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Acute stress facilitates habitual behavior in female rats

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ABSTRACT

Instrumental behavior can reflect the influence of goal-directed and habitual systems. Contemporary research suggests that stress may facilitate control by the habitual system under conditions where the behavior would otherwise reflect control by the goal-directed system. However, it is unclear how stress modulates the influence of these systems on instrumental responding to achieve this effect, particularly in females. Here, we examine whether a mild psychogenic stressor experienced before acquisition training (Experiment 1), or prior to the test of expression (Experiment 2) would influence goal-directed and habitual control of instrumental responding in female rats. In both experiments, rats acquired an instrumental nose-poke response for a sucrose reward. This was followed by a reinforcer devaluation phase in which half the rats in Stressed and Non-Stressed conditions received pairings of the sucrose pellet with illness induced by lithium chloride until they rejected the pellet when offered. The remaining rats received a control treatment consisting of pellets and illness on separate days (Unpaired). Control by goal-directed and habitual systems was evaluated in a subsequent nonreinforced test of nose poking. The results of Experiment 1 indicated that the Non-Stressed Paired group reduced nose-poking compared to the Unpaired controls, identifying the response as goal directed, whereas the Stressed Paired and Unpaired groups made a similar number of nose pokes identifying the response as habitual despite a similar amount of training. Results from Experiment 2 indicated habitual control of nose-poke responding was present when stress was experienced just prior to the test. Collectively, these data suggest that stress may facilitate habitual control by altering the relative influence of goal-directed and habitual processes underpinning instrumental behavior. These results may be clinically relevant for understanding the contributions of stress to dysregulated instrumental behavior in compulsive pathologies.

1. Introduction

Stress can cause perseveration of established behavioral responses and hinder the ability to adapt behavior to changing environments [1–3]. This influence on behavioral flexibility may play a role in maladaptive voluntary behaviors that characterize diverse psychiatric conditions, including substance use disorder, obsessive-compulsive disorder, and the enduring fear experienced in post-traumatic stress disorder [4–6]. Stress also has an important influence on instrumental behavior in the laboratory ([7]; reviewed in [8]). Instrumental responding can be goal-directed in the sense that it is sensitive to the current value of the reinforcing outcome. In contrast, responses can be insensitive to the current value of the outcome and therefore habitual [9–11].

In the laboratory, goal direction and habit can be identified with the reinforcer devaluation method. In a typical procedure, a rat, for instance, first acquires an instrumental response (e.g., a nose poke) paired with the contingent presentation of a reinforcing outcome. In a second phase, the response is not available to be performed and the reinforcer is devalued with taste aversion learning or specific satiety ([10]; reviewed in [12]). The following test phase consists of the opportunity to make the response without the outcome. If the behavior is goal-directed, rats that received the devaluation treatment will make fewer responses in comparison to a control group that received identical

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training but did not experience a change in the outcome value. In contrast, following devaluation, if responding does not differ from controls, then the response is not sensitive to changed value and meets criteria for habit.

Stress seems to facilitate control by the habit system in both humans and rodents [7,13]. However, while most human studies have used acute stressors [1,3,7], those conducted in rodents have primarily used chronic stress paradigms [13,14]. The extent to which the timing of the acute stressor (i.e., before response acquisition or before testing) modulates its influence on habit, also remains unclear. Further, while stress facilitated habit control in male rats [15], little is known about how stress influences control by goal-directed and habitual processes in female rats. This is especially relevant given women's higher risk for stress-related disorders [16], and the sexually dimorphic influences of stress on other learning processes [17–19].

The present experiments extended our recent work with female rats [20,21] to examine whether a mild acute stressor prior to acquisition training (Experiment 1) or before testing (Experiment 2) enhances habitual behavior when goal-directed behavior would otherwise be expected (diagrammed in Fig. 1). Based on studies of spatial learning in rodents and reward-based decision-making in humans (for review see [22,23]), we hypothesized that acute stress would provoke habitual responding in each experiment.

2. Material and methods

2.1. Subjects

Experiments 1 and 2 were conducted successively and each included a cohort of 36 Female Long Evans rats (Charles River, Quebec; 75–90 days old at the time of arrival). Sample size was based on a prior experiment in this laboratory that found sensitivity of nose-poke responding to outcome devaluation under training conditions identical to those described below [21]. Rats were pair-housed with unlimited access to water in a climate-controlled colony room maintained at 23 °C with a 12-hour light/dark cycle (7:00–19:00 light-on period). Rats were handled by an experimenter each day. Experimental sessions were conducted each day at approximately 08:30. Rats were maintained at 85 % of their free-feeding weights and were given supplementary feeding approximately 4 h after each session. During experimental phases that included acute restraint stress (see below), we conducted vaginal cytology to confirm that normal estrus cycling continued following the stressor. Vaginal lavage was performed on these days approximately 4 h after each experimental session, prior to food allotment delivery. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont.

2.2. Apparatus

The instrumental training equipment consisted of 6 standard rat operant chambers (model ENV-007; Med Associates, St. Albans, VT) housed within individual sound-attenuating cabinets. In the center of the right-facing chamber wall was a food cup (5 cm x 5 cm) into which a pellet dispenser (model ENV-203–45; Med Associates) could deliver 45-mg sucrose pellets (Bio-Serv). Entries to the food cup were recorded by an infrared beam. To the right of the food cup was a nose-poke response manipulandum (model ENV-114; Med Associates). The nose poke measured 2.5 cm in diameter and 2 cm deep and was located 6.3 cm (to center) above the chamber floor near the side panel that functioned as the chamber door. Entries into the nose-poke were recorded by an infrared beam. Experimental events were controlled and data were recorded by a computer located in an adjacent room.

