Published in final edited form as:

Appetite. 2019 January 01; 132: 131–138. doi:10.1016/j.appet.2018.10.016.

Stress as a context: Stress causes relapse of inhibited food seeking if it has been associated with prior food seeking

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Abstract

Three experiments with rats explored the hypothesis that inhibited food-seeking can be reinstated by stress if stress has been part of the context of earlier food-seeking. In all experiments, rats first learned to lever press for sucrose pellets and then had the response inhibited through extinction (where responding no longer yielded sucrose pellets). In a final test, inhibited responding was tested after exposure to a stressor or not. Previous research indicates that stress during testing does not normally reinstate extinguished food-seeking, although it reliably does so when animals are responding for drugs. In Experiment 1, stress caused a reinstatement of food seeking if and only if the rats had been exposed to stressors prior to sessions of lever press training. In Experiment 2, a new stressor that had not been associated with response acquisition also caused reinstatement if other stressors had been associated with response acquisition. Experiment 3 then established that stressors must be associated with the acquisition of lever pressing, rather than extinction, in order to allow a stressor to cause relapse of extinguished food seeking. The results support the view that stress can cause relapse of inhibited food seeking if it has been part of the context of original food seeking. The effect is therefore an example of the *ABA renewal effect* in which inhibited responding recovers after extinction when the response is returned to its training context. Implications for understanding relapse to overeating and other “addictive” behaviors are discussed.

Stress is a factor that is widely thought to produce relapse to human “addictive” behavior, such as drug use (Sinha 2001; Sinha, Shaham, & Heilig, 2011) and overeating (Oliver & Wardle, 1999; Torres & Nowson, 2007). The process has been studied in the laboratory with so-called *stress-induced reinstatement* procedures (Shaham & Stewart, 1995; Shaham, Erb, & Stewart, 2000). In those procedures, rats (for example) first learn to perform an instrumental response that is reinforced with a reward such as intravenous drug infusions (e.g., pressing a lever for an infusion of cocaine). The response is then extinguished in a second phase (i.e., lever pressing no longer produces cocaine). Drug-seeking behavior suppressed through extinction is then reinstated when animals receive a stressor (e.g., a footshock) before testing. Stress administered this way is widely known to reinstate

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extinguished drug-seeking (for a review, see Mantsch et al., 2016), although it does not appear to affect extinguished food-seeking the same way (Ahmed & Koob, 1997; Buczek, Le, Wang, Stewart, & Shaham, 1999; Koob & LeMoal, 1997). The present experiments were designed in part to test one possible reason why.

Explanations of stress-induced reinstatement of drug seeking often assume that exposure to stressors may activate the mesocorticolimbic dopamine (DA) system. For example, activation of the DA system may serve to enhance the incentive value of drug-related stimuli and cause a re-initiation of instrumental behavior that is motivated by them (see Robinson & Berridge, 1993). Another view suggests that stressors might reinstate behavior by merely disinhibiting extinguished drug responding (Shaham et al., 1997, 2000). Unfortunately, activation of the DA system, motivation by reinforcer-related stimuli, and disinhibition after extinction should also play a role in food seeking (Berridge, 2001; Brimer, 1970; Wise et al., 2004), and these explanations therefore do not appear to anticipate the peculiar absence of stress-induced reinstatement of food seeking.

An alternative possibility is that stress can cause relapse of inhibited behavior if it acts as a “context” that has been associated with instrumental responding. An effect called the *renewal effect* (e.g., Bouton, Todd, Vurbic, & Winterbauer, 2011; Nakajima, Tanaka, Urushihara, & Imada, 2000) is especially relevant. In renewal, if an instrumental response is first reinforced in one context (Context A) and then extinguished in a second one (Context B), the behavior suppressed during extinction recovers or *renews* when the animal is returned to the original context (ABA Renewal). Renewal also occurs if acquisition, extinction and renewal testing are conducted in Contexts A, B, and C (ABC Renewal) or A, A, and B (AAB Renewal). But in the present article we entertain the possibility that if stress has been present during the acquisition of instrumental behavior, it might function as a feature of the acquisition context (Context A) and cause ABA renewal when the organism is exposed to it again after extinction. Interestingly, drug reinforcers (but not food reinforcers) can cause cascades of stressful physiological and/or subjective effects (for a review, see Sinha, 2008). In this way, earning drug but not food reinforcers during acquisition may naturally produce an interoceptive stress state that can become associated with the instrumental response. Stress after the extinction of drug-seeking may therefore return the organism to a similar state. Stress-induced “reinstatement” of drug seeking may thus be another example of the ABA renewal effect.

