Acute psychophysiological stress impairs human associative learning

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Abstract

Addiction is increasingly discussed as a disorder of associative learning processes, with both operant and classical conditioning contributing to the development of maladaptive habits. Stress has long been known to promote drug taking and relapse and has further been shown to shift behavior from goal-directed actions towards more habitual ones. However, it remains to be investigated how acute stress may influence simple associative learning processes that occur before a habit can be established. In the present study, healthy young adults were exposed to either acute stress or a control condition half an hour before performing simple classical and operant conditioning tasks. Psychophysiological measures confirmed successful stress induction. Results of the operant conditioning task revealed reduced instrumental responding under delayed acute stress that resembled behavioral responses to lower levels of reward. The classical conditioning experiment revealed successful conditioning in both experimental groups; however, explicit knowledge of conditioning as indicated by stimulus ratings differentiated the stress and control groups. These findings suggest that operant and classical conditioning are differentially influenced by the delayed effects of acute stress with important implications for the understanding of how new habitual behaviors are initially established.

Keywords: associative learning, classical conditioning, operant conditioning, instrumental learning, reward learning, stress

1. Introduction

The ontology of addiction is often described as a series of associative learning processes (Everitt and Robbins 2005) involving both operant and classical conditioning. Operant conditioning is an active learning process that is initially driven by goal-directed behaviors involving actions leading to a rewarding outcome; however, over time the behavior becomes habitual and actions are performed irrespective of the outcome (Skinner 1938, Dickinson and Balleine 1994). In contrast, classical conditioning relies on passive learning of stimulus-outcome relations (Pavlov 1927). Addiction (e.g. drug use) is thought to be influenced by operant conditioning in the following way: Whereas initial drug use is driven by a voluntary goal-directed process reinforced by the rewarding properties of the drug, later stages of addiction are characterized by habitual and compulsive drug use that continues despite adverse consequences (Everitt and Robbins 2016). Pavlovian conditioning has been shown to interact with these operant conditioning processes through simple stimulus-outcome interactions, as drug-related cues predicting reward can enhance craving and compulsive tendencies observed in addicts. Thus, identifying the role of factors that facilitate initial operant and Pavlovian learning processes, which occur before habitual behaviors are established, is crucial for understanding individual variability in vulnerability to addiction.

Stress has long been known to be a major factor in the inception and development of addictive behavior, elevating drug self-administration and promoting relapse (Piazza and Le Moal 1998, Sinha 2008). Several human and non-human studies have demonstrated that habit formation, a key component in the emergence of addictive behaviors, is promoted by both chronic and acute stress (Koob 2008, Dias-Ferreira, Sousa et al. 2009, Schwabe and Wolf 2009, Graham, Yoon et al. 2010, Everitt and Robbins 2016). Building on these studies, research in humans has focused on effects of stress on favoring habitual over goal-related behavior. In a series of studies in human subjects, Schwabe and colleagues (2009, Schwabe and Wolf 2010) exposed participants to acute psychophysiological stress or a control condition either before or after operant training tasks. Participants in the stress group showed more persistent habitual performance even in the absence of reward both when stress was induced before and after contingencies were learned (Schwabe and Wolf 2009, Schwabe and Wolf 2010). A recent study (Pool, Brosch et al. 2015) further employed a Pavlovian-Instrumental Transfer (PIT) task to show that stress increases the craving for a rewarding outcome without affecting the pleasure of consuming it – an important characteristic of addiction (Everitt and Robbins 2016). The 3-stage

PIT task employed (Talmi, Seymour et al. 2008) taps three distinct processes implicated in habit formation. In the operant conditioning phase, the association between an action and reward is established via operant conditioning (Skinner 1938, Balleine 2011). In the second, Pavlovian learning phase, a passive association is made between a stimulus and reward. Finally, during the subsequent extinction phase, habitual or transfer behavior is measured by strength and persistence of instrumental action in response to the Pavlovian stimulus in the absence of reward. In the study by Pool et al. (2015), participants were exposed to an acute stress or a no-stress control condition after the learning phase. Here the stress group mobilized more effort in response to the now-unrewarded Pavlovian stimulus than the control group, which was interpreted as increased cue-triggered 'wanting' (Pool, Brosch et al. 2015). As this study focused on effects of stress on transfer, outstanding questions remain about effects of stress on learning processes that precede the establishment of habit, when simple associations between an action or a stimulus and a rewarding outcome are first acquired. Thus, the goal of the present study was to examine the effects of acute stress on the initial operant conditioning and Pavlovian conditioning stages of this 3-stage PIT task.

Based on previous research, there are a number of ways in which acute stress could influence initial reward learning. First, there is research suggesting that stress may have opposing effects on different phases of learning and transfer, reducing initial associative learning while enhancing reliance on habit once a habit has been formed. For example, a body of non-human animal literature suggests that stress reduces appetitive learning (Shors 2004, Pielock, Braun et al. 2013). Yet results in humans have been more equivocal. Schwabe and colleagues (2009) found no effect of stress on initial learning of probabilistic contingencies for different rewarding stimuli; however, additional evidence provided some preliminary indication that stress might have a detrimental effect (Schwabe and Wolf 2009). If stress has opposing effects on learning, given previous findings that stress enhances habit formation (Schwabe, Tegenthoff et al. 2010, Schwabe and Wolf 2011, Pool, Brosch et al. 2015), we would expect it to impair initial associative learning processes.

One reason for inconsistent findings with regard to effects of stress on learning may be that its effects on learning and memory do not depend only on the learning phase. They are also markedly influenced by the timing of the stressor relative to learning [for review see (Joels, Pu et al. 2006)]. An acute stressor activates two stress systems: 1) Immediate activation of a fast-acting stress system leads to a release of mostly catecholamines such as norepinephrine and dopamine.

Activation of this system facilitates cognitive processes at the time of stress induction [for review see (Schwabe, Wolf et al. 2010)]. 2) With a delay of up to one hour after stress induction, glucocorticoids (cortisol in humans) activate a gene-mediated pathway leading to an elevated processing threshold for incoming information (Herman, McKlveen et al. 2012). In other words, cognitive processes such as learning and memory are suppressed during this period (Kirschbaum, Wolf et al. 1996, de Quervain, Roozendaal et al. 1998). For consistency with the Pool et al. (2015) study, we aimed to examine effects of delayed stress on associative learning. As activation of the glucocorticoid pathway suppresses learning, we would again expect operant and Pavlovian learning processes to be suppressed by delayed stress.

Third, stress may not only differentially affect distinct stages of habit learning, but may also have different effects on learning rate and reward sensitivity as two independent components of reward-based learning (Huys, Pizzagalli et al. 2013). Previous research focusing on effects of stress on depression-related anhedonia suggests a detrimental effect of stress on reward responsiveness linked to learning - at least in some participants. When used as a stressor, threat of shock has been found to reduce preference for a high probability over a low-probability reward (Bogdan and Pizzagalli 2006). Other studies have observed such a pattern of reduced reward responsiveness under stress *only* in participants high in stress reactivity (Berghorst, Bogdan et al. 2013) or behavioral inhibition (Cavanagh, Frank et al. 2011). Yet, notably, the opposite pattern of improved reward responsiveness has been observed in those low in behavioral inhibition (Cavanagh, Frank et al. 2011). Thus, we also aimed to examine effects of stress on both learning rate and reward sensitivity.

