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A method for psychosocial stress-induced reinstatement of cocaine seeking in rats

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Abstract

We describe a novel preclinical model of stress-induced relapse to cocaine use in rats using social defeat stress, an ethologically-valid psychosocial stressor in rodents that closely resembles stressors that promote craving and relapse in humans. Rats self-administered cocaine for 20 days. On days 11, 14, 17, and 20, animals were subjected to social defeat stress or a non-stressful control condition following the session, with discrete environmental stimuli signaling the impending event. After extinction training, reinstatement was assessed following reexposure to these discrete cues. Animals reexposed to psychosocial stress-predictive cues exhibited increased serum corticosterone and significantly greater reinstatement of cocaine seeking than the control group, and "active" coping behaviors during social defeat episodes were associated with subsequent reinstatement magnitude. These studies are the first to describe an operant model of psychosocial stress-induced relapse in rodents and lay the foundation for future work investigating its neurobiological underpinnings.

Keywords

social defeat; cocaine; reinstatement; rat; relapse; stress

Introduction

A prominent feature of cocaine abuse and dependence disorders is the occurrence of relapse episodes even after prolonged periods of abstinence (1). Among the factors that induce

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relapse is exposure to psychological stress (2, 3), and drug-induced termination of negative emotional affect is one important factor that motivates long-term drug use and promotes relapse events (4-6).

Relapse has been modeled in animals using the reinstatement procedure, in which previously-extinguished drug-maintained behavior is “reinstated” by exposure to a drug-associated cue, a drug “prime”, or stress (7-9). However, the stressors typically employed in reinstatement studies are pharmacological or physical in nature (10-16), and may produce drug-seeking behavior via specific neural circuits that differ from those engaged by psychosocial stressors that more commonly provoke relapse in humans (7, 17-21). For example, exposure to yohimbine or footshock, the two most commonly employed stressors in reinstatement studies, produce brain activation patterns in rats that are distinct from those produced by psychosocial stressors (22, 23). Despite these findings, it is not known whether the activation of stress modality-specific circuits is an important determinant of subsequent drug-seeking behavior, in part because we lack a model of psychosocial stress-induced reinstatement. Psychosocial stressors in rats may more effectively recruit neural circuitry that is engaged in human stress-induced relapse processes, and thus their study could complement our current understanding of stress-induced relapse and help guide novel behavioral and/or pharmacological therapeutic efforts. Moreover, identifying physiological, affective, and/or behavioral markers that can predict relapse vulnerability in humans has been the focus of recent research (24-27). However, current reinstatement procedures fail to provide graded measurements during the stress exposure that can then be correlated with reinstatement magnitude in individual subjects because the behavioral responses to the stressors (e.g. footshock-induced freezing) are typically unidimensional.

To address these issues, we developed a novel reinstatement procedure in rats in which cocaine-seeking behavior is induced by anticipated social defeat stress using the resident-intruder paradigm (28, 29). We chose this paradigm because (1) both the stressor itself (i.e. conspecific social defeat by a dominant opponent) and the resultant behavioral sequelae exhibited by the subordinate are ethologically and ecologically valid (28-31), (2) compared with pharmacological or physical stressors, social defeat stress more closely recapitulates the psychosocial stress known to frequently precipitate relapse in human drug abusers (17-21), and (3) the behavioral responses exhibited by “intruders” during a social defeat encounter are diverse, operationally-defined, and can be segregated into “active” and “passive” coping strategies (28, 29, 32). Therefore, social defeat stress allows for a rich repertoire of behavioral sequelae for investigating the role of individual coping styles as a predictor of subsequent drug-seeking behavior.

Methods and Materials

Animals

Experimental subjects were 22 adult male Long-Evans rats (Charles River Laboratories Inc., Wilmington, MA; 150-175 g on arrival), individually-housed in polycarbonate cages (43 × 24 × 20 cm) under a reverse 12-hr light-dark cycle (lights off at 8:00am) with *ad libitum* access to rodent chow and water. A separate cohort of adult male Long-Evans rats (500-750 g) served as resident aggressors in social defeat studies and were pair-housed with a

sexually-receptive, tubally-ligated adult female Long-Evans rat within a larger Plexiglas enclosure (68 × 56 × 39 cm). Procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Emory IACUC.