Most often, the contexts in experiments on the renewal effect are different apparatuses or operant chambers that contain different visual, tactile, and olfactory cues. That is, the contexts differ in terms of their exteroceptive features. However, research also indicates that internal sensations or interoceptive cues (e.g., drug states, mood states) can also gain contextual control over behavior (Bower, 1981; Davidson 1993; for a review, see Bouton 1991). In recent experiments, Schepers and Bouton (2017) found that interoceptive stimuli associated with different degrees of food deprivation (i.e., hunger vs. satiety) could function as contexts in a renewal paradigm. Rats learned to press a lever for either sucrose or sweet-fatty pellets while they were satiated (Context A). They then received several extinction sessions in which they were food-deprived (after 23 hrs without access to homecage chow; i.e., Context B). Extinguished responding recovered in a test when the animals were satiated

again, suggesting ABA renewal controlled by satiety and hunger cues. Intuition and other views (e.g., Hull, 1943) would suggest that a shift to a satiated state from a hunger state should weaken, rather than enhance, responding for food. In contrast, the results offered a more nuanced view of behavioral control by hunger and satiety. They also provide a potential explanation of why some dieting attempts may ultimately fail: Overeating behaviors inhibited during a diet may renew when a simple lapse in the diet produces satiety stimuli that had been present when they were learned.

The current experiments were designed to test the hypothesis that stress can also play the role of context and cause a renewal of inhibited food-seeking. The new result is that extinguished food-seeking behaviors are susceptible to stress-induced reinstatement if and only if stressors are introduced during training to become part of the original context food seeking. The results may have implications for understanding why exposure to stress may result in the relapse of a variety of overeating and other problem behaviors (e.g., drug taking), even after they have been inhibited through treatment.

Experiment 1

Experiment 1 was designed to test whether extinguished food seeking can be made susceptible to stress-induced reinstatement if stress is associated with the acquisition of food seeking. One group of rats was given exposure to varying stressors immediately before daily sessions in which they learned to make a lever press response to earn sucrose pellets. Stressors were given using a Chronic Variate Stress (CVS) procedure that has been used and described elsewhere (e.g., Hammack, Cheung, Rhodes, Schutz, Falls, Braas, & May, 2009). A control group received identical lever press training, but did not receive the daily stressors. In a second extinction phase, the response was no longer reinforced in a series of sessions in which neither group received stressors. Finally, each rat was tested for responding in two separate sessions conducted in a counterbalanced order. In one session, responding was tested immediately after exposure to a stressor, and in the other, it was tested after the rat received approximately equal handling (but no stressor). As noted above, previous research indicates that stress exposure would not normally produce recovery of extinguished food seeking (e.g., Buczek et al., 1999; Koob & LeMoal, 1997). However, the hypothesis was that the addition of stressors prior to training sessions would render extinguished behavior susceptible to recovery after stressor exposure.

Method

Subjects—The subjects were 32 naive female Wistar rats ($n_s = 16$) purchased from Charles River Laboratories (St. Constance, Quebec). They were between 75 and 90 days old at the start of the experiment and were individually housed in a room maintained on a 16:8-h light: dark cycle. Experimentation took place during the light period of the cycle. Rats were deprived to 90% of their free feeding weight prior to the beginning of the experiment.

Apparatus—Two sets of four conditioning chambers housed in separate rooms of the laboratory were used. Each box was housed in its own sound attenuation chamber. All boxes were of the same design (Med Associates model ENV-008-VP, St. Albans, VT). The side walls and ceilings were made of clear acrylic plastic, while the front and rear walls were

made of brushed aluminum. They measured $30.5 \text{ cm} \times 24.1 \times 21.0 \text{ cm}$ ($l \times w \times h$). The first set of boxes had a 1.5 cm vertical gray stripe down the center of one acrylic side wall and the grids of the floor were spaced 1.6 cm apart (center-to-center). The other set of boxes had no adornment on the side walls and the floor consisted of alternating stainless steel grids with different diameters (0.5 and 1.3 cm, spaced 1.6 cm apart).

Recessed $5.1 \text{ cm} \times 5.1 \text{ cm}$ food cups were centered in the front walls approximately 2.5 above the level of the floor. Retractable levers (Med Associates model ENV-112CM) were positioned to the left and right of the food cup. These levers were 4.8 cm long and positioned 6.2 cm above the grid floor. The levers protruded 1.9 cm when extended (the right lever remained retracted throughout the experiment). A 28-V panel light (2.5 cm in diameter) was attached to the wall 10.8 cm above the floor and 6.4 cm both to the left and right of the food cup. Two identical panel lights were also mounted in the same positions on the back wall. The chambers were illuminated by one 7.5-W incandescent bulb mounted to the ceiling of the sound attenuation chamber, approximately 34.9 cm from the grid floor at the front wall of the chamber. Ventilation fans provided background noise of 65 dBA.