Taken together, previous studies suggest that the effects of acute stress on reward learning depend on the learning phase (acquisition vs transfer), the relative timing to the stressor (immediate vs delayed) as well as the reward learning component (learning rate vs reward sensitivity). Thus, the goal of the present study was to investigate the effect of *delayed* stress on initial stages of active operant and passive Pavlovian learning using a task that allows us to assess reward sensitivity. In particular we wished to determine the effects of stress on formation of associations that are distinct from, but contribute to, habitual behavior as operationalized in human PIT tasks (Talmi, Seymour et al. 2008, Pool, Brosch et al. 2015). For this reason, we examined effects of acute stress on behavior in the operant and classical conditioning tasks that comprised the first two stages of the 3-stage human PIT task described above (Talmi, Seymour et al. 2008). These tasks are distinct from those employed in many studies of operant conditioning

in that the associations learned are simple and learning occurs very rapidly (Talmi, Seymour et al. 2008, Pool, Brosch et al. 2015). For example, the association of an action and reward is learned after the first few encounters — very much as when a drug is taken for the very first time and the associated pleasurable experience is remembered immediately. Another advantage is that it allows us to investigate the willingness to exert physical effort rather than simply testing cognitive abilities. This is central to our goal of examining reward sensitivity because it allows us to measure how much work participants are willing to put into the task given a certain reward and whether this is affected by stress.

In the present study, two separate experiments investigated effects of acute stress on operant and Pavlovian learning as in (Pool, Brosch et al. 2015). In Experiment 1a and 1b healthy undergraduate students performed a simple operant conditioning task in which they learned to squeeze a hand-grip to obtain a low (Experiment 1a) or high (Experiment 1b) monetary reward (Talmi, Seymour et al. 2008). In Experiment 2 participants performed a simple Pavlovian learning task in which colored fractal patterns were associated with monetary reward. Both procedures were performed either following acute psychophysiological stress or in a stress-free control condition. For stress induction, participants were exposed to the commonly employed socially evaluated cold pressor test (SECPT) (Schwabe, Haddad et al. 2008, Pool, Brosch et al. 2015). We hypothesized that the delayed effects of acute stress during the first encounter of an action-outcome contingency would a) decrease the effort and frequency with which the behavior is performed to obtain that reward (that is reward sensitivity is reduced), and b) influence the extent of appetitive Pavlovian learning.

Experiment 1

2. Materials and Methods

2.1 Participants

Prior to data collection, a power analysis was performed in order to determine the number of subjects. Assuming an effect size of $\eta^2 = .15$ based on previous research (Pool, Brosch et al. 2015) and a repeated measures ANOVA, approximately 190 participants were necessary. A sample size of at last 200 allows for attrition, hence data collection was continued until the end of the academic term in which the minimum was reached.

214 participants (155 females, mean age: 21.59 ± 3.63 years) took part in Experiments 1a and 1b (102 and 112 participants respectively). All participants were compensated for their

participation by course credit. Participants were asked not to eat, consume alcohol or caffeine and exercise two hours before the experiment. Testing was completed between 9AM and 6PM (Table 1). Participants were randomly assigned to stress and control conditions (103 and 111 participants respectively). The study was approved by the Human Research Ethics Board of the University of British Columbia.

2.2 Materials

2.2.1 Stimuli and apparatus. For all stimulus presentation, the MATLAB (The MathWorks, Natick, Massachusetts, USA) toolbox Cogent 2000 was used.

2.2.2 Operant Conditioning. The visual stimuli viewed in this experiment were images of a thermometer with a real-time changing mercury level displayed on a gray background on a computer screen to indicate grip force and an image of a Canadian quarter to indicate reward (Fig 1). A handgrip apparatus was connected to a grip-force transducer (Powerlab, AD Instruments, Colorado Springs, CO, USA) that converted grip pressure into a voltage output. Variation in compression by the handgrip resulted in a voltage signal that was proportional to the force exerted. The dynamic value of the recorded signal provided participants with a real-time visual feedback that reflected the force on the handgrip, which was displayed as the "mercury" level moving up and down within the thermometer on the screen. Grip strength data (LabChart, AD instruments) was measured and stored in Newton (N).

2.2.4 Questionnaires. Participants were asked to complete a battery of questionnaires in order to control for possible interactions between psychopathology, life experience, and personality with task performance and stress response. In addition to a demographics questionnaire, we administered the Childhood Trauma Questionnaire (CTQ) (Bernstein, Fink et al. 1994), the State-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch et al. 1983), Beck Depression Inventory (BDI) (Beck, Ward et al. 1961), and the Big Five Inventory (BFI) (Benet-Martinez and John 1998).

2.3 Procedure

2.3.1 Overview. After obtaining written informed consent, we acquired initial saliva samples and blood pressure readings for baseline measures of physiological indicators of stress. This was followed by the SECPT in either the stress or control condition (Fig 2). To observe physiological reactions during stress induction we initiated continuous heart rate recording at the

beginning of the SECPT. The three-minute stress induction procedure was followed by immediate blood pressure measurements and the second cortisol sample. Successful stress induction was further assessed by the administration of the SECPT questionnaire – a three-item questionnaire measuring the subjective stress response (Schwabe, Haddad et al. 2008). Participants were further asked to fill out a battery of questionnaires in order to control for individual differences that may influence stress response or operant conditioning performance. The operant task started 25 minutes after the end of the SECPT allowing cortisol to reach peak levels (Schwabe, Haddad et al. 2008). Heart rate recording was stopped at this point as it is typically influenced by physical activity required for the operant task. After participants finished the task, blood pressure was measured for the last time and the third and last cortisol sample was taken. If participants did not complete all questionnaires in the 25-minute period before the learning phase, they finished them before the debriefing.

2.3.2 Stress procedure. In the stress condition, elevated stress levels were induced with the socially evaluated cold-pressor test (SECPT) (Schwabe, Haddad et al. 2008). First, participants were informed that their faces would be videotaped during the upcoming test for future evaluation of their facial expressions by researchers. Participants were then asked to put their dominant hand in ice water (0 – 4 °C) up to the wrist. They were told to keep the hand in the water for as long as possible while looking straight into the camera. The experimenter observed the participant at all times and recorded the time period during which each participant's hand remained in in the water. After 3 minutes participants were instructed to remove their hands from the water if they had not done so before. In the control condition the ice water was replaced by warm water (35 – 37 °C) and participants were neither videotaped nor watched by the experimenter. They were likewise instructed to keep their hand in the water and the experimenter made sure to look otherwise occupied.

2.3.3 SECPT questionnaire. To obtain a measure of subjective, psychological stress response we asked participants to rate how stressful, painful and unpleasant the SECPT was using a ten-point scale ranging from 1 ("not at all") to 10 ("extremely").

2.3.4 Heart rate. Heart rate was measured using LabChart software (AD Instruments) based on a finger pulse that was continuously measured with a pulse transducer (AD Instruments). In order to determine a baseline, heart rate was averaged within three subsequent one-minute time windows. Similarly, heart rate was measured throughout the three minute lasting stress procedure and averaged separately for three one minute time windows (Fig 2).

2.3.5 Blood pressure. Systolic and diastolic blood pressure were measured using a blood pressure monitor. Measurements were taken pre SECPT, post SECPT and post task. Data is missing for the first 30 participants.