Cocaine Self-Administration and Extinction

Rats self-administered cocaine (see *supplementary material*) for 20 days and on most days were immediately returned to their home cage after each session's conclusion. However, on days 11, 14, 17, and 20, two distinct environmental cues were presented within the operant chamber during the self-administration session. First, a stainless steel wire mesh box surrounded the animal while allowing for unobstructed access to levers and stimulus lights. Second, a cotton ball containing 0.5 ml of peppermint extract was placed out of reach of the animal. On these days, when the self-administration session ended, rats were removed from the operant chamber and immediately exposed to either social defeat stress (stress group, n = 12) or a clean cage (control group, n = 10). In this way, the compound tactile/odor stimulus was conditioned to signal impending exposure to either social defeat stress or no-stress conditions following the session's conclusion. Beginning on day 21, responding was extinguished in daily 2-h sessions during which responses on the active lever had no scheduled consequences. Responding was deemed extinguished once animals emitted ≤ 12 responses on the active lever per session across three consecutive sessions, after which reinstatement testing occurred. The experimental timeline is depicted in Figure S1.

Psychosocial Stress-Induced Reinstatement

The day after meeting extinction criteria, animals were reexposed within the operant chamber to the compound stimulus that had previously signaled impending social defeat stress or the no-stress condition, and responding was measured for 2 h. Immediately before and after this test session, blood samples were collected via the i.v. catheter and analyzed for levels of corticosterone (see *supplementary material*).

Social Defeat Stress

Following cocaine self-administration sessions on days 11, 14, 17, and 20, rats in the stress group were subjected to social defeat stress using the resident-intruder paradigm (28, 29, 33). Briefly, subjects ("intruders") were removed from the operant chamber and placed inside a "resident" male aggressor's home cage (previously prepared with peppermint odor on a cotton ball) from which the female rat had been temporarily removed. The physical interaction between the intruder and the resident was terminated if (1) the resident bit the intruder 3 times, (2) the intruder exhibited a supine submissive posture for 4 consecutive sec, or (3) 4 min elapsed, whichever occurred first. Immediately following the physical encounter, the intruder was placed within the same wire mesh box in which it was situated during the self-administration session and positioned inside the resident's home cage. The wire mesh box allowed for olfactory, visual, and auditory contact between the resident and intruder, but prevented further physical contact. After 5 min, the intruder was removed from the wire mesh box and returned to its home cage. To avoid habituation of aggression, intruders were never exposed to the same resident aggressor more than once.

Each social defeat episode was videotaped and scored by a trained observer to quantify the frequency and duration of operationally-defined species-appropriate behaviors exhibited by the intruder (28, 29, 32): *Self-defensive behavior* - lateral threat, defensive upright, attack, aggressive allogrooming, dominant posture; *Active avoidance* - retreat, flight; *Submissive behavior* - supine submissive posture, freezing. Operational definitions of each behavior can be found in the *supplementary material*. Data from social defeat encounters lasting < 30 sec were excluded from analyses because such a short duration of interaction did not allow for a rich behavioral repertoire to be exhibited. Of the 48 total defeat episodes recorded, 2 encounters were excluded from analysis based on this criterion.

No-Stress Control

Following cocaine self-administration sessions on days 11, 14, 17, and 20, rats in the control group were placed into a clean, unoccupied resident cage previously prepared with peppermint odor. Each animal was initially allowed unrestricted access to the cage for a duration yoked to the amount of time that a social defeat stress counterpart was unprotected in the resident cage (up to 4 min). Next, the animal was placed inside a wire mesh box within the unoccupied resident's cage for an additional 5 min before being returned to its home cage. In this way, the no-stress control subjects underwent all procedures identical to the stressed group, with the exception of exposure to a resident aggressor.

