The role of the prelimbic cortex in controllability-dependent stress-induced changes in effortful reward-seeking behavior

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Abstract

Humans are often faced with making decisions to exert effort and how this exertion can affect our motivation to perform these tasks. This cost-benefit analysis can be disrupted by feelings of stress that may hinder our ability to make these decisions, which can often lead to negative consequences including mental health disorders. Previously, it was seen that the prelimbic (PL) region was involved in modulating the behavior during effort tasks and for stressor controllability. By investigating what regions of the brain are able to modulate the behavioral effects following stress and our decision to expend effort, it can be significant to assist the population in mitigating the negative health outcomes. The present study was designed in order to evaluate the effects of stress on motivated reward-seeking behavior, and also assess the role of the prelimbic cortex in stress-related resiliency during effortful reward-seeking tasks. Male Sprague-Dawley rats were utilized in a weight lifting task with three groups consisting of escapable stress (ES), inescapable stress (IS), and homecage (HC) animals. We hypothesized that stressor controllability in the ES animals would protect them from a decrease in motivated behavior which was supported by the present results. A two-bottle choice test was also done in order to clarify that stress did not alter sucrose preference which was seen through the results that after ES and IS, rodents still preferred sucrose over water. We hypothesized that stimulating the PL region with DREADDS would reinstate the weight lifting behavior of the IS rats and create similar benefits to the ES group. The results did not support this and there was no protection from IS experience of weight lifting behavior in the animals. However, this data is still able to provide valuable insights into the regions and circuitry that may modulate stressor controllability-dependent changes in motivated reward-seeking behavior.

Introduction

As humans, we routinely experience how anticipating that we'll have to expend effort can reduce our motivation for otherwise rewarding outcomes. We may enjoy going to class but not studying for exams, or we may find value in going to work and obtaining an income but not getting assigned difficult tasks. Cost-benefit decision-making maintains the ease for us to continue to make decisions in our daily routine. However, this cost-benefit decision-making can be corrupted by unexpected experiences of stress. For example, the prioritization of education and work shifted early in the COVID-19 pandemic, and many individuals had difficulty coping with the uncertainty that suddenly plagued everyday life. Without the ease of understanding the costs, the ability to comprehensively analyze a decision is lost, and this can lead to negative outcomes. Stress may hinder the ability of humans to make appropriate decisions causing worse life outcomes, poor job performance, dissatisfaction in our work, and ultimately leading to many different health disorders including depression or anxiety (Frye, 2013). However, these negative outcomes from stress may be mitigated if we are able to feel a sense of control, which can protect us from the negative effects that stress has on effort-based decision-making.

I. Role of effort in motivated behavior

Effort can be physical, such as exercise intensity, or cognitive, such as algebra. In either definition, effort pertains to the extra exertion of work required to engage in a demanding task (Westbrook & Braver, 2015). However, the amount of work itself is not associated with being effortful. Effort is predominantly believed to be aversive, as it stimulates the sympathetic nervous system and often produces anxiety and frustration (Peters et al., 1998). The choice to engage in an effortful task, therefore, requires motivation, the driving force of behavior. Motivated "reward-seeking" refers to behaviors exerted towards obtaining rewards, such as

money or food. When faced with a decision between two actions providing the *same* reward outcome, behavioral economic theory and the principle of least effort (Hardy, 1982) suggest that humans and animals will choose the less effortful action. The ability that a reward has to motivate behavior decreases as the effort required to obtain it increases: a phenomenon known as "effort discounting". However, motivation is coupled with the concept of effort insofar as an increase in motivational value (for example, greater monetary reward) may lead to the exertion of more effort. Alternatively, if a reward is less motivating, it is likely that less effort will be exerted. As a result, effort can reliably discount how much motivation an animal is willing to exert to obtain rewards which allows us to estimate effort costs based on the behaviors the animals choose to perform under different effort and reward combinations.

Learning how to modulate behavioral strategies based on anticipated effort costs is conserved across species, and a number of behavioral models have been used to demonstrate these effects in rodents and provide insight into their neural correlates. One of the most commonly used methods to assess effortful decision-making is a lever-pressing task that assesses effort by using either a fixed ratio, in which rodents must press the lever a set amount of times or a progressive ratio, where the number of lever presses for a reward increases as time continues (Schweimer & Hauber, 2005). As the ratio of presses to rewards increases throughout the task, effort is thought to increase, and, at some point, animals reach a "breakpoint" in which they stop responding, presumably due to the effort cost being too high. A different effortful behavioral task is a T-maze barrier choice task. Here, a rodent is presented with the choice between getting a lower value reward on one side and a higher value reward on the opposite side. However, a barrier needs to be climbed over in order to reach the side with the higher value reward. In order

to increase the workload or the effort that needed to be put in, the barrier could be raised in height in order for the rodent to reach the reward (Salamone, 1994).