The apparatus for acute restraint stress consisted of a cylindrical restraining device 9 cm x 15 cm (D x H) placed in a brightly lit room

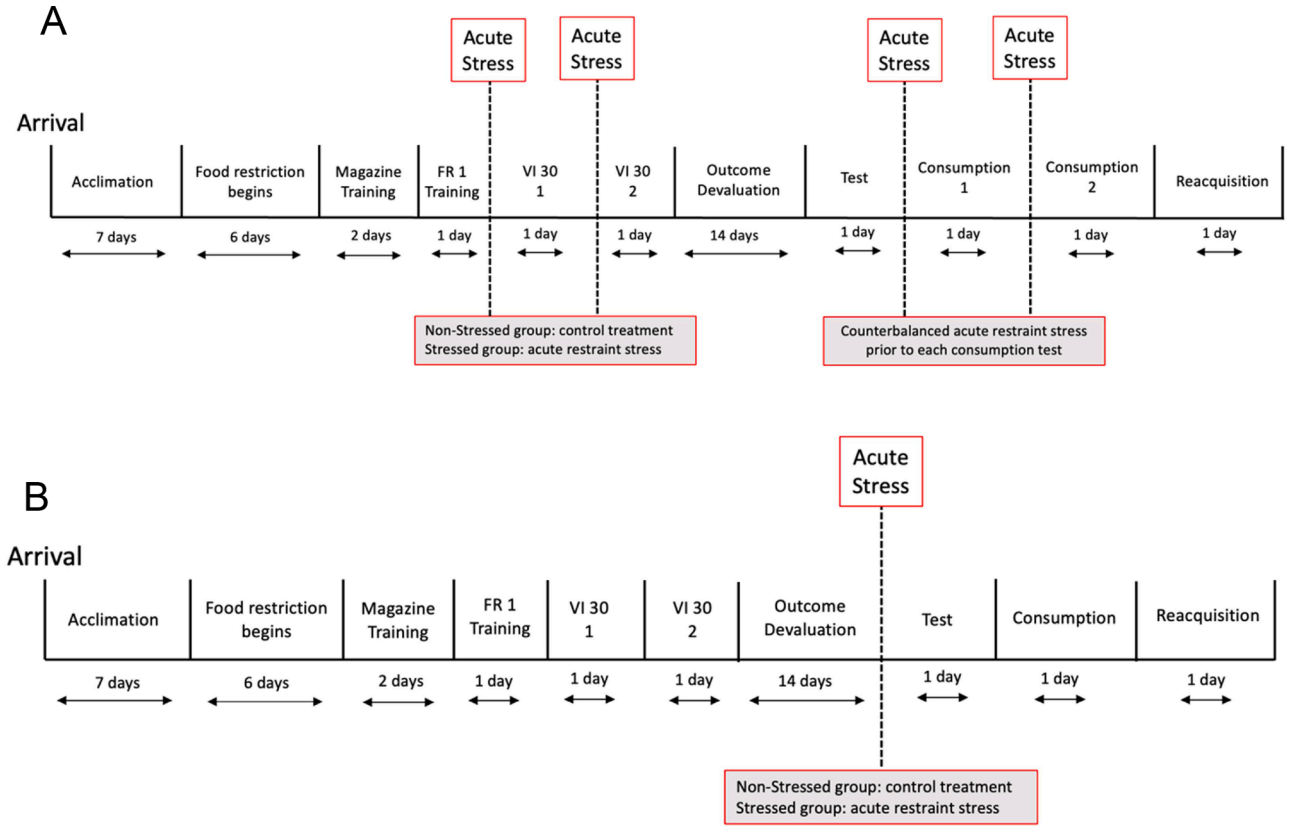

Fig. 1. Experimental timeline.
Note. A) Experimental timeline for Experiment 1: Stress before acquisition. B) Experimental timeline for Experiment 2: Stress before test.

upon the center of a countertop.

2.3. Procedure

2.3.1. Experiment 1

The timeline of Experiment 1 is diagrammed in Fig. 1a. Prior to the experiment, rats were assigned to an operant chamber in which they received all training and testing procedures. A house light located on the rear wall of the chamber signaled the beginning and end of each session.

2.3.1.1. Magazine training. On the first two days, all rats received 30 min sessions in which one sucrose pellet was delivered into the food cup every 60 s, on average (variable-time 60 s schedule; VT 60 s). Pellet delivery was signaled by the sound of the pellet striking the food cup; no other stimuli were presented. The nose-poke manipulandum was removed during magazine training.

2.3.1.2. Nose-poke training. The nose-poke device was installed in each chamber and rats received two days of free-operant nose-poke training. Each nose-poke response resulted in the delivery of a sucrose pellet (a fixed-ratio 1 schedule; FR 1). Sessions terminated after the delivery of 25 pellets or 1 h, whichever occurred first. No other stimuli were presented during these sessions.

2.3.1.3. Acute stress. Rats were randomly assigned to Stressed and Non-Stressed groups. Prior to the next 2 sessions, rats in the Stressed group received 60 min of acute restraint stress in a separate brightly lit laboratory room directly before each session. Acute restraint stress has previously been demonstrated to induce robust physiological and behavioral stress responses in rats [24]. Concurrently, the Non-Stressed group spent 60 min in a standard cage in a darkened room. For all rats, a variable-interval (VI) schedule arranged a sucrose pellet to become available contingent on a nose-poke response after 30 s, on average (VI 30 s). Sessions terminated after 40 reinforcers were delivered. The overall amount of instrumental training (130 reinforced responses over 4 sessions) was based on previous studies in the laboratory that found evidence of sensitivity to reinforcer devaluation, and hence goal direction, following the identical amount of training with female rats [21].

2.3.1.4. Outcome devaluation. On the following day, nose-poke manipulanda were removed from the operant chambers and the rats were randomly assigned to have the sucrose pellet devalued via pairings with an injection of lithium chloride (LiCl; Paired group) or to a non-devalued control group that received equivalent exposure to sucrose pellets and LiCl on different days (Unpaired group). Cage mates always received opposite devaluation treatments. The outcome devaluation procedure consisted of 2-day cycles (cf. [25]). On odd days (e.g., day 1, 3, 5, 7, 9, 11, 13), all rats received a 10 ml/kg intraperitoneal (i.p.) injection of 0.15 M LiCl immediately following the session. On even days, all rats received an i.p. injection of the same volume of 0.9 % physiological saline. On Day 1, the Paired group received 40 sucrose pellets according to VT 30 s, the Unpaired group was placed in the chamber but did not receive pellets. On Day 2, the Unpaired group received 40 sucrose pellets on VT 30 s and Paired group did not receive pellets. This 2-day cycle repeated for the remainder of the devaluation phase. The number of pellets eaten was recorded following each session by counting any pellets left in the food cup or chamber floor. The number of pellets delivered was adjusted each cycle to match the mean number of pellets consumed by the Paired group in the preceding cycle. Cycles repeated until the number of pellets consumed by rats in the Paired group reached zero.

2.3.1.5. Test. On the day following the final devaluation cycle, the nose-poke device was reinstalled in the operant chambers and all rats received a 12-min test. Rats were placed in the chamber and responses were recorded but had no programmed consequences.