Results

Cocaine Self-Administration

Animals acquired stable cocaine self-administration within the first 5 sessions with no differences between the stress and control groups. Two-way ANOVA of active lever responses throughout the 20 days of self-administration showed a main effect of session ($F_{(19,380)} = 4.19$, $p < 0.0001$) but not group ($F_{(1,20)} = 0.09$, $p = 0.77$) or interaction ($F_{(19,380)} = 0.71$, $p = 0.81$) (Figure S2). Two-way ANOVA of inactive lever responses yielded a similar pattern of results (session, $F_{(19,380)} = 3.41$, $p < 0.0001$; group, $F_{(1,20)} = 0.004$, $p = 0.95$; interaction, $F_{(19,380)} = 0.86$, $p = 0.63$) (Figure S2). The cumulative amount of cocaine self-administered between the stress and no-stress groups across the 20 days of self-administration was not significantly different ($p = 0.66$; Figure S3).

Extinction

The number of sessions required to reach extinction did not differ significantly between the stress and no-stress groups (stress group, 23.08 ± 3.34; control group, 28.00 ± 4.37; $p = 0.37$; data not shown).

Reinstatement

The reinstatement-inducing effects of reexposure to cues predictive of impending social defeat stress or no-stress control conditions are shown in Figure 1. Two-way ANOVA of active lever responses during extinction and reinstatement showed main effects of phase ($F_{(1,20)} = 30.73$, $p < 0.0001$) and group ($F_{(1,20)} = 4.75$, $p = 0.04$), and a strong trend for a phase x group interaction ($F_{(1,20)} = 3.98$, $p = 0.06$). Post hoc comparisons revealed that reinstatement responding in the stress group was significantly differently from extinction (p

< 0.001) and from reinstatement in the no-stress group ($p < 0.05$). Nearly half of all responding occurred within the first 30 min of the test session (data not shown). There was a trend for reinstatement responding to be higher than extinction levels in the no-stress group, but it did not reach significance. Two-way ANOVA of inactive lever responses revealed a main effect of phase ($F_{(1,20)} = 12.87$, $p < 0.0001$), but not of group ($F_{(1,20)} = 0.53$, $p = 0.47$) or interaction ($F_{(1,20)} = 0.27$, $p = 0.76$) (Figure S4).

Correlations of Stress-Induced Behaviors with Cocaine Seeking

The results of correlation analyses between behavioral elements exhibited by intruders during social defeat stress and subsequent reinstatement are shown in Table 1. Correlations were assessed only for those behaviors that were exhibited by at least half of the intruders (6/12). Reinstatement magnitude was positively and significantly correlated with time spent in a “submissive supine posture” (Figure 2A), traditionally considered a “passive” coping response. However, we observed that the correlations between reinstatement and 3 “active” coping responses, “aggressive allogrooming”, “dominant posture”, and “retreat”, narrowly missed significance (Table 1), and 5 of the 7 animals that displayed “dominant posture” yielded the highest number of responses during reinstatement. Because it seemed counterintuitive that both “passive” and “active” coping mechanisms could be associated with drug seeking, we hypothesized that an increased time spent in “submissive supine posture” was the result of frequent escape from pinning and thus multiple pinning episodes, which would reflect active, rather than passive, coping. When videos were rescored for the frequency of “pin breaks” (i.e. the number of times that an intruder was forced into a “submissive supine posture” but escaped prior to the 4 consecutive sec criterion for termination of the encounter), we found that increased time spent in the “submissive supine posture” was robustly and positively correlated with number of “pin breaks” (Figure 2B). Moreover, the amount of time engaged in “freezing”, the canonical measure of passive stress coping in rodents, failed to show an appreciable correlation with reinstatement (Table 1). These results suggest that active coping behaviors during social defeat stress were associated with increased drug seeking during reinstatement.

Corticosterone

In the no-stress control group, there were no differences in serum corticosterone levels sampled immediately prior to and immediately after the reinstatement test session (Figure 3A). By contrast, animals in the social defeat group showed significant elevations in corticosterone levels ($p < 0.05$; Figure 3B), suggesting that exposure to the cues predicting impending social defeat was stressful and providing a physiological correlate to the increased drug-seeking behavior during reinstatement.

