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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.