2.3.1.6. Consumption test. On days subsequent to the test day, rats received pellet consumption tests to confirm the total devaluation of the outcome. Rats were placed in the operant chamber and 10 pellets were delivered according to VT 30 s. The nose-poke devices were again physically removed from the chamber and pellet consumption was recorded. All rats received one consumption test session with 60 min acute stress immediately beforehand and one without (order counterbalanced) to assess whether the presence or absence of stress would influence the expression of the aversion to the pellets in the Paired group as indexed by free pellet consumption. Acute stress procedures were identical to those performed in acquisition.

2.3.1.7. Reacquisition. The next day, nose-poke manipulanda were again reinstalled in the chambers and rats received a 30-min session in which nose-poking could earn pellets according to VI 30 s.

2.3.2. Experiment 2

The timeline of Experiment 2 is diagrammed in Fig. 1b.

2.3.2.1. Training. Magazine and nose-poke training procedures were identical to Experiment 1. All rats received VI-30 s training on Days 3 and 4 similar to the Non-Stressed group in Experiment 1 except that stress was not administered before the session.

2.3.2.2. Outcome devaluation. Following instrumental training, half the rats were randomly assigned to Paired and Unpaired groups and received the same outcome devaluation procedure as described in Experiment 1.

2.3.2.3. Test. On the day following the final devaluation cycle, rats were randomly assigned to Stressed and Non-Stressed groups. The Stressed group received 60 min of acute restraint stress in a separate brightly lit laboratory room immediately before the test session. The Non-Stressed group spent 60 min in a standard cage in a darkened room. The test session was the same as described in Experiment 1. Rats were placed in the chamber and allowed to make the nose poke response for 12 min. As before, responses were recorded but had no programmed consequences.

2.3.2.4. Consumption and reacquisition. On the following days, rats received the same pellet consumption and 30 min reacquisition test sessions as described in Experiment 1 with the exception that consumption testing was carried out only once and was not preceded by stress.

2.4. Statistical analysis

Response rates (nose pokes per minute) during the VI 30-s training sessions were analyzed using analysis of variance (ANOVA). Stress (Stressed or Non-Stressed) and Devaluation (Paired or Unpaired) were entered as between-subject factors, and Session was entered as a within-subject factor. Counts of pellets consumed by Stressed Paired and Non-Stressed Paired groups in each outcome devaluation cycle were analyzed in a Stress by Devaluation by Session ANOVA.

Nose-poke and food-cup response rates from the test were analyzed as a proportion of baseline (nose-poke or food-cup entry rates at test divided by respective response rates in the final VI 30-s training session) with planned comparison tests of the Devaluation groups in each Stress condition [20,26–28]. The planned comparisons of devaluation groups for each stress condition were of *a priori* interest (cf. [29]). Pellets eaten in the consumption test and proportion of baseline response rates in the reacquisition test were analyzed with a Stress by Devaluation ANOVA.

Response rate microstructure during the final nose-poke training

session and the test were analyzed by parsing nonreinforced nose-poke responses not immediately followed by checking the food cup (discrete/independent responses) from nose-pokes that were followed by a food cup entry within the subsequent 2.5 s period (nose-poke to food-cup sequences; cf. [30]). These two types of response rate are illustrated in Fig. 3A. Because rats earned all possible rewards during the final training session, every animal exhibited approximately 40 reinforced action sequences and thus, only nonreinforced responses were analyzed. Independent nose-poke rates and nose-poke to food-cup sequences were calculated for the final VI 30-s training session and test session. Each rate measure from the test was analyzed as a proportion of baseline (i.e., divided by the respective rate from the final training session). In each analysis, separate Stress by Devaluation ANOVAs and planned comparisons of Devaluation groups compared independent nose-poke rates and nose-poke to food-cup sequences .

For all analyses, response rate outliers were operationalized as observations two standard deviations above or below the group mean (Z +/- 2; [31]). Exclusion criteria for Paired condition animals was consumption of an average of 4 or more pellets across the two consumption sessions (Experiment 1) or in the single consumption session (Experiment 2). Sphericity of the data was assessed using Mauchly's test ($p < 0.05$), Greenhouse-Geisser ($\varepsilon > 0.75$) or Huynh-Feldt ($\varepsilon < 0.75$) to determine if corrections of degrees of freedom were necessary. Effect sizes are reported when relevant to study hypotheses. Alpha was set at $p < 0.05$ for all tests. Bayesian independent samples t-tests were performed to quantify the evidence in favor of the null hypothesis when relevant [25,32]. Bayes factors were computed in JASP using a non-informative default Cauchy prior (JASP [33]). Data and materials are available upon request from the corresponding author.

3. Results

3.1. Experiment 1

3.1.1. Vaginal cytology

Our analysis of vaginal cytology found no evidence to suggest that acute stress disrupted the estrous cycle.

3.1.2. Nose-poke training

As shown in Fig. 2A, rats increased nose-poke response rates across the two training sessions and pre-session acute stress had no apparent effect on response rates in the Stressed group. This result was supported by a repeated measures ANOVA with a significant main effect of session, $F(1, 30) = 50.590$, $p < 0.001$, $\eta p^2 = 0.628$. Effects involving stress or devaluation did not approach significance, Fs < 1.

3.1.3. Outcome devaluation

Pellets consumed by Paired groups in each cycle of the devaluation phase are shown in Fig. 2B. Unpaired groups consumed all pellets (not shown). Paired groups reached the criterion of zero consumption of pellets by the seventh devaluation cycle. The ANOVA indicated no main effect of stress group or stress group by session interaction, Fs < 1, which