The apparatuses were controlled by computer equipment located in an adjacent room. Food rewards consisted of 45 mg sucrose pellets (TestDiet, Richmond, IN, USA).

Chronic Variate Stress—Stressed rats in Experiments 1-2 were exposed to a 7-day chronic variate stress paradigm adopting procedures used in the stress literature (e.g., Hammack et al., 2009). When the stress protocol was in effect, rats received 1 of 4 different types of stressor procedures on a given day:

Oscillation stress (O): Rats were placed inside a plastic chamber ($28 \times 17 \times 13 \text{ cm}$) that was secured to a clinical rotator (Fisher Scientific, Morris Plains, NJ) and were oscillated for 30 min at 30 rpm in a lighted room in the laboratory.

Footshock (F): Rats were placed inside a conditioning chamber (Med Associates, St. Albans, VT) measuring $30 \times 25 \times 35 \text{ cm}$ that was housed in a different room and differed from the chamber in which instrumental training occurred in terms of visual (i.e., designs on the walls), tactile (i.e., floor grids), and olfactory stimuli (i.e., scents). After a 5-min acclimation period, two 1.0-mA 5-s footshocks were delivered through the grid floor with a 1-min interval between them.

Restraint (R): Rats were placed in a $9 \times 15 \text{ cm}$ (D $\times$ H) Broome Style Rodent Restraint (Plas Labs, Lansing MI, 554-BSRR) and restrained for 60 min in a lighted room in the laboratory.

Pedestal stress (P): Rats were placed on an elevated $20 \times 20 \text{ cm}$ platform that was 60 cm from the floor for 30 min in a lighted room in the laboratory.

In practice, each stressor was repeated once over the 7 days with the exception of the Pedestal stressor, which was given only once. The order of stressors was ORFPORF for half the rats and ORFPOFR for the other half.

Procedure

Magazine training. On the first two days, each rat received a daily session in which it was placed in an operant chamber and pellets were delivered freely on average every 30 s. The response lever was retracted and unavailable during these sessions.

Acquisition. On each of the next 10 days, all rats received a single daily session in which lever presses resulted in a pellet delivery every 30 s on average (a VI 30-s reinforcement schedule). Sessions began with the insertion of the left-hand lever following a 2-min delay. Sessions ended with the retraction of the lever after 30 min. No special response shaping was necessary. Beginning on Day 4, rats in Group Acquisition Stress began receiving a stressor from the CVS protocol immediately prior to each daily session. Rats in Group No Acquisition Stress received approximately equal handling, but did not receive a stressor; they were similarly shuttled to the laboratory from the home cage and then returned immediately while the rats in Group Acquisition Stress received a stressor.

Extinction. On each of the next 5 days, all rats received a single daily session in which lever presses were available but had no programmed consequence. The extinction sessions also began when the lever was inserted following the 2-min delay. The sessions ended after 30 min when the lever was retracted from the chamber. Rats did not receive stressors prior to the extinction sessions.

Renewal Test. On each of the final 2 days, each rat received a single test session, identical to extinction, except that it was only 10 min in duration. Rats received one test session after stressor exposure and another after approximately equivalent handling. For half the rats in each group, the stressor was restraint (R) and for the other half it was footshock (F). For Group Stress Renewal, the test stressor was the same type of stressor that was received prior to the final acquisition training session. The Stressor was novel for rats in the No Acquisition Stress Group.

Data analysis. The results were examined with analyses of variance (ANOVAs) using a rejection criterion of $p < .05$. The renewal effect takes the form of an increase in extinguished behavior that occurs when the context is changed. In testing, we therefore also focused on planned comparisons (Fishers LSD Tests) that compared individual groups' lever pressing in the Stress and No Stress contexts.

Results

The results of acquisition, extinction, and testing are summarized in Figure 1. The chronic variate stress procedure given before acquisition sessions 4 through 10 did not affect the rate of lever pressing in acquisition (left panel) or extinction (middle panel). In contrast, stressors did have an effect during the final tests. Rats that had received the stressors during acquisition made more responses in the test preceded by a stressor than the test that was not. In contrast, rats that had not received stressors during acquisition responded at similarly low rates in both tests.