These previous models of quantifying effort have limitations in their methods that make it difficult to isolate effort as the variable. For both the barrier task and lever-pressing task, as the height of the barrier increases or the amount that needs to be pressed on the lever increases, there is the confounding variable of time between the task and the reward. For both, the amount of time it takes to complete the required amount of lever presses or overcome the height of the barrier will affect how long it takes to receive the actual reward. A newer model of effortful behavior is the weight-lifting task. Here, animals lift weights using a pulley system; every time they successfully pull the weight off the ground sufficiently, a reward tone plays and sugar is delivered to the food cup. Altering the weight, as opposed to the distance, that animals must lift, will isolate the role of physical effort from confounds such as time delays until reward delivery (Lang, 2020). The quantification of the amount of weight being pulled can be related to the expenditure of effort in the rodent models.

These three tasks have previously been used to delineate the neural correlates of effortful behavior, with research predominantly implicating the prefrontal cortex (PFC). In rodents, the PFC generally refers to the prelimbic cortex (PL), infralimbic cortex, and the anterior cingulate cortex (ACC). It has been previously proposed that the anterior cingulate cortex (ACC) is present in the modulation of effortful decision-making (Devinsky, 1995). However, PFC research has struggled to clearly define the boundaries of the three regions from each other in rodents (Laubach et al., 2018). Some recent effort work referring to ACC as cingulate gyrus area 1 (Cg1) demonstrated differential Cg1 firing to low- and high-effort behaviors (Hillman & Bilkey, 2010), and low-frequency Cg1 stimulation as sufficient to decrease effort on a weight-lifting task (Silva

et al., 2021). However, the involvement of the ACC appears to be specific to effort spent on specific tasks, namely barrier-crossing and weight-lifting but not progressive ratio lever-press. When the effort expenditure is gradually increasing over time, the prelimbic cortex (PL) appears to be necessary. Specifically, lesioning the PL lowers the breakpoint during progressive ratio testing (Walton, 2003). Collectively, these data suggest that the PFC is involved in the choice to engage with effortful reward-seeking behaviors, but PFC subregions, though experimentally difficult to parse apart in rodents, may hold specialized roles within that process.

II. Effects of stress on motivated behavior

More than half of the adults in the United States have claimed to suffer from the negative effects of stress (APA, 2020). The traumatic events experienced by stressed individuals can have profound effects on behavior and can decrease the motivation that people have to engage in activities they previously found rewarding. Exposures to mild stressors will often lead to a decrease in responsiveness to rewards and result in anhedonic behavior, one of the symptoms following stress and depressive disorders. Anhedonia is characterized by the inability to feel pleasure and is often behaviorally represented by reduced interest in physical activities (Willner et al., 1992). Anhedonic behavior is often associated with psychological comorbidities including depression and substance abuse. By having this decreased interest in something that one used to find rewarding, it increases the vulnerability to substance use initiation, regular usage, and then addiction through negative reinforcement (Destoop et al., 2019). These effects that are associated with anhedonic behavior can lead to societal problems such as an increase in substance abuse or addiction which is detrimental to the individual's health.

Translational models of stress-induced anhedonia have attempted to explain this loss of involvement in activities that were previously considered rewarding and pleasurable. For

example, in rodents, anhedonia is often associated with a reduction in the consumption of sucrose compared to water in a two-bottle choice task (Katz, 1982). Models of stress, varied in their intensity and chronicity (Liu et al., 2018) have been shown to reduce sucrose preference, and antidepressant treatment has been shown to restore that preference (Liu et al., 2015), highlighting the potential translational value of the model. However, given the ease at which rodents have access to sucrose in this task, sucrose consumption does not require much motivation. Sucrose preference over water may reflect the higher hedonic value of the sucrose, or how much the animals 'like' the sucrose (Meyerolbersleben et al., 2020). The subjective experience of 'liking' the sucrose is a unique process from 'wanting' in the incentive salience theory of motivated behaviors(Morales & Berridge, 2021). 'Wanting' sucrose implies that sucrose and sucrose-associated cues become attractive enough to drive motivated behaviors. Indeed, manipulations of reward-related neural circuitry alter these two systems separately. For example, opioid agonism in the nucleus accumbens (NAc) shell lowers 'wanting' but not hedonic 'liking' (Peciña & Berridge, 2005). The two-bottle choice task may be a better measure of how much animals "like" sucrose, while the extent to which this test can be used to measure "wanting", or demand for sucrose, is limited. While these tasks have been used to demonstrate the effects of stress on reward-associated neural systems, such as the midbrain dopamine system (Hollon et al., 2015), more demanding tasks are necessary to understand the effects of stress on prefrontal cortical control over motivated decision-making. For example, various uncontrollable stressors have been shown to diminish prefrontal control over neural firing in the striatum, and this stress-induced neural deficit was associated with changes in risky, reward-seeking behaviors within a T-maze choice task (Friedman et al., 2017).

While there is immense value in characterizing the pathways underlying stress-induced motivation deficits, traumatic experiences do not always lead to the development of anhedonia or aberrant decision-making. Many find ways to cope with stress and gain control over its long-term outcomes. The process of learning to control stress can actually produce resilience against future stressors (Maier & Watkins, 2010). In contrast, the so-called "learned helplessness" phenotype characterized by such features as anhedonia is predominantly imparted by experience with uncontrollable stress. Uncontrollable stress will create passivity in the organism that experiences it because there is a lack of ability to control the stressor or handle the face of trauma (Seligman, 1972). This sub-type of stressor dramatically predominates the translational stress literature.