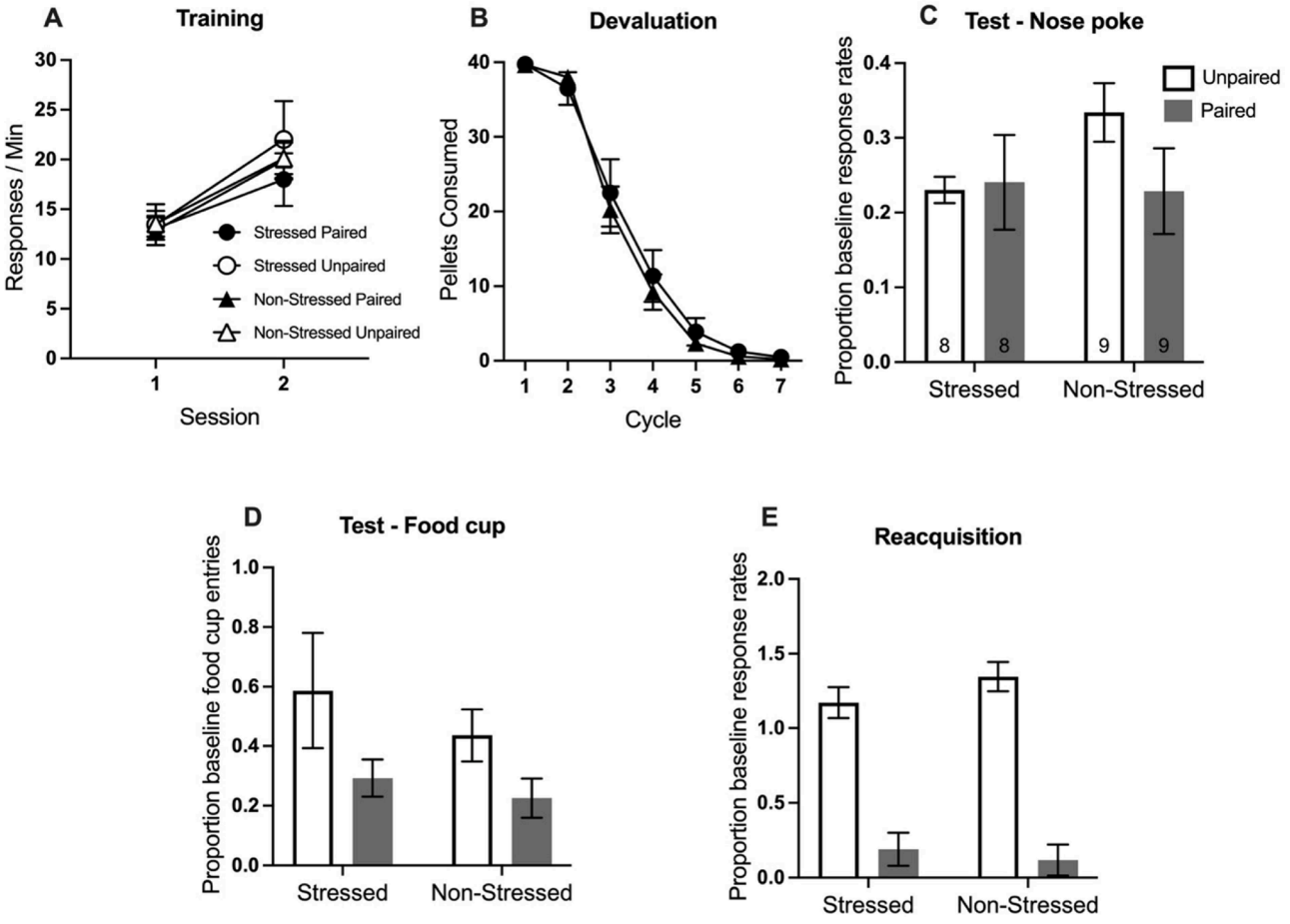

Fig. 2. Results of Experiment 1.
Note. A) Mean nose-poke rates (responses per minute) over the two sessions of training on a VI 30-s schedule. B) Mean number of sucrose pellets consumed by Paired condition animals during each cycle of the outcome devaluation phase. C) Mean nose-poke rates during the test as a proportion of nose-poke rate in the final VI 30-s session (proportion of baseline). D) Mean food-cup entry rates during the test as a proportion of baseline. E) Mean nose-pokes per minute during the reacquisition test as a proportion of baseline. Error bars denote standard error of the mean.

suggests that stress treatment did not significantly influence the outcome devaluation process.

3.1.4. Test

The results of the nose-poke test are shown in Fig. 2C. Two animals were identified as outliers and excluded from all analyses based on their performance in this test: One each from Group Stressed Unpaired, $Z = 2.24$, and Group Stressed Paired, $Z = 2.46$. Consistent with our hypothesis, Non-Stressed Paired rats exhibited lower proportion baseline response rates ($M = 0.23$, $SD = 0.17$) than their Unpaired counterparts ($M = 0.33$, $SD = 0.12$). In contrast, Stressed rats exhibited similar nose-poke rates across Paired and Unpaired conditions (respectively: $M = 0.24$, $SD = 0.18$; $M = 0.23$, $SD = 0.05$). Due to positive skew in this data, we applied a logarithm (base 10) transformation to normalize the distribution. In the Non-Stressed group, Bonferroni-corrected planned comparisons found significantly lower proportion of baseline response rates in Group Paired, $F(1, 30) = 4.733$, p=0.038, $\eta p^2 = 0.136$. In contrast, Stressed Paired and Stressed Unpaired groups did not differ statistically, $F(1, 30) = 0.264$, $p = 0.611$, $\eta p^2 = 0.009$. Further, a Bayesian independent samples t-test performed on the non-transformed data found anecdotal evidence for the null hypothesis of no difference between the Stressed Paired and Unpaired groups, $BF_{01} = 2.319$.

Food cup entry rates during the test are shown in Fig. 2D. These data were also positively skewed and again logarithm (base 10) transformed. Like the pattern observed for the nose-poke response, the planned pairwise comparisons with Bonferroni corrections confirmed a significant devaluation effect on food-cup entry rates in the Non-Stressed group, $F(1, 30) = 5.259$, $p = 0.029$, $\eta p^2 = 0.149$. The effect was not significant in the Stressed group, $p = 0.129$.

3.1.5. Consumption and reacquisition tests

Pellet consumption tests confirmed the success of the outcome devaluation procedure and that stress did not influence sucrose pellet valuation. In each pellet consumption test following stress and no stress, Stress and Non-Stressed Paired animals consumed close to no pellets, while those in the Unpaired condition consumed almost all pellets (see Table 1). No animals met exclusion criteria for pellet consumption. Results of the reacquisition test are shown in Fig. 2E. Unpaired groups reacquired nose-poke responding to circa-baseline levels while Paired groups made few responses. The analysis found a significant devaluation effect, $F(1, 30) = 118.317$, $p < 0.001$, $\eta p^2 = 0.798$). Effects involving stress did not approach significance, $Fs < 1$.