2.3.6 Salivary cortisol analysis. Saliva was collected pre SECPT, post SECPT and post task with a Salivette collection kit (Sarstedt AG & Co., Nümbrecht, Germany) and stored at -20 ^{o}C until the biochemical analysis of salivary levels of free cortisol. Analysis employed a luminescence immunoassay (IBL GmbH, Hamburg, Germany) performed by the lab of Prof. Dr. C. Kirschbaum, Dresden, Germany. Inter- and intra-assay variations were below 10 %.

2.4 Experiment 1a: Operant Conditioning Task

The operant conditioning paradigm was adapted from a Pavlovian Instrumental Transfer (PIT) test described in Talmi et al., (2008). In this procedure, participants learned to squeeze a handgrip in order to get a monetary reward (Fig 1). Because we wished to directly examine an earlier phase of the Pavlovian to instrumental transfer process indexing effects of stress on habit reliance tapped by Pool et al., (2015) we designed our operant conditioning task to be equivalent to the operant conditioning task used in that previous study. Another advantage of this design is that it allows us to measure willingness to perform physical effort to obtain a reward. This is distinct from operant conditioning tasks that rely on learning stimulus contingencies, which largely depend on cognitive abilities. Because the task is so simple, it can be performed equally well by all participants, ensuring that differences in performance are due to effort rather than differences in cognitive ability. This allowed us to evaluate reward sensitivity as we were able to measure how much effort participants were willing to exert for the given reward.

Participants were told that they could earn CAD 0.25 per successful grip in this operant conditioning task and that they would be given at end of the experiment in addition to the reimbursement for their participation. In a training trial, participants were asked to familiarize themselves with the handgrip. The grip force was visualized in real time by the level of the mercury displayed on the screen (Fig 1). Moreover, their maximum grip force was determined as criterion for their response during the main operant task. The training phase was followed by 24 operant conditioning trials each of which lasted 12 s with a 4 - 12 s fixation period as an intertrial interval (average duration 8 s). For each 12 s trial, participants were asked to squeeze the handgrip with their non-dominant hand to bring the mercury to its maximum and down again. They were told that there were up to three rewarded time windows. If they happened to reach

near maximum grip force, they would gain CAD 0.25 and a coin was displayed. It was emphasized that they should decide intuitively when to squeeze the handgrip and that the displayed coins represent a real monetary reward. In fact, there were always two rewarded time windows each lasting 1 s. Participants had to reach either 50 % or 70 % of their individual maximum grip force in the rewarded time windows in order to get the reward. The criterion for the maximum force changed every second to reduce predictability.

2.5 Experiment 1b: Operant Conditioning with high reward

A follow-up experiment was conducted to determine whether effects of stress on operant conditioning was due to reward sensitivity. In this study we used an identical procedure to that described above, with the exception that a higher rate of reward (CAD 1.00 per successful grip) was introduced.

2.6 Statistical Analysis

Two 24 x 2 mixed analyses of variance (ANOVAs) with trial as within- and stress group as between-subject factor were employed to independently test for effects of stress on operant conditioning in Experiment 1a and 1b. In a combined analysis a 24 x 2 x 2 mixed ANOVA was applied to the operant conditioning data with trial as within-subject factor and group (stress and control) and reward condition (low and high reward) as between-subject factors. Physiological data (heart rate, blood pressure and cortisol) were analyzed in a mixed ANOVA with time as within- and group (stress and control) as between-subject factors. All analyses were additionally performed with time of day - dichotomized as morning (testing between 9AM and 1PM) and afternoon (testing between 1PM and 6PM) – as a covariate. Greenhouse-Geisser corrections were applied if sphericity was violated. All analyses were performed with IBM SPSS Statistics 21.

3. Results

3.1 Control Variables

Exploratory correlations examining the relation between task performance and personality measures, state and trait anxiety, depression and childhood trauma did not reveal significant results. Furthermore, stress and control group did not differ with regard to age, sex, time of day, ethnicity and average levels of depression and anxiety (Table 1).

3.2 Stress manipulation

3.2.1 Experiment 1a

The effect of stress induction was assessed by both subjective ratings and physiological measures such as heart rate, blood pressure and cortisol.

On average, participants in the stress group kept their hands in ice water for 162.64 ± 42.93 s, and participants in the control group kept their hands in water for 175.00 ± 23.98 s. Subjective stress ratings (Table 2) confirmed that, compared to the control group, participants in the stress group perceived the SECPT as more stressful, $t(69.07) = 8.08$, $p < .001$, painful, $t(50.18) = 14.96$, $p < .001$, and unpleasant, $t(90) = 9.84$, $p < .001$ than participants in the control group.

3.2.1.1 Heart rate. Analysis of heart rate (including a baseline measurement and recordings during the three minute stress induction) revealed a main effect of time, $F(1.87, 162.41) = 8.73$, $p < .001$ as well as a time by stress group interaction, $F(1.87, 162.41) = 5.48$, $p = .006$. Post hoc tests using Bonferroni correction showed that in the stress group, heart rate significantly increased in minute 1, $p < .001$, and minute 2, $p = .001$, of the stress test relative to baseline. Thus, only the stress group showed a stark increase in heart rate as a result of stress induction (Table 2).

3.2.1.2 Blood pressure. For systolic blood pressure the analysis revealed a main effect of time, $F(2, 126) = 8.17$, $p < .001$, showing that systolic blood pressure dropped after the SECPT in both groups.

3.2.1.3 Cortisol. The analysis of cortisol showed a main effect of time, $F(1.22, 63.50) = 4.81$, $p = .010$, as well as a time by stress group interaction, $F(1.22, 63.50) = 17.12$, $p < .001$. Post-hoc comparisons revealed that cortisol levels measured 50 minutes after stress induction were significantly elevated relative to pre-stress measurements in the stress, $p = .001$, but not in the control, $p = .252$, group. The direct comparison of stress and control group further showed that cortisol levels are significantly higher in the stress group 50 minutes after stress induction, $p = .002$. In conclusion, peak cortisol levels measured 50 minutes after stress induction were significantly elevated only in the stress group demonstrating the effectiveness of the stress induction.

3.2.2 Experiment 1b

Participants in the stress group kept their hands in ice water for 155.12 ± 49.05 s. All participants in the control group kept their hands in water for the maximum of 180 s. Participants in the stress group perceived the SECPT as more stressful, $t(61.38) = 9.23$, $p < .001$, painful, $t(50.96) = 16.93$, $p < .001$, and unpleasant, $t(89.03) = 5.87$, $p < .001$ than participants in the control group indicating the success of stress induction as measured subjectively.

3.2.2.1 Heart rate. The analysis of heart rate showed a main effect of time, $F(2.41, 195.10) = 4.76$, $p = .003$ as well as a time by stress group interaction, $F(2.41, 195.10) = 9.56$, $p < .001$. Post hoc tests using Bonferroni correction revealed that in the stress group, heart rate significantly increased in minute 1, $p < .001$, and minute 2, $p = .016$, of the stress test relative to baseline. Thus, as in Experiment 1a only the stress group showed an increase in heart rate due to stress induction (Table 2).

3.2.2.2 Blood pressure. For systolic blood pressure the analysis revealed a time by stress group interaction $F(2, 216) = 3.07$, $p = .048$. Post hoc comparisons showed a marginal difference in the stress group between time points 2 and 3, $p = .055$. Significant differences between stress and control group were visible before stress induction, $p = .039$, as well as 50 min after, $p = .012$. The analysis of diastolic blood pressure showed a time by stress group interaction, $F(2, 216) = 5.11$, $p = .007$. Post hoc analyses showed that in stress group there was a drop in diastolic blood pressure from the time of the SECPT to 50 minutes after, $p = .005$. Moreover, the control group had significantly higher blood pressure than the stress group at the end of testing, $p = .001$. While the pattern of results is different from Experiment 1a, the difference in blood pressure 50 minutes after stress induction is likely to be attributed to factors other than the SECPT. It might be the result of completing the task and is not likely to reflect the activation of the fast-acting stress system.