In order to evaluate the effect of controllability on stress in rodents, a mild tail shock paradigm can be used known as "escapable stress/ inescapable stress", or ES/IS. Here, two animals are "yoked" to one another such that any shock one receives, the other receives, making their physical experience of stress (shock intensity and duration) identical. However, one of the rodents serves as the inescapable stress (IS) animal while the other rodent that the first is yoked to serves as the escapable stress (ES) animal. During ES, the rodent can terminate the shock by turning a wheel, which also stops the shock for the yoked IS animal. This creates a sense of controllability within the ES animals, while the IS animal has no such perception of control. This model has previously been used to demonstrate that IS will produce learned helplessness, which will create a decrease in responsiveness and anhedonic behavior that is most commonly seen in individuals suffering from chronic stress (Maier and Watkins, 2005). Alternatively, ES-experienced animals are protected from these negative outcomes.

Research facilitated by the ES/IS model of stressor controllability has enriched our understanding of the neural correlates of resilience. The prelimbic cortex (PL) is associated with stress resilience during times of anxiety or depression (Jing et al., 2021). Stressor controllability activates the PL, and this activation is necessary for trans-situational protection against stress-induced behavioral deficits (Amat et al., 2005). Consistent with this, inactivating the population of PL neurons that project to the dorsal raphe nucleus (DRN) prevents the protective effects seen in ES animals, demonstrating the necessity of this pathway for generating resilience and protection against anhedonia. As previously discussed, the PFC also monitors and evaluates effortful behavior to create a bias within the decision-making (Porter et al., 2019). Collectively, this overlapping neural circuitry between stress and effortful behavior suggests that alterations in PL activity during stress may facilitate subsequent changes in effortful reward-seeking. Given that subregions of the PFC may be involved in discrete aspects of motivated behavior, PL activation during ES may similarly modulate different aspects of decision-making. Therefore, the present study examines the effects of ES/IS on both sucrose preference and effortful behavior. Moreover, we address with preliminary data the potential involvement of the PL in stress-mediated changes in behavior.

To assess whether this region is also important for controllability-related changes in motivational effort, the experiment chemogenetically alters prefrontal activity using DREADDS, or Designer Receptors Exclusively Activated by Designer Drugs (Ferguson, 2014). This vector-driven approach can be used to selectively target populations to express a unique receptor (here, hM3D(Gq)) that can only be activated by an exogenous compound called clozapine-*N*-oxide (CNO) when injected into the animal. This allows for selective stimulating of cells in the brain without having off-target effects on other cells, even in the same brain region.

This system can be used to specifically excite glutamatergic neurons in the prelimbic cortex, and this methodology has previously been used to reversibly modulate neural activity for two to six hours.

There is significant evidence that suggests that a lack of control over a situation (as in IS) can elicit susceptibility to future stressors, but that having control over that stressful situation (as in ES) can provide resiliency to future stressors. However, less is known about the effects of IS and ES and how they may alter non-stressful future situations including the expenditure of effort on motivational tasks. To test this, I use stressor controllability to assess whether IS induces anhedonia in a rewarded motivation task, and further, whether ES experience will prevent this anhedonic response. Using the rope pulling effort task described above, the rats were trained to pull a rope attached to increasing amounts of weight to earn sugar reward pellets. After this, rats were assigned a single session of ES, IS, or no stress (home-cage) controls. Following the stressful experience, the rats were returned to the testing chamber and allowed to pull the rope again under different weights to assess whether they are less motivated to expend effort to gain rewards than prior to the controllability experience. We hypothesized that IS will induce deficits in both sucrose preference and effortful, motivated behavior assessed via the weight-lifting assay. We furthermore anticipated that ES will protect against these stress-induced changes in behavior.

In the second study for this thesis, I assessed the sufficiency of the PL region for this effect. Prior work has demonstrated that inactivating the PL and the PL-DRN pathway can prevent the rats from gaining stress resilience from an ES experience. However, there is nothing known about the opposite situation: can stimulating the PL in an IS experience induce changes in DRN activity that would confer resilience to the rat in subsequent motivational situations? If this were to happen, the present study would be the first demonstration of PL being sufficient to

induce resilience in animals after an IS stressful experience. In order to test this, we infused the PL with either an AAV coding for the excitatory DREADD hM3DGq-EYFP or a control AAV coding only for the reporter (EFYP). The rats were trained as in the first study on the rope pulling task. However, for this second study, 30 minutes prior to IS, the rats were given an injection of CNO, which will activate PL neurons in the "on" but have presumably no effects in controls. I then tested these animals on the rope task post-IS experience to assess how this altered motivation, and whether the same neuronal mechanisms that mediate the detection of behavioral control may, in turn, modulate motivational outcomes. In summary, we predicted that activation of the PL using chemogenetics during the experience of IS will protect animals against IS-induced anhedonia and therefore produce similar behavioral outcomes to the ES experience.