Acquisition and Extinction—A 2 (Group) $\times$ 10 (Session) ANOVA confirmed that response rate increased over the acquisition sessions, $F(9, 270) = 122.40$, $MSE = 13.94$, $p < .001$. Neither the group effect nor the session by group interaction was significant, $F_s < 1$, suggesting that stress did not have an unconditional effect on response rates during lever press training. Similarly, a 2 (Group) $\times$ 5 (Session) ANOVA found that responses decreased during extinction, $F(4, 120) = 52.26$, $MSE = 173.25$, $p < .001$. Non-significant group and interaction effects suggested no differences in the rate of extinction, $F_s < 1$.

Test—A 2 (Group) $\times$ 2 (Session: No Stress vs. Stress) ANOVA indicated a main effect of session, $F(1, 30) = 7.33$, $MSE = 5.03$, $p = .01$, $\eta_p^2 = .20$. There was also a significant session by group interaction, $F(1, 30) = 11.71$, $MSE = 5.03$, $p = .002$, $\eta_p^2 = .28$, and a main effect of group $F(1, 30) = 28.36$, $MSE = 4.81$, $p = .036$, $\eta_p^2 = .49$. The interaction indicated that the groups exhibited a different pattern of responding over the tests. Fisher's LSD tests confirmed that Group Acquisition Stress made more responses in the test session preceded by stressor exposure than the non-stressed test, $p < .001$. Responding in Group No Acquisition Stress did not differ between the test sessions, $p = .62$.

Discussion

Rats that had received stressors prior to lever press training sessions responded more in a test that was preceded by a stressor than in one that was not. In contrast, rats that had not received stressors during training responded at similar rates in both test sessions. This lack of recovery in Group No Acquisition Stress is consistent with the extensive literature indicating that stress does not generally reinstate extinguished food seeking (e.g., Buczek et al., 1999; Koob & LeMoal, 1997). In a preliminary way, the contrasting recovery of responding in Group Acquisition Stress suggests that stress exposure during training may have allowed stress to play the role of Context A in an ABA renewal design. That is, animals may have learned that lever responses were reinforced in the context of stress (Context A) and were extinguished in its absence (Context B). On this view, stress exposure produced stress-induced reinstatement by returning the rat to the context that had been associated with conditioning.

Extinction responding in Group Acquisition Stress was not affected by the removal of pre-session stressors that occurred at the start of the extinction phase. In typical instrumental renewal paradigms, instrumental responding during extinction is reduced when its onset coincides with a shift to a new physical context (see Bouton & Todd, 2014). Interestingly, the absence of a context shift effect here is reminiscent of its absence following a change in another interoceptive context, namely, a shift from satiety to hunger (see Schepers and Bouton, 2017).

It should be noted that the renewal effect here was produced after exposures to a stressor of a type that had also preceded two of the training sessions. That is, rats tested with footshock or restraint stressors had also received them prior to lever training on acquisition sessions 6 and 10. This made it possible that animals had learned about (and responded to) the presence of the interoceptive or exteroceptive components of specific stressors rather than general

interoceptive stress stimuli that might be common to all of them. Furthermore, while care was taken to provide equal handling between the stressed and unstressed test sessions, it was also possible that rats had learned to use exteroceptive cues that were not uniquely related to stressor exposure. That is, on days when stressors were delivered (and thus reinforcers could be earned), the rats received relatively lengthy exposures to various exteroceptive stimuli (e.g., restraint tubes, plastic boxes, etc.). In contrast, extinction was in effect when the rats were placed more directly into the conditioning context without any prior apparatus stimulation. Animals may have simply learned that responses were reinforced in sessions when they had received a relatively lengthy exposure to a context (the stressor situation) before being placed in the conditioning chamber.

Experiment 2

Experiment 2 was designed to examine whether the renewal effect in Experiment 1 was the result of renewal in a general context produced by stress or whether it depended on the presence of a specific stressor that had been directly associated with reinforcement during acquisition. There were three groups that all received stressors prior to lever press acquisition but not extinction. During testing, the different groups were tested after (1.) a stressor that had been directly associated with acquisition (Group Paired), (2.) another stressor that had not been associated with acquisition (Group Unpaired), or (3.) exposure to a neutral plastic box in a darkened room (Group Control). If the renewal effect in Experiment 1 had been produced by learning about stress rather than a specific stressor associated with conditioning (see Figure 2a), then it should occur equivalently in Groups Paired and Unpaired. If it was contrastingly caused by learning only about the specific stressors (see Figure 2b), it should occur only in Group Paired. Finally, if the effect was merely a result of exposure to another apparatus in a different room immediately before testing, the effect should also be evident in Group Control.