3.2.2.3 Cortisol. As in Experiment 1a, analysis of cortisol revealed a time by stress group interaction, $F(1.54, 168.19) = 3.41$, $p = .035$. Post-hoc comparisons showed that stress and control group were marginally different at baseline, $p = .082$, as well as right after stress induction, $p = .080$. They further revealed that cortisol levels in the control group dropped (presumably due to circadian rhythm) while cortisol levels in the stress group increased 50 min after stress induction demonstrating a change in cortisol levels due to stress induction.

In summary, while not all indicators of the fast-acting stress system reflect successful stress induction, cortisol levels indicate that delayed effects of acute stress were present at the time of testing.

352

353 3.3 Behavioral Results

354 3.3.1 Experiment 1a: Operant Conditioning

355 In order to determine whether stress and control group differed in degree of operant
356 conditioning, the number of handgrips reaching 50 % or more of the participant's maximum grip
357 strength (Talmi, Seymour et al. 2008, Pool, Brosch et al. 2015) was compared between groups.

358 A mixed ANOVA revealed that irrespective of experimental condition, all participants
359 readily learned to squeeze the handgrip in the first few trials: The analysis revealed a main effect
360 of trial, $F(8.62,861.47) = 4.03$, $p < .001$, such that grip frequency increased with the progression
361 of the experiment. Crucially there was a main effect of stress group, $F(1, 100) = 7.34$, $p = .008$,
362 indicating overall fewer grips in the stress relative to the control group (Fig 3a). This set of
363 findings suggests that while action-outcome relations were learned instantaneously in both
364 groups, acute stress led to a reduction in grip rate possibly due to reduced willingness to work for
365 the reward.

366

367 3.3.2 Experiment 1b: Operant Conditioning with high reward

368 To ensure our findings did not simply reflect lack of motivation with low levels of
369 reward, we aimed to replicate the main findings with higher levels of reward. As a follow-up to
370 Experiment 1a, Experiment 1b employed 4x higher reward levels with a new set of participants.
371 Again a main effect of trial, $F(7.47,821.99) = 2.55$, $p = .011$, indicated that all participants
372 learned how to perform the task immediately. Moreover, a main effect of stress group, $F(1, 110)$
373 $= 8.52$, $p = .004$, again indicated reduced response rates under stress (Fig 3b). Thus, we were able
374 to replicate the main findings from Experiment 1a in an independent sample.

375

376 3.3.3 Experiment 1 a and b combined analysis

377 We further wished to examine whether the reduced response rate in Experiment 1a
378 reflected reduced reward sensitivity. Because the pattern of behavioral results was equivalent
379 across studies 1a and 1b, we combined the results from both studies and included reward level as
380 a between-subjects factor. A mixed ANOVA with trial as within as well as stress group and
381 reward condition as between-subject factors was employed to assess the effects of all factors and
382 their interaction. The analysis revealed a main effect of trial, $F(8.53,1790.20) = 5.16$, $p < .001$,
383 showing increasing grip frequency over the course of the experiment in all groups. There was a

main effect of stress group, $F(1, 210) = 14.32$, $p < .001$ indicating overall fewer grips in the stress relative to the control group. Importantly, there was also a main effect reward condition, $F(1, 210) = 4.81$, $p = .029$, indicating fewer grips in the low relative to the high reward condition (Fig 4). There was no interaction between stress and reward level, $p > .2$. In summary, those under stress and those working for lower reward similarly demonstrated reduced willingness to work for reward immediately following initial learning, consistent with predictions that stress reduces reward sensitivity.

In order to control for any effects of testing at different times of the day, the above reported analyses of behavioral data were also performed with time of day as a covariate. No significant interactions with time of day were observed (ps > .320) and the pattern of significant results did not differ from those presented above.

Experiment 2

4. Materials and Methods

4.1 Participants

63 participants (48 females, mean age: 20.27 ± 3.04 years) completed enough trials for behavioral analyses. Nine participants were excluded due to insufficient task completion. All participants were compensated for their participation by course credit for undergraduate psychology courses. Participants were asked not to eat, consume alcohol or caffeine and exercise two hours before the experiment. Testing was completed between 9AM and 6PM (Table 1). Participants were randomly assigned to stress and control conditions (25 and 38 participants respectively). The study was approved by the Human Research Ethics Board of the University of British Columbia.

4.2 Materials

4.2.1 Pavlovian Conditioning. Stimuli were comprised of visual images of green, blue or purple fractal patterns displayed on a computer screen. These were randomly paired with sounds of cello, flute and trumpet to create three compound Pavlovian stimuli. The three compound stimuli were randomly selected to serve as CS+, CS- or baseline conditions. Monetary reward was indicated by presenting a Canadian quarter in the middle of the screen (Fig 1).

4.2.2 Questionnaires. See section 2.2.3.

4.3 Procedure

After obtaining written informed consent, we acquired initial saliva samples and blood pressure readings. This was followed by the SECPT in either the stress or control condition (Fig 2). To observe physiological reactions during stress induction we initiated continuous heart rate recording at the beginning of the SECPT. The three-minute stress induction procedure was followed by blood pressure measurements, a cortisol sample and subjective stress ratings. The task started 25 minutes after the end of the SECPT allowing cortisol to reach peak levels (Schwabe, Haddad et al. 2008). Heart rate was continuously recorded. After participants finished the task, blood pressure and cortisol were tested one more time. For a more detailed description of the stress procedure and indicators of the stress response, see 2.2 Procedure for Experiment 1.

4.4 Classical Conditioning Task

Each participant completed 36 'task on' blocks with 4 s intertrial intervals or 'task off' blocks, during which the baseline stimulus was presented. The 'task off' or baseline period serves as a control condition for gathering initial likeability ratings not affected by reward expectations. Each 12 s 'task on' block was either a CS+ or a CS- trial characterized by the continuous presentation of the Pavlovian compound stimulus. Each 12 s block consisted of three 4 s time window each of which started with the random onset of the presentation of a gray patch, the cue (Fig 1). Participants were instructed to press a key to remove the patch in order to see whether it was hiding a reward. Participants were further told that the cue appeared three times per trial leaving to up to three possible rewards. In contrast to the operant task, participants were well aware of the fact that their action, i.e. the button press, had no influence on the outcome. No action was required during 'task off' blocks. Conditioning was assessed by reaction time in CS+ and CS- trials as well as likeability ratings of CS+, CS- and baseline stimulus.

4.5 Statistical Analysis

A mixed analysis of variance (ANOVA) was applied to the reaction time data with trial and CS type (CS+ and CS-) as within-subject factor and group (stress and control) as between-subject factor. Stimulus ratings were analyzed with a mixed design ANOVA with stimulus type (CS+, CS- and baseline) and stress group as factors. Physiological data (heart rate, blood pressure and cortisol) were analyzed in a mixed ANOVA with time as within- and group (stress and

control) as between-subject factors. All analyses were additionally performed with time of day - dichotomized as morning (testing between 9AM and 1PM) and afternoon (testing between 1PM and 6PM) – as a covariate. Greenhouse-Geisser corrections were applied if sphericity was violated. All analyses were performed with IBM SPSS Statistics 21.