Methods

Rodent Model

Sprague Dawley rats were used as the rodent model all weighing around 300-330g. Following instructions from the Institutional Animal Care and Use Committee (IACUC), animals were individually housed and kept in a controlled vivarium. They experienced a 12-hour light-dark cycle (lights on at 0700 hr). The experiments took place during the light cycle. During periods with no food restriction, water and rat chow (ENVIGO, Indianapolis, IN) were available to the rats *ad libitum* prior to the start of the weight lifting experiments. Rats were provided with enrichment (red tubes for hiding and paper twists) in the cages. During periods of food restriction, rats maintained no less than 95% free feed body weight and were provided 15-20g of rat chow in the home cage. Procedures done on rats were in accordance with IACUC at CU-Boulder protocols.

Stressor Controllability "Escapable/Inescapable Stress" Training

Two yoked rats were placed in separate compartments, and they were subjected to identical numbers and intensities of shocks (100 1mA tail-shocks every 60-s (±30-s). One rat per pair was able to terminate the tail-shock by turning a wheel (1-4 full turns, with the requirement increasing every 2 successful trials until the maximum of 4 turns is reached) which terminated the shock for both rats. For each pair of rats yoked, a third rat remained in the home cage and unhandled to serve as a no-stress control.

Two Bottle Choice Test

Rats were food-restricted but not water-restricted for the duration of this experiment. During the one-day acclimation period, animals were given overnight (dark phase) access to a 5% sucrose solution, during which time standard water bottles were removed. 48 hours later, 4 days of pre-testing began to establish a baseline sucrose preference. In the rat's home cage, two 50mL bottles were placed into the cage: one filled with water and the other a 5% sucrose solution, made fresh daily. Bottles were weighed prior to and after a 4 hour testing period. Bottle location was counterbalanced daily, and testing occurred approximately 8 hours into the inactive period. The following day, animals underwent ES or IS training, or were deemed home-cage (HC) controls. 24 hours later, post-tests began, occurring on days 1, 2, 3, 7, and 14 post-stress. Sucrose preference was calculated using the equation:

$$Sucrose\ preference\ = \frac{(Sucrose\ Consumed)}{(Total\ Liquid\ consumed)} \times\ 100$$

Weight Training

Rats went through magazine training for 1-2 days where pellets were delivered to the food cup as rats became familiar with banana sugar pellets. Rats were Pavlovian conditioned for 5 days to associate a song with food rewards. The pellets were delivered randomly as the song played to cue the reward and allow it to become a conditioned reinforcer. After, the rope was introduced to the behavioral box and fitted with a 40g weight 2.5 inches away from the sensor. The training proceeded for 7 days to shape the rat to pull the rope and as the weight with a flag hit the sensor, the conditioned reinforcer played along with dispensing the reward.

Weight Lifting

One end of the rope on the weight lifting system leads into the operant chamber for around 2 inches. On the other end outside the chamber is a 40g fishing weight attached to a flag. There is a sensor located five inches above the starting position for the flag, and, in order for the sugar reward to be dispensed, the weight needs to be pulled to the sensor. After it hits the sensor, the tone plays, at the end of which the reward is dispensed into a food cup on the opposite side of the chamber from the rope. Each successful pull of the weight past the sensory and subsequent delivery of food is termed a “rep”. After the seven days of weight training, the rat begins progressive weight lifting. Modeled on a well-established behavioral method called the progressive ratio (Salamone et al., 2009) rats start with a low weight, and then progressively more weight is added until the rat stops doing the task. This endpoint is called the "break point", and is used to determine the point of maximum effort the rat is willing to exert for a given reward. For this task, we added 40g every day during training, up to 160 g. Then we did pre-testing to determine the maximum effort breakpoint for each subject. Starting at 40 g, 40 g was added every 5 reps, and the greatest weight rewarded was recorded for each animal.

Bilateral Viral Injection Surgeries

The Sprague Dawley rats were allowed to habituate after their arrival in the vivarium for seven days. These rats were handled for 3 days prior to the start of the surgeries. Rats were anesthetized with 1-3% isoflurane. The rodent’s head was fixated and a vertical incision was made. The skull was cleaned and holes were drilled at target sites using stereotaxic measurements. The viral vectors, pAAV-CaMK2a-hM3D(Gq)-mCherry (1 µL; titer= 4x10^11 vg/mL; Addgene, Watertown, MA) DREADDS or pAAV-CaMK2a-mCherry (1 µL; titer =

1.4x10^12; Addgene, Watertown, MAs), were stored at -80 degrees Celsius and diluted in sterile PBS just prior to injection. They were injected bilaterally onto the cells of the prelimbic cortex with the following coordinates (Anterior/Posterior: +3.0, Medial/Lateral:±0.6, Dorsal/Ventral: -3.9). An automated microinjection syringe pump (World Precision Instruments, Sarasota, FL) was used to inject the virus as 100 nL per minute, and the needle was kept in place for an extra ten minutes to promote diffusion. The skin was sutured closed. After the viral injection, the rats were allowed to recover for 5 days prior to starting training.