3.1.6. Response rate microstructure

Fig. 3B shows the nonreinforced independent nose-pokes per minute during the final session of VI 30 s training. A stress by devaluation ANOVA found no significant effects of stress, devaluation, or interaction, largest $F(1, 30) = 1.145$. Fig. 3C shows nonreinforced nose-poke to food-cup sequence rates during the final training session. The same ANOVA found a significant effect of stress, $F(1, 30) = 8.287$, $p = 0.007$, $\eta p^2 = 0.216$, and no effects involving devaluation, largest $F(1, 30) = 1.210$.

Proportion of baseline independent nose-poke rates during the test are shown in Fig. 3D. This data was positively skewed and logarithm (base 10) transformed prior to analysis. Bonferroni corrected pairwise comparisons of Paired and Unpaired groups did not approach significance in the Stressed condition, $F < 1$. The same comparison of Non-Stressed Paired and Unpaired groups approached significance, $F(1, 30) = 3.529$, $p = 0.070$, $\eta p^2 = 0.105$. Proportion of baseline nose-poke to food-cup sequence rates during the test are shown in Fig. 3E. These data were also positively skewed and logarithm transformed. Planned comparisons with Bonferroni corrections found evidence of higher proportion baseline nose-poke to food-cup sequence rates in the Unpaired condition for Stressed, $F(1, 30) = 4.876$, $p = 0.035$, $\eta p^2 = 0.140$, and Non-Stressed groups, $F(1, 30) = 11.137$, $p = 0.002$, $\eta p^2 = 0.271$.

Table 1
Results of experiment 1 consumption tests.

Group	Unstressed consumption	Stressed consumption
Stressed unpaired	10.00 (0.00)	9.75 (0.71)
Stressed paired	0.88 (1.13)	0.38 (0.74)
Non-stressed unpaired	10.00 (0.00)	10.00 (0.00)
Non-stressed paired	0.11 (0.33)	0.56 (1.33)

Note: Mean (standard deviation) pellet consumption during the unstressed and stressed consumption tests by stress and devaluation group.

3.2. Experiment 2

3.2.1. Vaginal cytology

As in Experiment 1, we found no evidence of disruption to normal estrous cycling following acute restraint stress.

3.2.2. Nose-poke training

Fig. 4A shows nose-poke response rate in the first and second VI 30-s training sessions. There was a significant effect of session, $F(1, 31) = 65.934$, $p < 0.001$, $\eta p^2 = 0.680$, and no effect of stress, devaluation, or interaction, $Fs < 1$.

3.2.3. Outcome devaluation

Pellets consumed by Paired groups in each cycle of the devaluation phase are shown in Fig. 4B. Unpaired groups consumed all pellets (not shown). The analysis found a significant effect of stress, $F(1, 16) = 5.495$, $p = 0.032$, $\eta p^2 = 0.256$, and no interaction with session, $F(1.606, 25.697) = 2.596$, $p = 0.104$, $\eta p^2 = 0.140$. All rats met the criterion of complete cessation of pellet consumption by the end of devaluation.

3.2.4. Test

The Experiment 2 test results are shown in Fig. 4C. One rat from Group Non-Stressed Unpaired was identified as an outlier and excluded from all analyses ($Z = 2.62$). Stressed Paired rats had slightly lower proportion baseline response rates than Stressed Unpaired rats (respectively: $M = 0.24$, SD=0.12; $M = 0.33$, $SD = 0.13$). This difference was more pronounced in the Non-Stressed group (Paired: $M = 0.15$, $SD = 0.07$; Unpaired: $M = 0.31$, $SD = 0.09$). Planned comparisons with Bonferroni corrections indicated that Paired and Unpaired groups did not differ significantly in the Stressed condition, $F(1, 31) = 2.857$, $p = .101$, $\eta p^2 = 0.084$. A Bayesian independent samples t-test found anecdotal evidence in favor of the null hypothesis of no devaluation effect in the Stressed group, $BF_{01} = 1.199$. Paired and Unpaired groups significantly differed in the Non-Stressed condition, $F(1, 31) = 8.808$, $p = 0.006$, $\eta p^2 = 0.221$.

Proportion of baseline food cup entry rates during the test are shown in Fig. 4D. In Group Non-Stressed, proportion of baseline food cup entry rates were lower in Paired compared to Unpaired animals. Food cup rates appeared to not differ in Group Stressed. Planned pairwise comparisons with Bonferroni corrections found that Paired and Unpaired groups differed in the Non-Stressed condition, $F(1, 31) = 12.223$, $p = 0.001$, $\eta p^2 = 0.283$, and not in Group Stressed, $F(1, 31) = 1.080$, $p = .307$, $\eta p^2 = 0.034$.

3.2.5. Consumption and reacquisition tests

The results of the consumption test confirmed the success of the outcome devaluation procedure. Paired groups rejected nearly all pellets ($M = 0.83$, $SD = 1.42$). Unpaired groups consumed all pellets offered ($M = 10$, $SD = 0$). In the reacquisition test, stress condition did not influence the observation that Paired groups clearly failed to reacquire the nose-poking for the sucrose pellet while the Unpaired reacquired nose-poking to circa-baseline levels (see Fig. 4E). There was a significant Devaluation effect, $F(1, 31) = 108.798$, $p < 0.001$, $\eta p^2 = 0.778$, and no effects involving stress group approached significance, $F's < 1$.