Method

Subjects and Apparatus—The subjects were 32 naive female Wistar rats of the same age and from the same vendor as those in the previous experiments. The apparatus and maintenance conditions were also the same with the exception of two new “stressor” apparatuses described below.

Procedure

Pre-exposure.: During each of the first two days of a pre-exposure period, all rats were individually placed into transparent plastic shoebox containers (39.4 cm × 30 cm) with woodchip bedding on their floors in a darkened room of the laboratory for 35 min before being returned to their home cage. During days 3 and 4, half the rats received the usual footshock stressor and the other half received restraint (both as previously described). Following stressor exposure, rats were returned to the home cage. This procedure was used so that the stressors used during testing were not novel in any of the groups.

Magazine training.: Magazine training then proceeded as in Experiment 1.

Acquisition.: Lever press training was then conducted as described in Experiment 1. All rats received stressor exposures prior to training sessions 4-9. In this experiment, a new stressor, 5 min of exposure to an open field ("X"), was given to all animals prior to Session 4. This involved placing the rats in a 232-cm square opaque container with 60.96-cm sidewalls in a lighted room in the laboratory for 5 min. The open field stressor replaced the restraint or footshock stressor that was removed to allow the paired and unpaired testing conditions. Half the rats in each group received stressors in the sequence XOFPOF and half received XORPOR. One rat was removed from the experiment following the 3rd session after having failed to make a single lever response.

Extinction.: Extinction training then proceeded exactly as described in Experiment 1.

Renewal Test.: Test sessions proceeded as described in Experiment 1. However, here the groups differed in whether the tested stressor had been received prior to actual acquisition sessions (Group Paired) or during the days of pre-exposure (Group Unpaired). As before, half the rats in Groups Paired and Unpaired received restraint and half received footshock. For Group Paired ($n = 10$), that stressor had also been received prior to two training sessions; that is, rats tested with F had received XOFPOF and rats tested with R had received XORPOR. For Group Unpaired ($n = 11$), however, the tested stressor had only been received during pre-exposure, and was thus not connected with lever press training. Here, rats tested with F had received XORPOR during instrumental training and rats tested with R had received XOFPOF. Prior to their "stressed" test, rats in Group Control ($n = 10$) were simply placed into the familiar control shoebox with woodchip bedding in the darkened room for 35 min.

Results

The results of acquisition, extinction, and testing are shown in Figure 3. The groups learned (left panel) and extinguished (middle panel) lever pressing similarly. The results of the test (right panel) indicated a stress-induced recovery in both the Paired and Unpaired Groups.

Acquisition and Extinction—A 3 (Group) $\times$ 9 (Session) ANOVA indicated that response rate increased over acquisition sessions, $F(8, 224) = 74.21$, $MSE = 20.68$, $p < .001$; the main effect of group and the session by group interaction did not approach significance, $F_s < 1$. A 2 (Group) $\times$ 5 (Session) ANOVA similarly found that responding decreased over extinction training, $F(4, 112) = 73.82$, $MSE = 8.78$, $p < .001$; there was no group effect or interaction, largest $F = 1.49$.

Test—A 3 (Group) $\times$ 2 (Session: No Stress vs. Stress) ANOVA indicated a main effect of Session, $F(1, 28) = 33.83$, $MSE = 167.09$, $p < .001$, $\eta_p^2 = .55$. The group by session interaction approached significance, $F(2, 28) = 3.14$, $MSE = 15.53$, $p = .059$, $\eta_p^2 = .18$, although there was no main effect of group, $F < 1$. Planned comparisons indicated that Groups Paired and Unpaired both made more responses in the session following stressor exposure $p_s < .001$. In contrast, there was no change in responding in the Control Group over sessions ($p = .17$). A subsequent analysis including only Groups Paired and Unpaired

found a session effect, $F(1, 19) = 38.85$, $MSE = 4.83$, $p < .001$, $\eta_p^2 = .60$, but no group effect or a group by session interaction, $F_s < 1$.