DREADD Activation and Validation

Using the viral injection (pAAV-CaMK2a-hM3D(Gq)-mCherry) in the prelimbic cortex, the glutamatergic neurons were selectively silenced via intraperitoneal injection of clozapine-*N*-oxide (CNO) obtained from the NIDA Drug Supply Program. CNO was dissolved in 150µL DMSO and then saline to a 3 mg/mL concentration just prior to injection. Animals received 1 mg/kg injections of CNO i.p. 30 minutes prior to ES/IS/HC treatment. To validate DREADD activation, animals were additionally injected with either 1 mg/kg CNO or saline 90 minutes prior to sacrifice.

Histology

After the behavioral testing was complete, rodents were anesthetized using 3-5% isoflurane. The rats were trans-cardially perfused with 250 mL each ice-cold 0.9% NaCl followed by a 4% paraformaldehyde. The brains were dehydrated with 20% sucrose and then flash-frozen with 2-methylbutane. (Note: For the validation experiment, CNO-induced activation will be validated using co-labeling via immunohistochemistry for *Fos* protein, but this has not occurred yet and thus cannot be discussed in this paper.)

Statistics

Statistical procedures were carried out with GraphPad Prism 8 (San Diego, CA). The training data across days (measured by lifts per 30-minute session) were analyzed using one-way analysis of variance (ANOVA), while the correlation between lifting and bodyweight used simple linear regression analyses. When looking at only single session post-test data, one-way ANOVAs were used to examine potential stress (ES, IS, HC) differences. In many cases, we instead examined stress effects over time (i.e. pre-and post-test of progressive weight lifting; pre-and post-tests of sucrose preference and consumption), in which cases, two-way repeated-measures ANOVAs were used. Similarly, effects of CNO treatment compared across dummy and DREADD-expressing animals were examined using two-way ANOVAs.

Results

Experiment 1: Effects of Controllability on Effort Motivation

Weight Training

A total of 40 animals were trained to lift varying weights. 2 animals were removed from the study for failing to lift anything at the lowest weight requirement (40 g). During the weight training period, the animals were required to lift 40 g, 80 g, 100-120 g, and 160 g, and each animal was given 30 min to lift freely. A mixed-effects analysis of variance (ANOVA) indicated a main effect of weight requirement ($F_{(2.6,95.2)}$=6.124, $p < 0.0001$, *Fig. 1a*). Follow-up comparisons with Šídák's corrections indicated that at 120 g ($p = 0.013$) and 160 g ($p = 0.005$), rats lifted less than they did at 80 g. To examine whether the decrease in the number of successful lifts at higher weights was mediated by body weight, such that larger rats were able to lift more, simple linear regressions were used to examine the correlation between body weight and successful lifts at 40 g and 160 g. At both weight requirements, these regressions were significant (40 g: ($F_{(1,37)}$=9.67, $p = 0.0036$, $R^2 = 0.21$; 160 g: $F_{(1,38)}$= 4.86, $p = 0.0337$, $R^2 = 0.11$). The slopes of these regressions were not significantly different from each other ($p = 0.51$, *Fig. 1b*), but the y-intercepts were different ($p = 0.006$). These data support the idea that independent of body weight, rats lift more at 160 g. Bodyweight likely leads to more lifts at a given weight without interfering with rats' ability to keep up with weight requirements in the task. The data reflected in *Figure* 1 are the rats' first attempts at each weight requirement. However, in order to pass onto the next weight requirement, the animal needed to successfully lift 25 times at the previous weight, thus this data does not reflect the final attempt of that weight.