Discussion

While traditional yohimbine- and footshock-induced reinstatement procedures have yielded important insights into the mechanisms underlying drug-seeking behavior following exposure to stress, the information they can provide may be limited because they do not employ psychosocial stressors that have been suggested to more closely approximate the types of stress that promote drug seeking and relapse in humans (17-21). In particular,

psychosocial stressors in rodents engage neural circuitry that is distinct from that recruited by other stress modalities, yet whether these brain regions contribute to stress-induced drug seeking has not been investigated due to the lack of an appropriate model. The present study was undertaken to develop and characterize a rat model of psychosocial stress-induced reinstatement of drug seeking. We demonstrate that previously-extinguished cocaine self-administration may be reinstated following exposure to discrete environmental stimuli that signal impending social defeat stress, and that the magnitude of the drug-seeking response was associated with behaviors indicative of an "active" coping response to stress.

As the goal of this study was to design a reinstatement model that employs psychosocial stress to trigger drug seeking, one might imagine that the simplest procedure would be to mirror footshock or pharmacological stress-induced reinstatement by exposing animals to an acute episode of social defeat stress immediately prior to a reinstatement test session. Indeed, acute exposure to social defeat stress produces reinstatement of previously-extinguished place preference to cocaine or morphine in mice (21, 34). However, previous attempts by us and others to reinstate previously-extinguished operant self-administration using acute social defeat stress have been unsuccessful (our unpublished data; 35). One explanation for these failures is that drug self-administration took place in a context (i.e. operant chamber) that was different from the context in which social defeat stress exposure occurred (i.e. resident aggressor's home cage), and context specificity is critical for reinstatement produced by other stressors (9). In addition, the ability of stress to induce drug-seeking behavior follows an inverted-U "dose-response" curve (36), and social defeat stress is an especially powerful and salient stressor that may be on the descending limb of this curve and disrupts, rather than promotes, operant behavior. Consistent with this hypothesis, acute social defeat stress reduced ongoing alcohol self-administration and also suppressed extinction responding (35), suggesting a nonspecific rate-suppressant effect of social defeat. Our current model overcomes each of these obstacles. First, the cues used to signal impending social defeat stress within the operant chamber (wire mesh enclosure and peppermint odor) are also present during each social defeat stress exposure within the resident aggressor's cage. In this way, the self-administration context becomes "mobile" and transfers between the self-administration and stress environments, thus preserving context specificity. Second, rather than use social defeat stress itself to promote reinstatement, the trigger is reexposure to *cues that signal impending* social defeat stress. We speculate that presentation of these cues is stressful enough to promote a behavioral response (e.g. drug seeking, which is supported by the increase in corticosterone), but not so stressful that it disrupts operant behavior. Indeed, exposure to an odor cue paired with previous social defeat exposure was able to produce a modest reinstatement of alcohol seeking (35), although their cue was not predictive of impending defeat per se.

The observation that animals in the no-stress group exhibited a modest (though nonsignificant) level of cocaine seeking when reexposed to cues that were predictive of a non-stressful control condition likely reflects the fact that the tactile/odor cues were classically conditioned not only to signal impending placement into the stress or no-stress environment, but also to signal cocaine availability (akin to "context-induced" reinstatement). The finding that corticosterone levels in the no-stress animals did not increase during the test session further indicates that the weak reinstatement effect was not

associated with a stress response. By contrast, the stress group showed significant elevations in corticosterone during the test session and exhibited a reinstatement magnitude that was significantly higher than that exhibited by the control animals, suggesting that the defeat-exposed animals were indeed stressed by reexposure to the defeat-predictive cues and that this underlies their robust reinstatement.

One advantage of social defeat stress over conventional stressors is the diverse repertoire of behavioral responses exhibited by intruder animals during the stress itself and the level of individual variability that lends itself to correlation analyses (28, 29, 32). We found that cocaine-seeking behavior induced by cues predicting impending social defeat stress was positively correlated with one “passive” coping behavior (supine submissive posture). However, the supine submissive posture requires special consideration. If an animal spends 4 consecutive seconds engaged in this behavior, the defeat episode is immediately terminated. Therefore, animals that are predominantly “passive” would be expected to exhibit a maximum of 4 seconds in this posture. By contrast, animals that are predominantly “active copers” would be expected to break free from submissive pinning, and thus show higher durations spent in the submissive posture due to the accumulation of time spent in multiple supine posture bouts. In support of this idea, we found a significant correlation between the amount of time animals spent in submissive supine posture and the number of times they broke free, suggesting that “active” copers actually spent more total time in the submissive supine posture than their “passive” counterparts. Additionally, the association of three “active” coping mechanisms (retreat, aggressive allogrooming, and dominant submissive posture) with reinstatement magnitude nearly reached significance. Combined, our results suggest that “active” coping during social defeat stress was associated with a greater drug-seeking response when impending stress was signaled. We offer that stress-induced relapse in humans could also be viewed as active coping since it requires volition to seek, acquire, and take drug.