Discussion

The results of Experiment 2 suggest that the present renewal effect does not depend on testing with a stressor that had been directly associated with the instrumental lever-press training during the acquisition phase. Rather, equally familiar stressors that had not been connected with training (Group Unpaired) also produced a recovery that was similar to the one observed in rats that had received the tested stressor prior to acquisition sessions (Group Paired). Furthermore, a lack of renewal in the control group suggests that response recovery cannot be simply attributed to mere placement in a different apparatus before testing. The results are thus most consistent with the hypothesis that the stress-induced reinstatement effect produced here is created by a common interoceptive stress state that is produced by the different stressors present during acquisition training and then absent during extinction (see Figure 2a). Thus, renewal occurs upon returning the animal to that general interoceptive stress state or context that had been associated with training.

Experiment 3

Another question concerning the renewal results of Experiments 1 and 2 was whether the renewing effects of stress during testing actually depended on exposure to stressors immediately prior to the acquisition of lever pressing, as the ABA renewal account would imply. It is instead possible that the response-enhancing effects of stress during testing in Experiments 1 and 2 depended only on prior exposure to stress, rather than its specific connection with lever pressing. Experiment 3 was therefore designed to address this issue. As usual, Group Acquisition Stress received exposure to stressors prior to reinforced acquisition sessions and not extinction sessions. But a new group, Group Extinction Stress, received the reverse: This group was exposed to the same sequence of stressors prior to extinction sessions rather than acquisition sessions. As in Experiment 2, responding was then tested with or without a stressor that had not been directly associated with acquisition or extinction. If stress-induced reinstatement depends on stress's connection with acquisition, we should observe it only in Group Acquisition Stress. Indeed, for Group Extinction Stress, stress might provide a context for extinction, rather than acquisition, perhaps creating more responding during testing in the absence rather than the presence of stress.

Method

Subjects and Apparatus—The subjects were 32 naive female Wistar rats ($ns = 16$) of the same age and from the same vendor as those in previous experiments. The apparatus and maintenance conditions were the same as described in the previous experiments.

Procedure

Pre-exposure.: As in Experiment 2, during each of the first 2 days rats were pre-exposed to their eventual test stressor. For half the rats this was the footshock stressor and for the other

half it was restraint (as previously described). Following each stressor exposure, rats were returned to the homecage.

Magazine training. On each of the next two days, a daily magazine training session proceeded as in the previous experiments.

Acquisition. Over the next 9 days, lever-press training and stressor exposures proceeded exactly as described in Experiment 2 for rats in the Acquisition Stress group. Beginning with Session 4, half of those rats received stressors in the sequence XOFPOF and half received XORPOR. Rats in the Extinction Stress Group received similar handling, without stress, prior to each acquisition session. Two rats in Group Extinction Stress were removed from the experiment due to an experimenter error in which they were exposed to a stressor during acquisition.

Extinction. Then, over the next 6 days, all rats received daily extinction training sessions as described in Experiments 1 and 2. However, for this experiment, rats in the Extinction Stress group were also exposed to a stressor before each session. These rats received the same stressors in the same orders (i.e., XOFPOF or XORPOR) as rats in the Acquisition Stress Group had received before acquisition sessions.

Renewal Test. Test sessions proceeded exactly as described in Experiment 2. All animals were tested with the stressor they had received during pre-exposure, which had not occurred before an acquisition (Group Acquisition Stress) or extinction (Group Extinction Stress) session. As usual, half the rats received one test after restraint and the other half after a footshock. Before the other test rats were not exposed to a stressor but received similar handling.

Results

The results of acquisition, extinction, and testing are shown in Figure 4. Both groups learned the response, which increased in rate over the acquisition sessions (left panel). Then, both groups extinguished the response over extinction (middle panel). Visual inspection of the test data (right panel) suggested that each group showed evidence of the predicted renewal effects. That is, rats responded more during a session when their pre-session treatment (Stress or No Stress) was consistent with their acquisition treatment, and less when the pre-session treatment (No Stress or Stress) was consistent with their extinction treatment. The difference was statistically reliable in the Acquisition Stress Group.

Acquisition and Extinction—A 2 (Group) $\times$ 9 (Session) ANOVA indicated that lever presses increased over acquisition sessions, $F(8, 224) = 60.99$, $MSE = 30.06$, $p < .001$. The main effect of group was not significant, $F(1, 28) = 1.34$, $MSE = 468.11$, $p = .257$. However, a significant session by group interaction, $F(8, 224) = 4.40$, $MSE = 30.06$, $p < .001$, indicated that response rate differed over sessions between the groups. Follow-up analyses revealed that rats in the acquisition stress group responded at a greater rate in the 8th session, $p = .01$. Significant differences in responding did not occur in any other session.