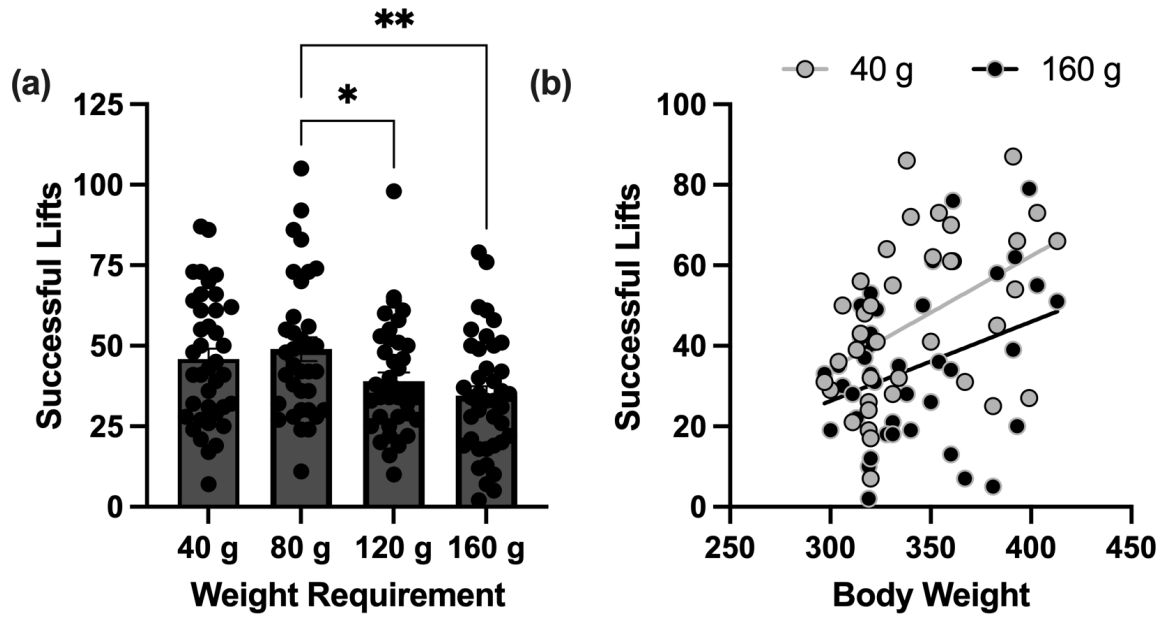

Figure 1. **Weight training. (a)** Rats were trained prior to pre-testing at 5 different weight requirements. The number of lifts completed at each weight decreased as the weight requirement increased above 80 g. Rats lifted less at 120 g and 160 g than they did at 80 g. **(b)** The rats' abilities to complete lifts at low (40g) and high (160g) weight requirements were not correlated with body weight. The y-intercepts were significantly different, supported by (a), but the slopes were not. *p<0.05 **p<0.01

Escapable and Inescapable Stressors Differentially Alter Effortful Behavior.

32 rats were studied to assess the effects of stress on weight lifting performance. After completing the 160 g lift session, rats were pre-tested to determine the maximum weight they were willing to lift, which could go as high as 280 before the pulley could no longer hold more weight. Rats were again given 30 minutes, and weight was increased by 40 g after every 5 successful (and rewarded) lifts. Then the animals underwent ES (n = 9), IS (n = 10), or HC (n = 10) experiences. 2 days following ES, IS, or HC experiences, a post-test was performed to re-determine the maximal weight lifting for each group. 2 rats showing motor deficits following ES/IS were removed from the study. A two-way, repeated-measures ANOVA reflected that there was a significant interaction between performance pre- and post-stress and stress-type (ES, IS, or

HC) ($F_{(2,22)} = 22.47$, $p < 0.0001$, *Fig. 2a*). Follow-up comparison with Šídák's corrections showed that only IS animals had a significantly lower maximum weight lifted. Both ES and HC groups did not significantly differ in weight lifting maximum in the post-test so they performed similarly. Furthermore, in comparison to pre-test levels, only the IS animals showed a significant difference between the pre-and post-test in the amount of weight lifted ($p < 0.0001$). Similarly, looking at the post-tests only, IS animals performed differently from both HC ($p < 0.0001$) and ES ($p = 0.0014$) animals, creating a main effect of stress experience ($F_{(2,24)} = 22.18$, $p < 0.0001$, *Fig. 2b*).