It is well-established that different stress modalities engage distinct neural circuits (22, 23). For example, some neuroanatomical and neurochemical substrates required for footshock-induced reinstatement (37-39) are dispensable for yohimbine-induced reinstatement (40). Furthermore, social defeat stress engages subcircuits within a behavioral alarm/defense system that is either weakly activated, or not activated at all, by other stress modalities such as footshock, restraint, or forced swim (41-43), and incorporates hypothalamic (ventromedial and dorsal premammillary nuclei), amygdalar (medial amygdaloid nucleus), and midbrain (periaqueductal gray) regions. The procedure described here provides a framework to identify the circuitry underlying psychosocial stress-induced reinstatement, which likely involves neuroanatomical substrates that have not yet been implicated in relapse-like behavior, and therefore could lead to the development of novel biomarkers for addiction vulnerability and/or targets for therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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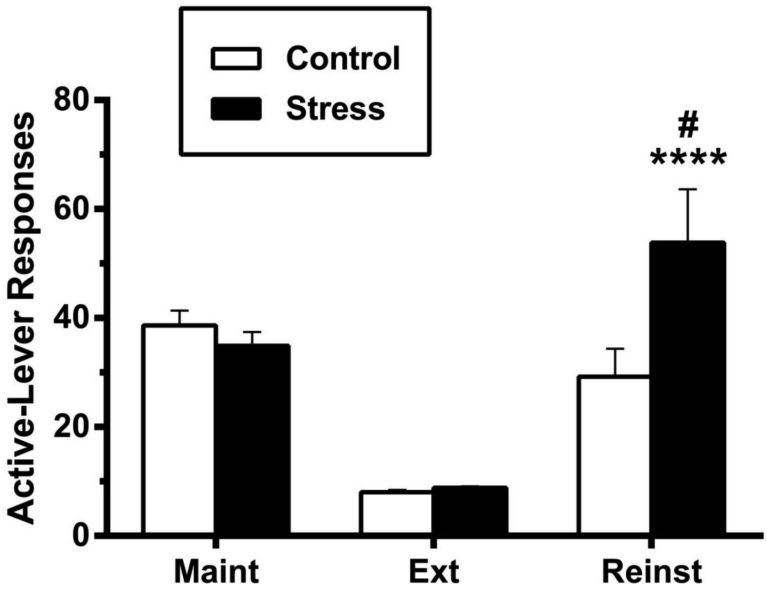

Figure 1. Reexposure to cues predictive of impending social defeat stress produces reinstatement of cocaine seeking

Shown are the levels of responding on the active-lever during the maintenance phase of self-administration ("Maint"), extinction ("Ext"), and reinstatement following reexposure to discrete cues that signaled impending social defeat stress (filled bars, n = 12) or a non-stressful control condition (empty bars, n = 10) ("Reinst"). Data represent the mean (± SEM) number of responses during the final 3 days of self-administration prior to the onset of stress sessions (i.e. sessions 8-10, "Maint"), the final 3 days of extinction ("Ext"), and a single reinstatement test session ("Reinst"). ****$p<0.0001$ compared to extinction; $^{\#}p<0.05$ compared to reinstatement in the control group. Abscissa, phase of experiment. Ordinate, number of active-lever responses.