A 2 (Group) $\times$ 6 (Session) ANOVA on the extinction data indicated that responding declined over extinction, $F(5, 140) = 89.05$, $MSE = 6.35$, $p < .001$. The main effect of group was not significant, $F < 1$, although there was a group by session interaction, $F(5, 140) = 2.40$, $MSE = 6.35$, $p = .040$. Follow up analyses indicated that the groups did not differ significantly during any session, smallest $p = .224$.

Test—A 2 (Group) $\times$ 2 (Session) ANOVA on the test data revealed the crucial significant Group $\times$ Session interaction, $F(1, 28) = 6.52$, $MSE = 3.02$, $p = .016$, $\eta_p^2 = .19$. Thus, the effect of stress during testing did depend on when it had been received during training. Neither the main effect of session nor of group was significant, $F_s < 1$. Planned comparisons revealed that Group Acquisition Stress made more responses in the test after a stressor had been given ($p = .027$), replicating the results of Experiments 1 and 2. There was no difference between the stressed and nonstressed conditions in Group Extinction Stress ($p = .202$). However, the data shown in the right panel of Figure 4 suggest that when stress was absent, Group Extinction Stress tended to respond more than Group Acquisition Stress, perhaps providing evidence that the absence of stress cued responding in Group Extinction Stress. The difference between groups in that session was reliable, $F(1, 30) = 4.33$, $MSE = 8.15$, $p = .046$, $\eta_p^2 = .13$. The groups made a similar number of responses in the session after stress, $F < 1$.

Discussion

The results suggest that the capacity of stressors to promote relapse differs depending on whether they are present during response acquisition versus extinction. As in Experiments 1 and 2, stressor exposures prior to acquisition rendered behavior subject to renewal when stress was re-encountered at test. In contrast, no such effect was observed when the stressors had preceded extinction training instead of acquisition training. The results thus suggest that stress must be associated with acquisition to allow it to produce response recovery in the present paradigm.

General Discussion

The results of these experiments suggest that stress cues, like external stimuli that comprise a physical environment, can play the role of context and exert control over the performance of instrumental behavior. In each experiment, food-seeking that was learned in sessions that followed exposure to stress, and was inhibited through extinction while stress was absent, renewed when a stressor was encountered before a test. In contrast, food-seeking remained inhibited after the test stressor if stress had not preceded acquisition sessions. The results of Experiments 2 and 3 further provide evidence that the context that controlled behavior generalized across specific stressors (see Figure 2a). First, renewal did not depend on testing with a stressor that had been directly associated with instrumental training. Second, spending time in a neutral (nonstressful) apparatus before testing did not renew responding, suggesting that animals had not merely learned that being placed in another apparatus before an instrumental session signaled reinforcement. Together, the results have direct implications

for understanding the effects of stress on inhibited food seeking, and more tentatively, perhaps the effects of stress on inhibited drug seeking.

Experiment 3 went on to suggest that renewal after stress depended on animals receiving stressors before the actual instrumental training sessions. Stress failed to renew behavior if the same stressors had been given prior to extinction instead of acquisition. Although responding in Group Extinction Stress did not differ during the stressed and non-stressed test sessions, the group's responding in the absence of stress was significantly higher than that in Group Acquisition Stress, suggesting some evidence that the presence and absence of stress controlled performance even in Group Extinction Stress.

That result may be relevant in considering another account of the stress-induced reinstatement observed here. It is possible that the results could be due in part to a process known as *incentive learning* (e.g., Dickinson & Balleine, 1994). According to such an account, during acquisition the animals might have learned that sucrose pellets made them feel better after recent exposure to stress. As a consequence, stressor exposure prior to the test could have motivated the animal to respond for sucrose because it increased the value attributed to sucrose while under stress. For example, in a well-known example of incentive learning, Balleine (1992) found that food deprivation (hunger) had no effect on instrumental responding for food pellets during extinction unless the rats had previously eaten the food-pellet reinforcer in the hunger state. That is, hunger caused an increase in lever press rate only if the rats had eaten the pellets while hungry and presumably learned about their incentive value while hungry. Rats in the present experiments could have analogously learned the value of the sucrose pellet after stress and therefore responded more when tested after a stressor. However, any evidence of contextual control of performance by stress in Group Extinction Stress in Experiment 3 would be difficult to attribute to incentive learning, because stressors received during extinction could not affect the value the rat attributes to sucrose pellets—which are entirely absent during extinction. Instead, response recovery in the absence of stress in Group Extinction Stress would suggest that stress played the role of an extinction context.