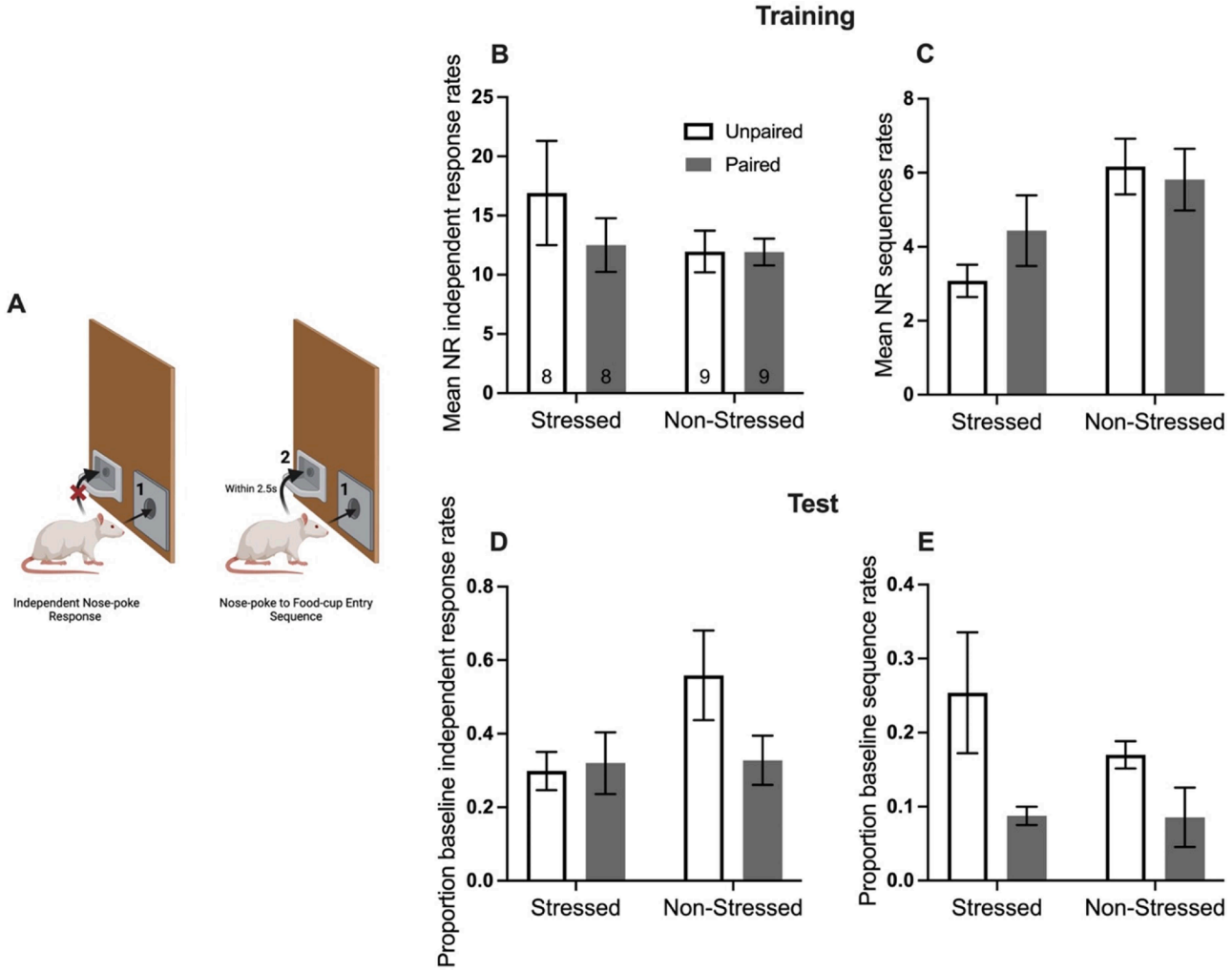

Fig. 3. Results of Experiment 1 response sequence analyses.
Note. A) Graphic depicting independent nose-poke responses and nose-poke to food-cup entry sequences. B) Mean nonreinforced (NR) independent nose-poke responses per minute during the final VI 30-s training session. C) Mean NR nose-poke to food-cup entry sequences per minute during the final VI 30-s training session. D) Mean independent nose-poke responses per minute during the test as a proportion of the independent response rates from the final VI 30-s training session (proportion of baseline). E) Mean nose-poke to food-cup entry sequences as a proportion of mean sequence rate in the final VI 30-s training session (proportion of baseline). Error bars denote standard error of the mean.

3.2.6. Response rate microstructure

Mean nonreinforced independent nose-poke rates in the final training session are shown in Fig. 5A. These rates did not differ between groups at this point in the experiment, F's < 1. As shown in Fig. 5B, there were also no differences in nonreinforced nose-poke to food-cup entry sequence rates, F's < 1.

Fig. 5C shows the proportion of baseline independent nose-poke rates per minute during the test. Independent nose pokes were lower in Paired compared to Unpaired Non-Stressed groups and supported by a Bonferroni-corrected planned comparison, $F(1, 31) = 4.183$, $p = 0.049$, $\eta p^2 = 0.119$. These rates did not differ significantly in the Stressed group, $F < 1$. The proportion of baseline nose-poke to food-cup entry sequence rate, shown in Fig. 5D, was lower in the Paired group in the Non-Stressed condition, $F(1, 31) = 10.550$, $p = 0.003$, $\eta p^2 = 0.254$. Devaluation did not influence sequence rates in the Stressed group, $F < 1$.

4. Discussion

Acute psychogenic stress resulted in habitual behavior, as evidenced by insensitivity to outcome devaluation, at a training level that otherwise maintained goal-directed behavior. In two experiments utilizing the outcome devaluation method and intact female rats, we found that acute restraint stress prior to training, and stress directly before the test both rendered a nose-poke response insensitive to outcome devaluation. These findings suggest two ways in which acute stress can alter the flexibility of decision-making. Experiment 1 suggests that acute stress facilitated habit learning, whereas Experiment 2 suggests that stress experienced after learning and just before testing may bias instrumental responding in female rats toward being expressed as habit.

Studies with human participants indicate that acute stress may shift control of instrumental behavior from goal-directed to habitual ([3,7,34]; see also [35,36]). Nonhumans studies have found evidence of habit following chronic stress [13,14] and acute stress [15] in males. To our knowledge the present study is the first to examine facilitation of habit by acute stress in female rats.

Although across-experiment comparisons should be interpreted with caution, it does appear that male and female rats may differ in sensitivity to the severity of the stress event. The present study found that 60 min of restraint stress facilitated habitual responding in female rats. In contrast, Braun & Hauber [15] found that males rats remained goal-directed after 60 min of restraint stress and habit was observed only after multiple stress exposures in male rats. Further work including direct comparisons of female and male rats under the same conditions is needed to assess a