5. Results

5.1 Control Variables

Exploratory correlations examining the relation between task performance and personality measures, state and trait anxiety, depression and childhood trauma did not reveal significant results. Furthermore, stress and control group did not differ with regard to age, sex, ethnicity and average levels of depression and anxiety.

5.2 Stress manipulation

The effect of stress induction was assessed by both subjective ratings and physiological measures such as heart rate, blood pressure and cortisol.

Participants in the stress condition kept their hand for 145.20 ± 54.70 s in ice water, while all participants in the control group kept their hand in water for 180 s. In addition, participants in the stress group perceived the SECPT as more stressful, $t(33.09) = 5.74$, $p < .001$, painful, $t(27.49) = 9.45$, $p < .001$, and unpleasant, $t(61) = 5.70$, $p < .001$ than participants in the control condition (Table 2).

5.2.1 Heart rate. The analysis revealed a main effect of time, $F(3, 135) = 21.78$, $p < .001$ (Table 2) indicating that both groups showed an increase in heart rate as a result of the SECPT.

5.2.2 Blood pressure. No significant differences between stress and control group were found, $p > .2$ (Table 2).

5.2.3 Cortisol. The analysis of salivary cortisol (Table 2) revealed a time by condition interaction, $F(1.20, 73.29) = 10.12$, $p < .001$. Post-hoc comparisons show such that the control group showed a significant drop in cortisol levels at the end of the experiment, $p = .001$, whereas cortisol levels in the stress group remain unchanged ($p = .574$). Thus, while under control conditions cortisol levels dropped presumably due to circadian rhythm, this effect was not detected in the stress group since the stress induction might have counteracted the observed drop.

Taken together, physiological indicators of acute stress do not deliver enough evidence to conclude that the fast-acting stress system was activated as a result of the SECPT, but differences

in cortisol levels allow us to conclude that differences in cortisol levels were present at the time of testing, which was the intended effect.

5.3 Classical conditioning

In this experiment participants were asked to complete a total of 36 trials (18 CS+, 18 CS- trials in randomized order). However, most participants failed to respond in one or more trials, leaving the majority of participants with at least 14 completed trials for each condition. Thus, for the analysis, the first 14 completed trials for each condition (CS+, CS-) were taken from each individual and subjected to a mixed design ANOVA in order to compare response times in CS+ and CS- trials between participants under stress and control conditions.

The analysis revealed a main effect of trial, $F(9.10, 555.26) = 3.76$, $p < .001$, showing that reaction times decreased over the course of the experiment (Fig 5). Crucially, there was a CS type (CS+ and CS-) by stress interaction, $F(1, 61) = 10.67$, $p = .002$. Post-hoc comparisons revealed that participants in the stress condition were slower to respond to CS+ relative to CS-, $p = .003$. No effect was observed in the control group ($p = .184$). Thus, appetitive classical conditioning was affected by delayed acute stress induction such that typically observed reaction time indices of conditioning were reversed by stress.

Subjective ratings of likability for experimental stimuli were also examined. Here there was a main effect of stimulus type, $F(2, 112) = 21.11$, $p < .001$, such that all participants liked CS+ stimuli better than baseline stimuli, and liked both stimuli better than the CS- fractal pattern after conditioning (Fig 5). This confirms that conditioning did indeed occur in both groups. There was also an effect of stress group, $F(1, 56) = 4.79$, $p = .033$, such that participants in the stress group had higher likeability ratings relative to the control group. There was no significant stimulus type by group interaction ($p = 0.31$). This opposing pattern of results for likeability ratings and behavioral response could suggest that these two indicators of conditioning measure different aspects of learning (e.g. outcome vs cue directed learning).

Again to control for potential effects of time of day on learning, all of the analyses reported above were also performed with time of day included as a covariate. Once again, no significant interactions between time of day and other factors were observed (ps > .692) and the pattern of significant results did not differ from that reported above.

Taken together the behavioral results suggest that despite the fact that both stress and control group did experience a conditioning effect, as evidenced by stimulus ratings, overall

response times were markedly slowed under delayed acute stress. Such findings indicate a dissociation between effects of stress on implicit relative to explicit measures of Pavlovian learning.

6. Discussion

The aim of the current study was to investigate the influence of delayed acute stress on simple appetitive associative learning processes in humans. Results showed that stress administered by means of the SECPT reduced operant responding as well as behavioral indices of Pavlovian learning. While the ability to learn contingencies in the operant task was unaffected by stress, following stress induction participants were overall less willing to work for a reward than they were in the no-stress control condition, and this was true regardless of whether participants received higher or lower levels of reward. In the no-stress condition, comparison of high and low reward showed that, in the absence of stress, participants were also less willing to work when the amount of reward was substantially lower. Furthermore, in the Pavlovian conditioning study likeability ratings indicated that both stress and control groups similarly developed explicit emotional associations. Yet the stress group showed an opposing behavioral pattern such that response times were faster in response to unconditioned relative to conditioned stimuli.

Our operant task results revealed that overall stress reduces the willingness to work for a reward at a very early stage of habit formation, providing novel evidence that such early stages are susceptible to the detrimental effects of stress. Our study was designed to assess such effects of stress in relation to findings from a previous study (Pool, Brosch et al. 2015). In the study by Pool and colleagues (2015), after performing equivalent operant and classical conditioning tasks to those we employed, participants were presented with Pavlovian stimuli while performing the operant task in extinction. Results revealed that, in the stress relative to the control condition, participants were more likely to show *increased* responding (i.e. number of handgrips) when presented with the CS+. The authors concluded that under stress people are more prone to rely on habitual behavior irrespective of the rewarding value of the outcome. That is, once habits are established, craving a reward guides participants' behavior - an effect that is enhanced by stress. In contrast, our examination of the operant conditioning phase of the task allowed us to probe effects of stress on the establishment of instrumental responses. Such associations are required for the subsequent habitual transfer of Pavlovian associations to operant responding measured by Pool and colleagues (2015). Our findings support the conclusion that, whereas stress may

increase reliance on existing habits, initial stages of habit formation driven by the reinforcing properties of the reward are negatively affected by stress.

Another line of research has emphasized the notion that acute stress promotes the switch from goal-directed to habitual behavior (Schwabe and Wolf 2011) For that purpose, operant paradigms are used in which an initially rewarded action is trained until a habit is established, i.e. participants keep completing the action despite a lack of reinforcement or devaluation of the outcome. (Schwabe and Wolf 2009). Critically, this shift from initial goal-directed or reward-oriented behavior towards habitual responding is facilitated by acute stress (Schwabe and Wolf 2009, Schwabe and Wolf 2010). In contrast, in the present study, we measured behavior that was not overtrained to the point that habits were strongly established. Thus, whereas previous studies provide evidence for reduced behavioral flexibility under stress, as indicated by reduced goal-directed behavior after devaluation, our findings further suggest that stress reduces reward-oriented behavior or the willingness to work for a reward before habit formation can occur — at least in a simple task where learning is very rapid.