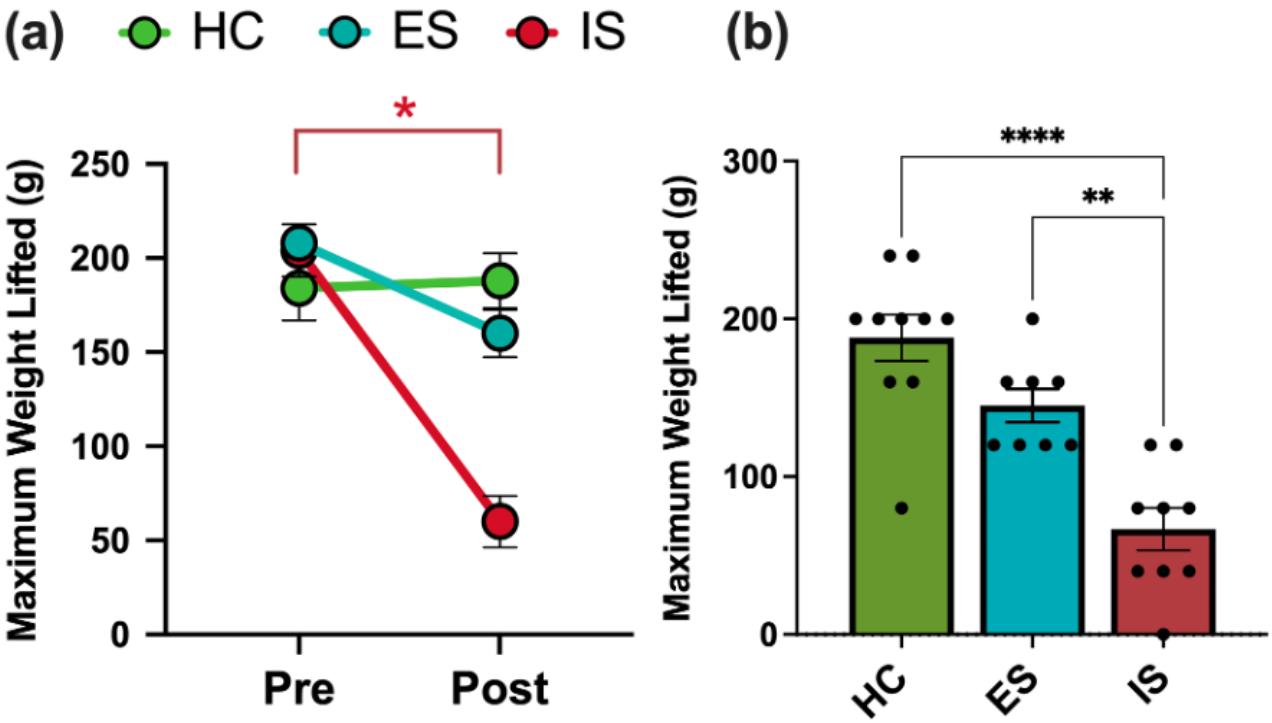

Figure 2. **Stress effects on weight lifting. (a)** Compared to pre-stress baselines, only rats that endured IS experience showed a decrease in the maximum weight lifted 48 hours later. **(b)** During the 48-hour post-test, IS-experienced rats lifted significantly less weight than both HC and ES counterparts. *p < 0.05, ***p < 0.001, ****p < 0.0001

Experiment 2: Prefrontal Chemogenetic Activation During Inescapable Stress

PL Activation with DREADDs

12 animals were included in the chemogenetic manipulation experiment, but the experiment is ongoing. 2 animals were removed due to IS-induced motor deficits, and 2 animals are yet to be run. However, thus far, our results are demonstrating no interaction between DREADD activation and group on post-tests of either progressively increasing weight or 40 g lifting (HC or IS; $F_{(1,2)} = 0.00$, $p > 0.99$, Fig 3). There is, thus far, the main effect of DREADD activation of the PL on post-test weight-lifting in either group compared to counterparts that received dummy injections ($F_{(1,2)} = 2.38$, $p < 0.0001$), but the DREADD expression unexpectedly appeared to make post-experience appearance worse.

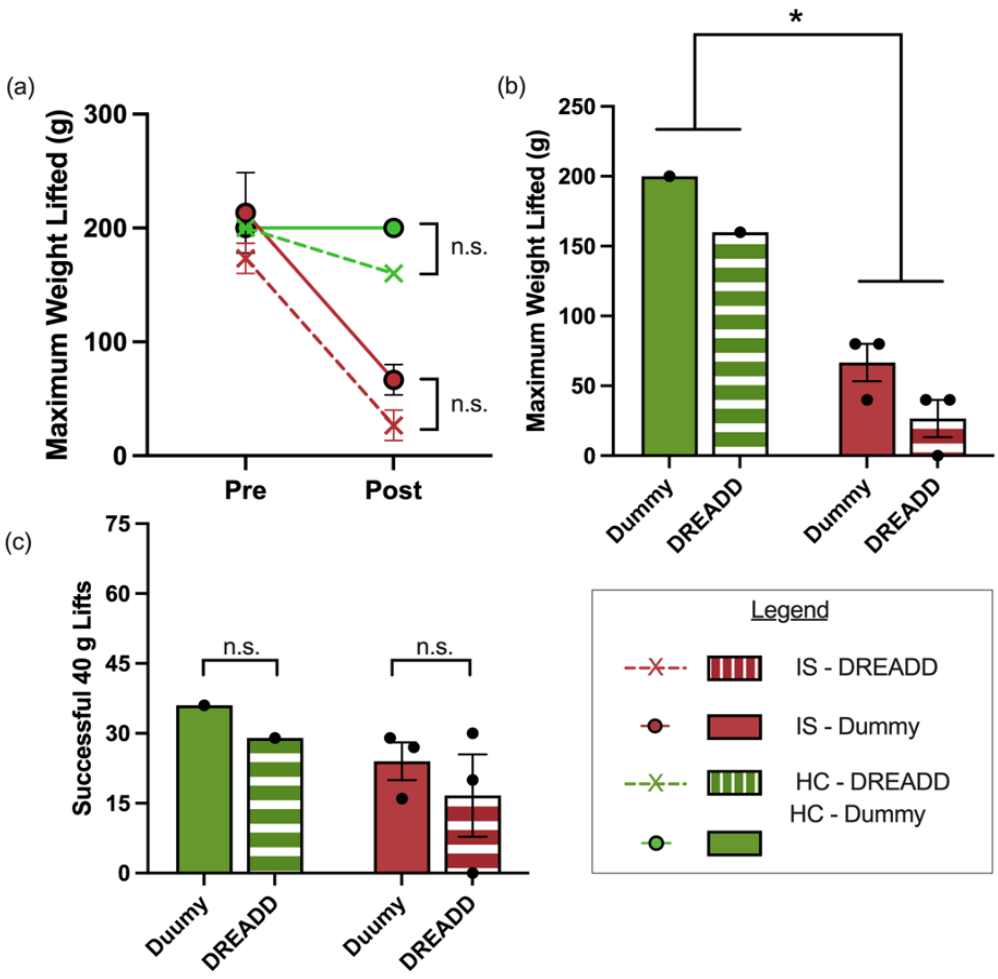

Figure 3. **Prefrontal chemogenetic activation during inescapable stress. (a)** Animals with CAMKII-HM3D-Gq expression in the PL that received treatments of clozapine-*N*-oxide prior performed similarly to dummy counterparts, indicating no change in IS-induced benefits due to DREADD activation. **(b)** After stress, there was a significant difference in maximum weight between HC and IS, but not within-groups due to DREADD expression. **(c)** There was no difference between HC and IS animals during the 72 hour test at exclusively 40 g weight, the lowest effort requirement experienced. (*p<0.05)