Another potential problem for an incentive learning account arises from the training parameters used in the current experiments. Incentive learning is thought to affect the value of the organism's representation of the reinforcer, and thus depends on the response being a goal-directed action, i.e., a behavior that is mediated by the animal's knowledge of the reinforcer's value as well as the response-reinforcer relation (e.g., Balleine & Dickinson, 1998). Other previous research suggests that the amount of instrumental training given here might have favored the development of habit rather than goal-directed action (e.g., Dickinson, 1985). Habitual instrumental behaviors are thought to be automatic and independent of the animal's representation of reinforcer value. Thus, lever pressing that has become a habit (and is performed independently of the value of its outcome) may not be influenced by earlier incentive learning. Dickinson, Balleine, Watt, Gonzales, and Boakes (1995) reported that the effects of an incentive learning treatment that were present after rats earned 120 pellets on an RI 30-s reinforcement schedule were no longer present after extended training in which they had earned 360 pellets, an amount previously shown to produce habitual behavior. In our own laboratory, Thrailkill and Bouton (2015) have also

shown the development of habit after rats earned 360 pellets on the present VI 30-s reinforcement schedule. In the current experiments, rats earned more than 500 pellets on VI 30 over the acquisition sessions (e.g., $M = 534.09$ in Experiment 2). Furthermore, exposure to stress or drugs has also been shown to accelerate habit formation (Nelson & Killcross, 2006; Schwabe & Wolf, 2009). Thus, after the extensive training and the stress exposure involved here, lever pressing may have transitioned to habit, thus reducing any possible role for incentive learning.

Together, the current experiments suggest that the stimuli produced by stress, like other interoceptive stimuli, are sufficient to exert control over behavior (e.g., Besheer, Palmatier, Metschke, & Bevins, 2004; Brener & Jones, 1974; Sample, Jones, Hargrave, Jarrad, & Davidson, 2016; Schepers & Bouton, 2017; Schuster & Brady, 1971), thus making it susceptible to renewal in their presence after extinction.

The present results suggest that so-called stress-induced “reinstatement” of instrumental behavior (Shaham & Stewart, 1995) may sometimes be attributable to ABA renewal. They have especially direct relevance for understanding the effects of stress on behavior reinforced by food. Obesity-related illness and death are a substantial financial burden on health care systems in the United States and around the world. As of 2014, an estimated 35% of men and 40% of women in the United States were considered obese. Ubiquitous access to and excessive intake of highly palatable and energy-dense foods has been described as major contributor to the obesity epidemic (Flegal et al., 2016). Moreover, frequent exposure to stress may compound the problem. In 2013, 38% of adults reported that stress caused them to overeat or consume unhealthy foods over the previous month (American Psychological Association, 2013). The current experiments suggest that even though palatable food-seeking behaviors can be suppressed through extinction, if they had been learned in the presence of stress they may be subject to relapse when stress is reencountered. Consistent with an incentive learning hypothesis, “stress-eating” has been suggested to develop in a manner consistent with a self-medication hypothesis. That is, “comfort foods” may be especially valued after stress in that they may alleviate some of its effects (Ulrich-Lai, 2016). However, the present results suggest that learning to perform the behavior itself in the context of stress may also be a critical factor. In other words, relapse may occur when stress becomes linked with the performance of the original instrumental behavior itself (e.g., eating), rather than any specific outcome that it had produced (e.g., comfort food). Recent clinical research suggests that treatments that target stress, such as mindfulness meditation, may be effective in helping to control weight and reduce emotional eating (Katterman, Kleinman, Hood, Nackers, & Corsica, 2014; Mason et al., 2016). The present results suggest that one reason why stress management strategies may be effective is that they might reduce a stress-induced renewal effect.

Acknowledgement

This research was supported by NIH grant DA 033123 to MEB.

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Figure 1. Results of Experiment 1. Mean lever responses per minute during each 30-min session of Acquisition (left), and Extinction (middle). The right panel shows the mean responses per minute during the first 5 min of the test sessions.

Figure 2ab.

Panel a describes the proposed account of interoceptive stress control over behavior. Stress stimuli that are common across different stressors produce an interoceptive context of stress which comes to exert contextual control over the behavior. Panel b describes an alternative account in which individual stressors are directly and separately associated with the performance of the behavior.

Figure 3. Results of Experiment 2. Mean lever responses per minute during each 30-min session of Acquisition (left), and Extinction (middle). The right panel shows the mean responses per minute during the first 5 min of the test sessions.

Figure 4. Results of Experiment 3. Mean lever responses per minute during each 30-min session of Acquisition (left), and Extinction (middle). The right panel shows the mean responses per minute during the first 5 min of the test sessions.