Our manipulation of reward value revealed a pattern of results consistent with research suggesting that stress reduces reward sensitivity — at least in susceptible individuals (Bogdan and Pizzagalli 2006, Cavanagh, Frank et al. 2011, Berghorst, Bogdan et al. 2013). We assessed reward sensitivity by not only manipulating stress but also investigating effects of reward value. We suggest that, as the reduction in operant responding observed with stress mirrored that observed with lower levels of reward, the unwillingness to work for reward under stress may reflect reduced reward sensitivity. Theories of depression propose that stress induces an anhedonia-like state – an effect known as *learned helplessness* (Overmier & Seligman 1967; Shors & Dryver 1992). As a condition characterized by decreased reward sensitivity and motivation to pursue rewards, learned helplessness has been used as an animal model for depression (Klein, Fencil-Morse et al. 1976). While previous animal studies induced inescapable, traumatic shock, the current results are consistent with human literature showing effects that are not restricted to uncontrollable, traumatic stress (Bogdan and Pizzagalli 2006).

It should be noted in this study we employed a very simple operant conditioning task. Here learning was instantaneous, and no stress-related differences in learning rate were observed. This had both advantages and limitations. Our task not only allowed us to compare our findings to those of previous studies, but our measure of willingness to work for reward was not confounded by individual differences in the ability to learn complex reward contingencies. The

simplicity of the task also effectively models common situations in which human learning is instantaneous and the action-outcome relation is encoded after the first encounter (e.g., experiencing pleasant effects of a novel drug on the first encounter). In this way we were able observe the effects of stress on this type of salient instantaneous learning, with implications for understanding how stress may contribute to trajectories toward habitual drug taking. However, further studies should employ a more difficult learning task that manipulates reward contingencies, allowing assessment of stress on learning rates over time.

The results of the classical conditioning task further revealed a dissociation between explicit responses and behavior: Likeability ratings indicated successful learning of reward associations in both stress and control groups. However, response times were slower for CS+ than CS- trials under stress. In contrast, no difference between CS+ and CS- was observed in controls, suggesting that only implicit measures of conditioning were influenced by acute stress. Our results are consistent with findings in non-human animals indicating that, in classical conditioning, effects of acute stress on implicit learning are dissociable from effects on explicit learning processes (Shors and Servatius 1997). Another possible interpretation of the data can be found in the animal literature on individual differences in associative learning (Flagel, Akil et al. 2009): *Goal-trackers* prioritize rewarding outcomes without developing emotional associations with the CS+. In contrast, *sign-trackers* develop strong emotional associations with the cues signaling the reward, even at the cost of interest in the rewarding outcome (Hearst and Jenkins 1974). In the current study, we can speculate that acute stress induction made participants more likely to act like sign-trackers, who give more weight to the associated cue and less to the rewarding outcome. Future research should be conducted to investigate sign- and goal-tracking in humans especially under the influence of environmental factors such as stress.

The pattern of results observed here (i.e. reduced operant responding) may depend in part on the timing of the associative learning tasks in relation to the acute stressor. In the present study, we employed a delay following the stress induction to capitalize on effects of glucocorticoids on behavior. Non-human animal research has suggested that stress typically enhances learning whether training begins immediately after stress induction or with a delay (Shors, Weiss et al. 1992, Servatius and Shors 1994), although this finding has not been found be generalizable to all stressor types or tasks and also depends on the sex of the animal (Shors 2004). Research in humans suggests that acute stress impairs explicit learning mediated by glucocorticoid action, while learning is enhanced when it occurs in close temporal proximity to

608 the stressor, a process that is thought to be mostly driven by norepinephrine (NE) (Joels, Pu et al.
609 2006). Recently, studies demonstrated that glucocorticoid action via mineralcorticoid receptors
610 (MR) is critical for a shift from hippocampus-based 'cognitive' to dorsal striatum-dependent
611 'habit' learning strategies [for review see (Vogel, Fernandez et al. 2016)]. In line with that, the
612 present findings suggest that goal-directed or 'cognitive' behaviors were impaired under
613 glucocorticoid driven delayed stress effects. An important follow-up to the present study will
614 involve investigating effects of stress when learning occurs directly after stress induction to
615 differentiate the effects of glucocorticoid and NE activation and to demonstrate the involvement
616 of the LC-NE system in more (complex) forms of reinforcement learning. Norepinephrine is not
617 only a key modulator of the stress response, but the locus coeruleus norepinephrine (LC-NE)
618 system is also known to be generally activated in response to salient or
619 emotionally/motivationally relevant stimuli (Aston-Jones and Bloom 1981, Bouret and Sara
620 2002). Despite these facts, the influence of the LC-NE system on reward and reinforcement
621 learning has been largely neglected (Weinshenker and Schroeder 2007). Recent investigations
622 however, provide evidence for a link of the LC-NE system and reward-based learning (Bouret
623 and Richmond 2009, Bouret and Richmond 2015, Sadacca, Wikenheiser et al. 2016) as well as
624 for the role of stress and the NE system in the flexible development of habits (Wirz, Wacker et al.
625 2017).

626 In both experiments, for a number of different measures including psychophysiology
627 (heart rate, blood pressure), cortisol and subjective parameters, significant stress group
628 differences indicated that the stress manipulation was successful. Nonetheless it should be noted
629 that heart rate and blood pressure measurements were not available for the time of stress
630 induction, which is the time when differences would be expected to be largest. Yet the fact that
631 differences were observed even after the stress induction suggests that these differences were
632 present during the SECPT. The same holds true for the cortisol samples taken right after stress
633 induction as well as an hour after (at the end of the experimental procedure). While we did not
634 assess peak cortisol ~25min after SECPT, elevated levels by the end of task completion indicate
635 that cortisol levels were elevated during behavioral experiments. Moreover, heart rate and blood
636 pressure changes due to stress induction were not visible in all Experiment 2 indicating that the
637 fast-acting stress system might not have been activated or alternatively that the measurements
638 were not able to capture those changes due to timing. However, group differences in cortisol

levels were present in all experiments suggesting that the effects of delayed stress targeted in the present study were in effect.

In conclusion, the current study showed that delayed effects of acute stress reduce operant responding presumably due to reduced reward sensitivity as one aspect of reinforcement learning. Further, stress prevented the translation of learned emotional associations into reward-oriented behavior. Thus, consistent with what is known from stress and learning research, it seems that appetitive learning processes subsequently leading to the establishment of new habits, are suppressed for a certain period after stress induction, an effect thought to be driven by glucocorticoid processes. These findings add to our understanding of the influence of stress on early stages of habit formation relevant for the development of addictive behaviors. Future research will be necessary in order to show whether immediate, NE-driven stress effects enhance reward-based learning promoting the establishment of maladaptive habits and relapse related to addiction.

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762 Tables

763 **Table 1.** Mean and standard error for demographics as well as personality measures,
764 depression, state and trait anxiety, depression and childhood trauma. Time of Day was
765 dichotomized as 'morning' (M) with testing before 1pm and 'afternoon' (A) with testing
766 after 1pm. No frequency differences (demographics) between groups or significant
767 correlations ($p < 0.05$) with task performance were found.