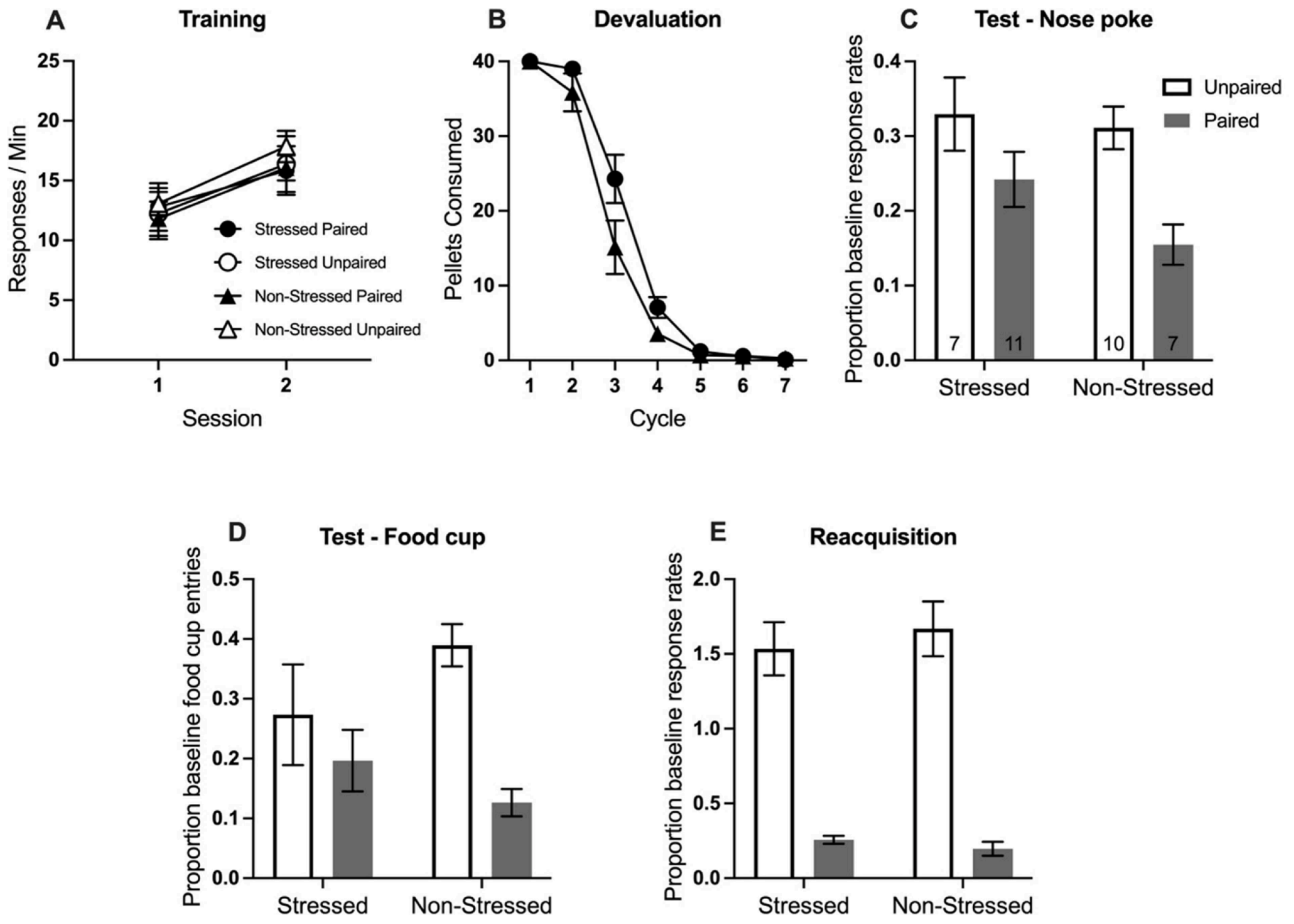

Fig. 4. Results of Experiment 2.

Note. A) Mean nose-poke rates (responses per minute) in the two VI 30-s training sessions. B) Mean number of sucrose pellets consumed by Paired condition animals during each cycle of the outcome devaluation phase. C) Mean nose-poke rates during the test as a proportion of nose-poke rate in the final VI 30-s training session (proportion of baseline). D) Mean food-cup entries per minute during the test as a proportion food-cup rates in the final VI 30-s training session. E) Mean nose-pokes per minute during the reacquisition test as a proportion of baseline. Error bars denote standard error of the mean.

potential sex difference.

Other work on sex differences suggests that female rats exhibit similar or greater corticosterone responses to restraint stress than males [17,37,38], as well as different effects of restraint stress on spatial [17] and Pavlovian memory (reviewed in [39]). These differences, along with ovarian hormone effects in females, may mediate the differential effects of stress upon these behavior systems in males and females. Indeed, studies of ovariectomized female rats suggest that the behavioral effects of acute stress can be reversed in the absence of ovarian hormones, and that corticosterone is not required to produce stress-induced learning deficits in females [19,40]. Similarly, recent evidence from this laboratory suggests that ovarian hormones are required for habit formation in female rats (unpublished data), and cyclic estrogen and progesterone are both required to facilitate this process [20]. Further research is needed to ascertain whether females are more prone to expressing habit following stress compared to males and whether such changes are mediated by gonadal hormones or adrenocortical reactivity.

Prior work has shown that, like other interoceptive states, stress may acquire a modulatory function over instrumental food-seeking behaviors [41,42]. That is, nose-poking to earn the sucrose pellet while stressed could allow rats to encode the stress state as modulating the relationship between nose-poking and pellets. We assessed this potential influence in Experiment 1. Here, rats in the Stressed group received VI 30 s training for the reward in the presence of stress (note all rats received initial response training under identical nonstressed conditions). Devaluation occurred under nonstressed conditions for all rats. Given prior exposure to sucrose pellets in a stressed state, it was possible for Stressed Paired rats to have learned that stress signaled nondevalued sucrose pellets. We assessed such a possibility by testing the consumption of sucrose pellets under stressed and non-stressed conditions. The results clearly suggest that stress did not influence the value of sucrose in the consumption test. Therefore, insensitivity to devaluation observed in the Stressed groups is not complicated the possibility of the value of the sucrose pellet being modulated by stress.

While such comparisons should be made with caution, it is potentially notable that different overall levels of responding were observed in the Stressed groups in Experiments 1 and 2. In Experiment 1, responding in the Paired and Unpaired Stressed groups was similar to the Paired Non-stressed group (Fig. 2C). Some work has suggested that the behavioral output of the animals during the test is a composite of both goal-directed and habitual components [25,43]. In this way, it is possible that stress before acquisition may have resulted in insensitivity to reinforcer devaluation by reducing responding in both groups. This interpretation is speculative given the lack of influence of stress on instrumental responding during the training sessions (Fig. 2A); the two groups were virtually identical. However, it remains possible that the effect of stress on instrumental control occurred during a post-training consolidation period or interfered with retrieval at the test. These results contrast with the those in Experiment 2, where stress before the test did not appear to produce an overall reduction in the Stressed groups. Altogether, these comparisons suggest directions that could clarify the possibly diverse influences of stress on instrumental behavior when