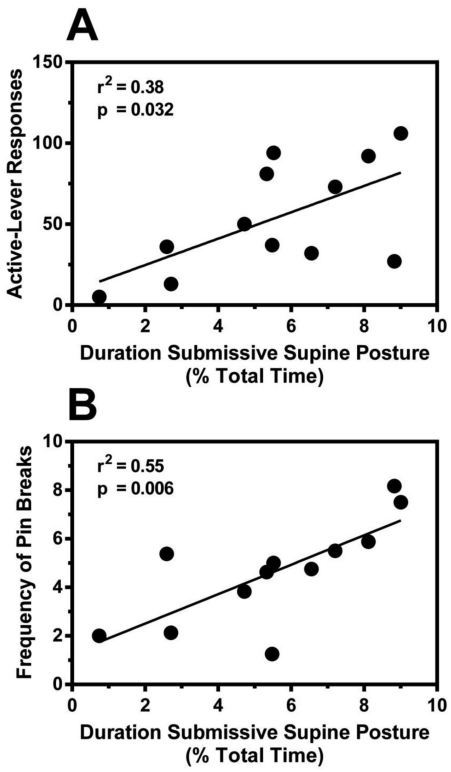

Figure 2. Specific behaviors during social defeat episodes correlate with drug-seeking behavior during reinstatement

Shown are scatter plots of statistically significant correlation analyses with best-fit lines. For each animal, the duration of time spent engaged in each behavioral element was averaged across the four social defeat episodes to determine a mean duration value. Reinstatement magnitude was positively correlated with the amount of time spent engaged in the "submissive supine posture" (2A). The mean duration engaged in the "submissive supine posture" was also positively correlated with the frequency of "pin breaks", i.e. escapes from being forced into the supine posture (2B). Abscissae, mean duration of time spent in the "submissive supine posture", expressed as the percentage of the total duration of the social defeat encounter. Ordinates, number of active-lever responses during reinstatement test (2A), or the frequency of escapes from the "submissive supine posture" (pin breaks, 2B).

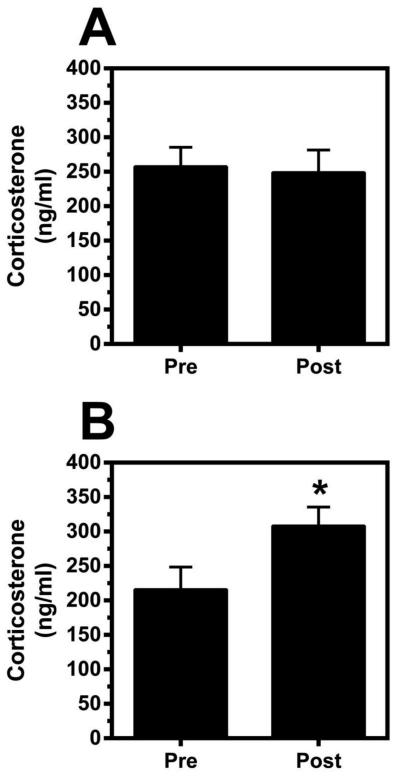

Figure 3. Cues predictive of social defeat increase serum corticosterone levels during reinstatement

Shown are mean ± SEM serum corticosterone levels taken immediately prior to (Pre) and immediately after (Post) reinstatement test sessions in which animals were exposed to cues predictive of impending no-stress control condition (top panel, n = 5) or impending social defeat stress (bottom panel, n = 10). Abscissae, sample time relative to reinstatement session. Ordinates, ng corticosterone per ml serum.

Table 1

Correlation Analyses of Individual Behaviors Observed During Social Defeat Stress Encounters and Subsequent Reinstatement Magnitude

Behavioral Measure	# Animals Exhibiting	r^2	p-value
Active Coping: Self-Defense			
Lateral Threat	0/12	ND	ND
Defensive Upright	12/12	0.04	0.531
Attack	2/12	ND	ND
Aggressive Allogroom	6/12	0.25	0.098
Dominant Posture	7/12	0.21	0.133
Active Coping: Avoidance			
Retreat	12/12	0.32	0.056
Flight	12/12	0.02	0.652
Passive Coping			
Submissive Supine	**12/12**	**0.38**	**0.032***
Freezing	12/12	0.10	0.313

Behaviors that significantly correlated with reinstatement are in bold. ND, not determined.

* $p < 0.05$.