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	Experiment 1a		**Experiment 1b**		**Experiment 2**	
	Control	**Stress**	**Control**	**Stress**	**Control**	**Stress**
Demographics						
Age	21.0±0.4	21.3±0.5	22.2±4.4	21.1±3.7	19.9±2.5	20.7±3.4
Sex (% female)	77 %	71 %	72 %	69 %	76 %	80 %
Time of Day (% M)	51 %	39 %	42 %	58 %	45 %	33 %
Ethnicity (% Asian)	69 %	59 %	60 %	69 %	62 %	77 %
Big Five Inventory: Personality						
Openness	3.6±0.5	3.4±0.6	2.8±0.1	2.8±0.1	3.0±0.1	3.3±0.1
Conscientiousness	3.5±0.5	3.4±0.7	3.0±0.1	3.1±0.1	3.9±0.8	3.3±0.1
Extraversion	3.1±0.7	3.1±0.7	3.0±0.1	3.1±0.1	3.2±1.0	3.2±0.1
Agreeableness	3.8±0.5	3.6±0.5	2.8±0.1	2.7±0.1	3.1±0.1	4.0±0.1
Neuroticism	2.9±0.8	3.0±0.8	2.8±0.1	2.9±0.1	3.4±0.9	3.1±0.1
Beck's Depression Inventory (BDI)						
Depression	9.3±1.1	10.1±1.4	8.7±1.2	10.5±1.4	11.5±1.3	12.7±2.3
State-trait anxiety inventory (STAI)						
State anxiety	38.3±1.6	42.8±1.5	35.9±1.3	37.7±1.5	37.6±1.6	40.0±2.2
Trait anxiety	39.2±1.6	43.9±1.6	43.1±1.4	43.0±1.6	44.5±1.8	44.0±2.5
Childhood Trauma Questionnaire (CTQ)						
Emotional Abuse	7.6±0.5	8.4±0.5	9.5±0.6	7.8±0.4	7.3±0.4	9.3±1.2
Emotional Neglect	9.3±0.6	8.9±0.6	10.4±0.6	9.2±0.7	8.9±0.5	9.7±0.9

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778 **Table 2.** Subjective stress ratings, heart rate (beats per minute), systolic and diastolic
779 blood pressure and cortisol in Experiment 1a (operant conditioning, low reward),
780 Experiment 1b (operant conditioning, high reward) and Experiment 2 (classical
781 conditioning) in Control and Stress group. [1] indicates significant differences between
782 stress and control group, [2] indicates significant differences between time points.
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	Experiment 1a		Experiment 1b		Experiment 2	
	Control	**Stress**	**Control**	**Stress**	**Control**	**Stress**
Ratings						
Stressful	1.7±0.2	4.8±0.3[1]	1.4±0.1	5.0±0.4[1]	1.6±0.2	4.9±0.5[1]
Painful	1.1±0.1	6.3±0.3[1]	1.0±0.1	6.8±0.3[1]	1.2±0.1	6.1±0.5[1]
Unpleasant	4.4±0.3	8.3±0.3[1]	3.4±0.3	9.3±0.2[1]	2.5±0.4	6.5±0.5[1]
Heart Rate [BPM]						
Baseline	76.4±3.4	67.7±3.7	76.3±1.5	74.5±1.8	74.3±1.7	73.7±2.3
SECPT Min 1	79.0±3.6	85.2±3.9[2]	75.2±1.5	79.8±2.0[2]	79.5±1.7[2]	81.4±2.3[2]
SECPT Min 2	76.7±4.2	83.3±4.5[2]	76.0±1.6	78.1±2.0[2]	79.9±1.8[2]	80.5±2.5[2]
SECPT Min 3	74.1±4.0	76.4±4.3	76.1±1.6	75.8±2.0	80.6±1.8[2]	79.0±2.5[2]
Systolic BP [mm/Hg]						
Pre SECPT	116.6±2.8	117.2±2.7	118.8±2.2	112.2±2.3[1]	109.8±2.3	108.5±2.7
Post SECPT	110.4±2.6[2]	112.9±2.6[2]	115.1±2.2	113.4±2.4	106.2±2.0	107.2±2.3
Post Task	115.8±2.5	114.8±2.5	116.5±2.1	108.8±2.2[1,2]	108.9±2.3	107.4±2.8
Diastolic BP [mm/Hg]						
Pre SECPT	79.0±1.5	77.4±1.5	76.1±1.3	74.8±1.4	75.0±1.4	74.6±1.7
Post SECPT	77.7±1.6	78.3±1.6	76.8±1.2	75.8±1.3	72.3±1.4	74.5±1.7
Post Task	79.7±1.4	78.7±1.4	77.7±1.1	72.4±1.1[1,2]	74.4±1.3	75.2±1.5
Cortisol [nmol/l]						
Pre SECPT	6.7 ±0.9	5.3± 0.9	6.5±0.6	4.9±0.6[(1)]	7.4±0.8	5.4±0.9
Post SECPT	6.1±0.7	4.8±0.7	6.0±0.5	4.7±0.5[(1)]	7.1±0.7	5.2±0.9
Post Task	5.1±0.8	9.0±0.9[2]	5.6±0.5	5.6±0.6	4.6±0.7[2]	6.0±0.8

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792 Figures

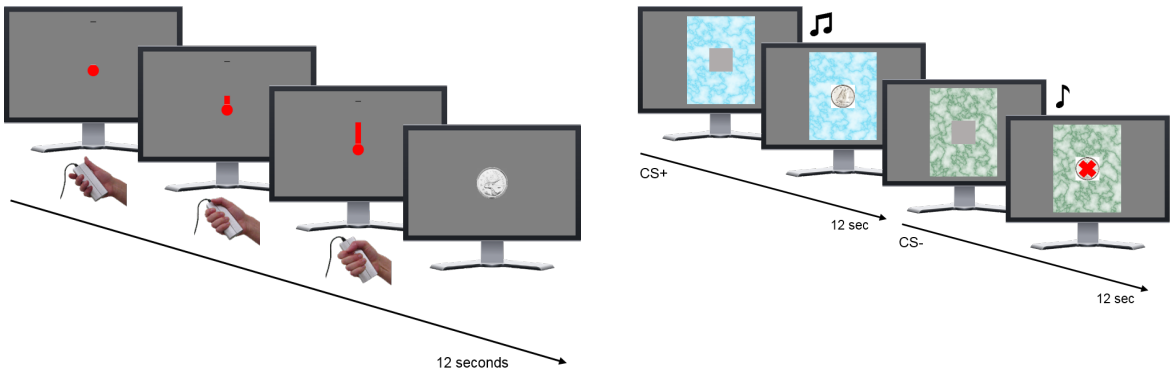

Figure 1. Overview of experimental design for operant (left) and classical (right) conditioning task. In Experiment 1, the operant conditioning task, participants squeezed a handgrip to get a monetary reward. In Experiment 2, the classical conditioning task, participants learned to associate compound stimuli (fractal pattern and tone) with reward or no reward.

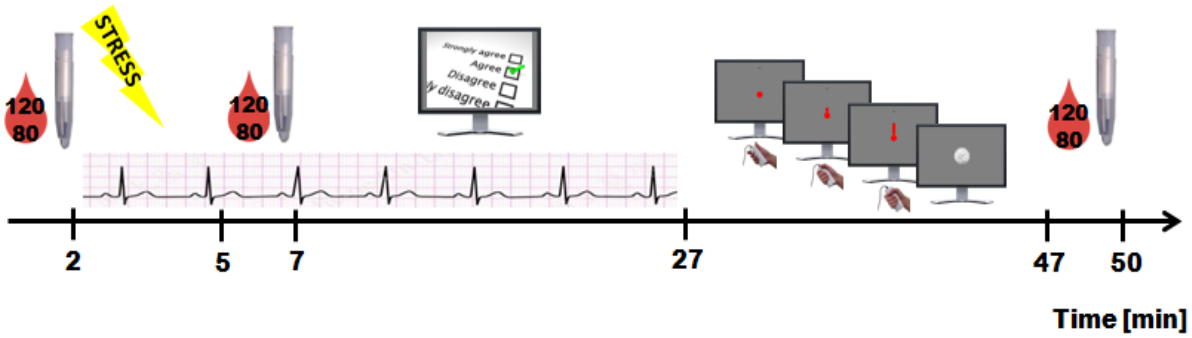

Figure 2. Overview of experimental procedure. Blood pressure and cortisol samples were taken before and after stress induction by means of the socially-evaluated cold pressor test (SECPT). Heart rate was continuously measured throughout the three minute stress test as well as while answering questionnaire. Twenty minutes after stress induction, the operant or classical conditioning task was performed followed by final blood pressure and cortisol samples.

