is important to note that this effect was limited to expression and not acquisition of CD and muscimol in the lateral septum increased aggression independent of whether the animals had been previously defeated or not [58].

The mechanisms underlying CD learning, and indeed even the critical stimuli for this conditioning, are not fully understood. It is possible that the acquisition of CD involves aspects of both Pavlovian and instrumental fear conditioning. Pavlovian fear conditioning entails the contingent pairing of a neutral conditioned stimulus (CS), such as a tone, with an aversive unconditioned stimulus (US), such as a footshock, that elicits a reflexive or unconditioned response (UR), such as freezing. Through multiple CS-US pairings, the CS comes to elicit conditioned fear responses (CR). In contrast, instrumental fear conditioning involves an aversive stimulus, such as social defeat, that is paired contingently with an animal's response, such as flight. Although CD may mimic some aspects of Pavlovian fear conditioning, it is clear that the potential CSs change fairly dramatically from training to testing in CD. Specifically, the hamster is defeated in the home cage of a larger resident aggressor during CD training, but then it is tested in its own home cage with a smaller, non-aggressive intruder. The present finding that post-training infusions of propranolol impair the consolidation of CD is consistent with the finding that post-training infusions of propranolol impair both Pavlovian contextual fear conditioning [16,32,44] and instrumental fear conditioning [28,50,77]. Our present findings are also congruent with evidence indicating that the memory-modulating effects of post-training manipulations on fear conditioning are observed when both instrumentally- and Pavlovian-conditioned responses are involved [78,88], but not when Pavlovian-conditioned cued responses are the only option [49,90,91].

The present study did not reveal the brain regions through which propranolol affects consolidation of CD memory. We did determine in Experiment 3, however, that the effect of propranolol on the consolidation of the memory of social defeat is likely due, at least in part, to its blockade of central beta-adrenergic receptors. There are several brain regions wherein beta-adrenergic receptors may influence the consolidation of social defeat. For instance, post-training, intra-amygdala injections of a beta-adrenergic antagonist produce memory deficits in a shock avoidance [26,51] and a water maze task [33]. Moreover, intra-amygdala infusions of propranolol, at doses that do not affect memory alone, block the memory-enhancing effects of systemic infusions of epinephrine [60]. Finally, systemic administration of propranolol to mice exposed to chronic social defeat stress reduces the amount of Fos protein within the basolateral amygdala [19]. Consistent with previous findings showing that the amygdala is important for conditioned fear [4,17,20,34,47,48,68,73,76], temporary inactivation of the amygdala impairs the acquisition and expression of CD [42] and overexpression of cyclic AMP response element binding protein (CREB) in the basolateral amygdala enhances the acquisition of CD after a sub-optimal defeat [43]. Finally, we have shown that injections of the protein synthesis inhibitor anisomycin into the basolateral amygdala [54] or the nucleus accumbens ([85].) block the acquisition of social defeat. Together, the data suggest that the basolateral amygdala is a central site wherein noradrenergic modulation of CD consolidation might occur. Additionally, we have shown that the bed nucleus of the stria terminalis and the ventral, but not dorsal, hippocampus are important elements of the neural circuit mediating CD [55,56]. Each of these areas may also contribute to the effects of propranolol, and future studies will explore these possibilities.

In summary, the present results provide convincing evidence that beta-adrenergic receptor activation is involved in the consolidation of the memory for social defeat. Interestingly, these findings are consistent with the notion that CD is an ethologically relevant model of fear conditioning that encompasses elements of both instrumental and Pavlovian fear conditioning and suggest that CD is a valuable model to study the processes by which emotion and stress modulate memory. Finally, the current data also suggest that the popular conception that the anxiolytic effect of propranolol in humans is primarily due to the drug's peripheral effects may need to be reconsidered.

Acknowledgments

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