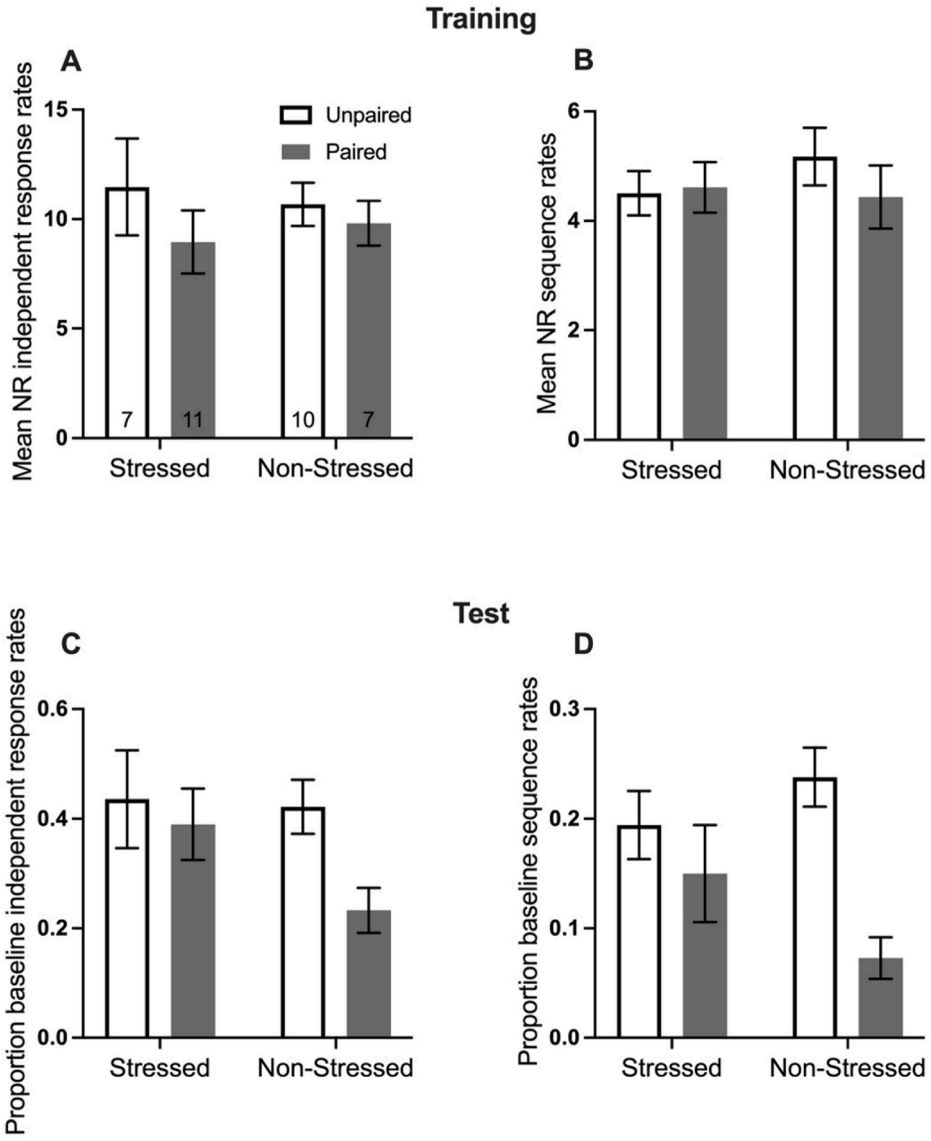

Fig. 5. Results of Experiment 2 response sequence analyses.
Note. A) Mean NR independent nose-poke responses per minute during the final VI 30-s training session. B) Mean NR nose-poke to food-cup entry sequences per minute during the final VI 30-s training session. C) Mean independent nose-poke responses per minute during the test as a proportion of baseline independent response rates from the final VI 30-s training session. D) Mean nose-poke to food-cup entry sequences as a proportion of baseline sequence rates from the final VI 30-s training session. Error bars denote standard error of the mean.

experienced before training versus before testing.

To further explore these possibilities, we compared the execution of discrete nose-poke responses and nose-poke-food-cup entry sequences [44–46]. Discrete reward-seeking responses not immediately followed by checking the food cup may be more adaptive under ambiguous reinforcement circumstances than seeking-retrieval sequences which are more resource costly [30]. On the other hand, in a reward rich environment (as in training) rats follow their reward-seeking response with an attempt to retrieve the reward more often. Previous research suggests that reinforcer devaluation can reduce performance of discrete seeking responses but spare seeking-retrieval sequences [30]. That is, following devaluation treatment, discrete actions may remain goal-directed while action sequences may be more apt to become habits [47]. Discrete reward-seeking responses and seeking-retrieval sequences may also be dissociable at the neural level [30,48].

Following this logic, in Experiment 1, if acute stress before training diminished goal-directed behavior, then Paired and Unpaired groups should not differ in their discrete nose-poking rates. Likewise, if stress had a weaker influence on the habitual component of responding, then nose-poke-food-cup sequence rate should remain sensitive to reinforcer devaluation. This is indeed what we found. When stressed prior to training, only the discrete nose-poke responses were insensitive to devaluation (Fig. 3d-e). Interestingly, this was not the case when acute stress occurred before the test of expression in Experiment 2. Here, both discrete nose-poke and nose-poke-food-cup sequences were insensitive to the devaluation treatment (Fig. 5c–d). Taken together, these results may suggest that stress before training impaired expression of goal-direction, whereas stress before the test enhanced habitual control. Further studies are needed to investigate the diverse influence of stress on aspects of instrumental behavior, particularly with female rats.

To summarize, two experiments with minimally-trained female rats found that acute stress administered either before instrumental conditioning or before the test resulted in the expression of habit in instrumental behavior, albeit by distinct mechanisms. These results may have important implications for understanding how stress may lead to behavioral inflexibility in disorders associated with compulsive

responding to stimuli. This knowledge could help us engineer interventions for reinstating goal-directed control, or instituting productive habitual control, and reducing problematic behaviors in these pathologies.

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The authors report there are no competing interests to declare.

CRediT authorship contribution statement

Russell Dougherty: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Eric A. Thrailkill:** Funding acquisition, Methodology, Supervision, Writing – review & editing. **Zaidan Mohammed:** Data curation, Investigation, Supervision, Writing – review & editing. **Sarah VonDoepp:** Investigation. **Ella Hilton-Vanosdall:** Investigation. **Sam Charette:** Investigation. **Sarah Van Horn:** Investigation. **Adrianna Quirk:** Investigation. **Adina Kraus:** Investigation. **Donna J. Toufexis:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

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