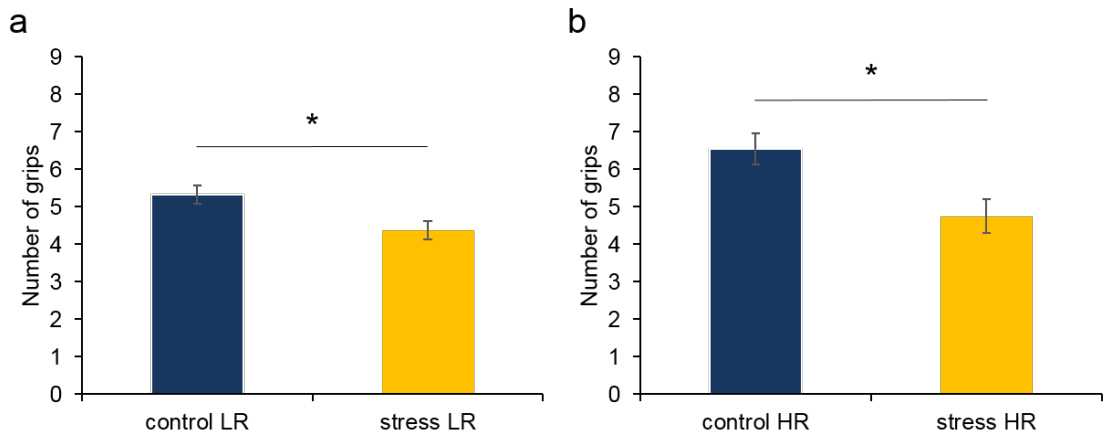

Figure 3. Operant conditioning results displayed separately for Experiment 1a (LR = low reward) and Experiment 1b (HR = high reward). The results show that acute stress induction reduced overall number of grips under both a) low reward and b) high reward conditions. Error bars indicate standard error of the mean. Asterisks indicate significance differences between stress and control group.

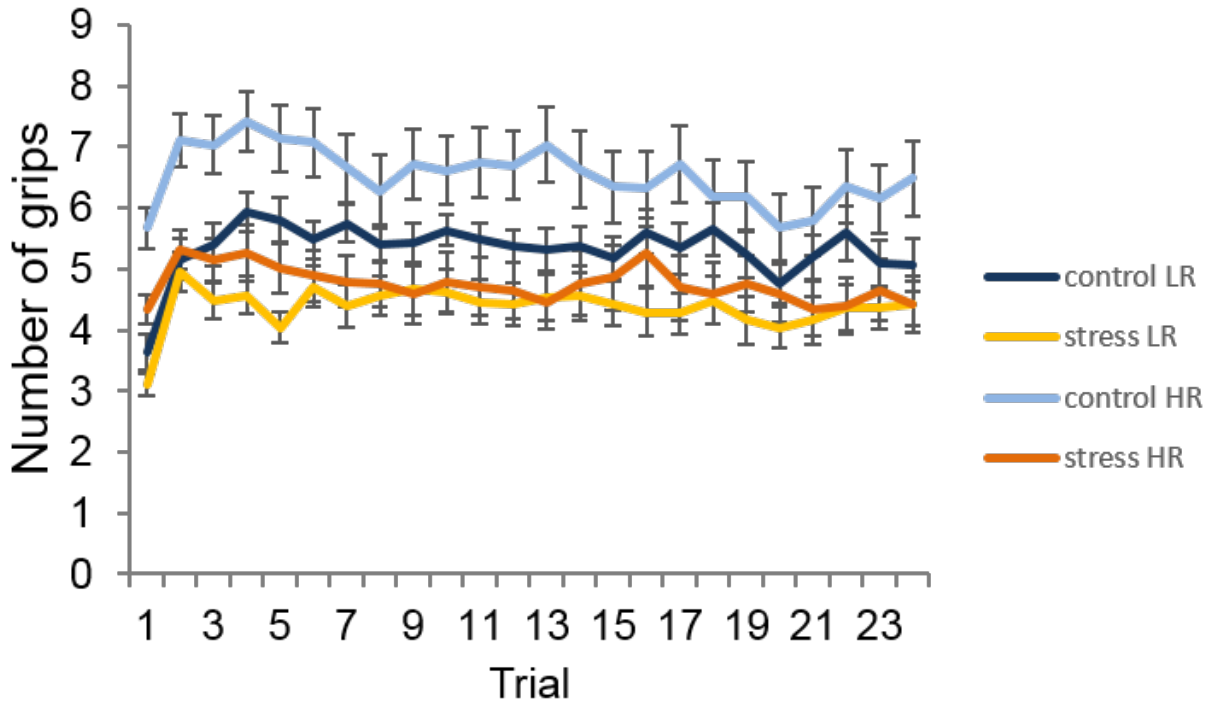

Figure 4. Operant conditioning (Experiment 1) results displayed separately for control and stress group as well as for low reward (LR) and high reward (HR) groups show that mean number of grips reaching criterion force is reduced by acute stress induction and reduction of reward. Error bars indicate standard error of the mean.

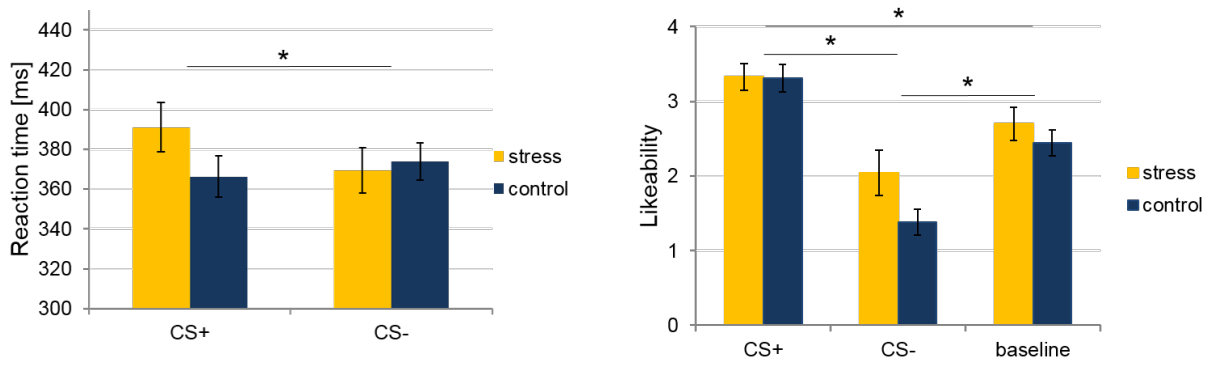

Figure 5. Results of classical conditioning (Experiment 2) study show reduced reaction time in CS- relative to CS+ trials under acute stress. Likeability ratings suggest successful conditioning in stress and control group with overall higher ratings under stress. Error bars indicate standard error of the mean. Asterisks indicate significance differences.