Two Bottle (sucrose vs. water) choice test

24 animals were included in this experiment, and 2 were removed for drinking no liquid of either kind at pre-testing. Sucrose preference was measured by calculating a difference score for each animal, where: (total sucrose consumption/ total liquid consumption) * 100. A mixed linear effects ANOVA showed no main effect of stress experience on sucrose preference over

water ($F_{(2,19)} = 0.51$, $p = 0.61$, *Fig. 4a*). However, for overall sucrose consumption, a two-way ANOVA revealed main effects of session ($F_{(2.59,49.26)} = 13.88$, $p < 0.0001$) and stress experience ($F_{(2,19)} = 3.75$, $p = 0.043$). Follow-up comparisons with Šídák's corrections revealed that ES ($p = 0.015$) and IS ($p = 0.03$) reduced consumption within 48 hours post-stress (*Fig. 4b*).

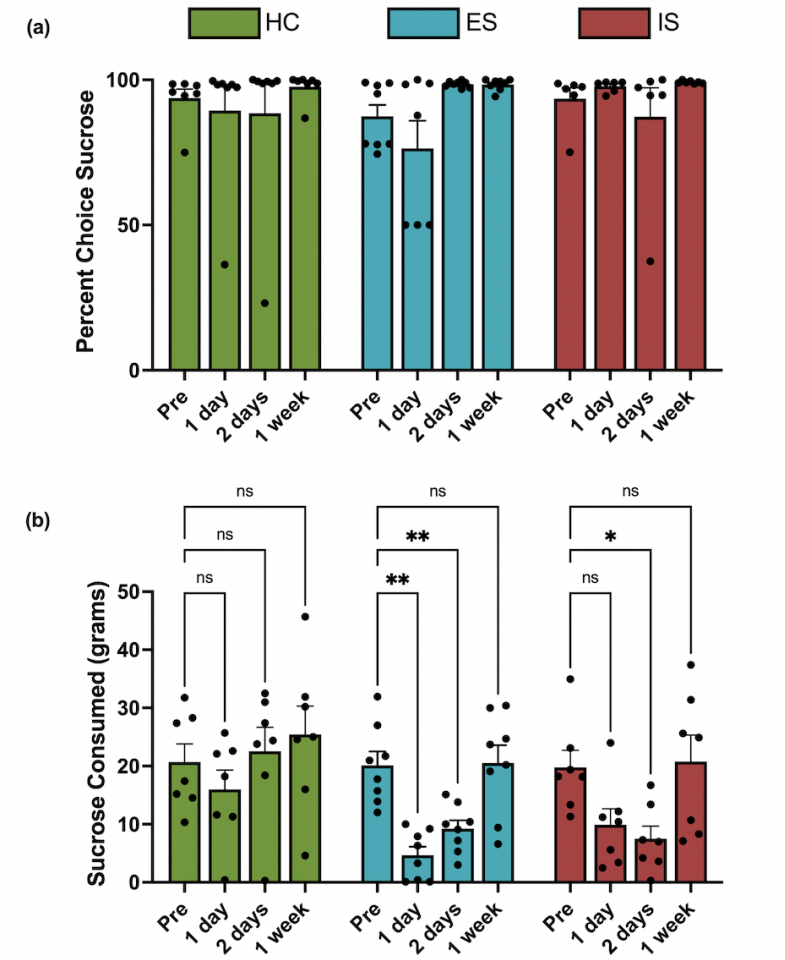

Figure 3. **Two-bottle (sucrose vs. water) choice task. (a)** When given 4 hours to freely consume water and/or sucrose, rodents drank significantly more sucrose than water. Sucrose accounted for nearly 100% of the total liquid consumed by the end of the session, both before and after stress. **(b)** Total sucrose consumption was measured by weighing bottles before and after the session. At 24 and 48 hours after escapable stress, sucrose consumption declined compared to pre-stress. At 48 hours after inescapable stress, but not 24 hours, sucrose consumption declined compared to pre-stress. Following both stress experiences, consumption returned to pre-stress levels by 1 week. *$p < 0.05$, **$p > 0.01$

Discussion and Future Directions

In the present study, we were able to examine the behavioral effects of stressor controllability on a motivated effort exertion task. Stressor controllability is known to have protective effects from the prolonged negative consequences of stress, but less is understood regarding the role of stress in hedonic and reward behaviors. Given previous work from our lab demonstrating ES protections against IS-induced deficits in reward-seeking, we predicted that escapable stress (ES) rodents would lift more weights and perform similarly to the homecage (HC) groups in comparison to the inescapable stress (IS) group. The weight-lifting experiments supported this hypothesis.

Beginning with Pavlovian conditioning, the rats were first trained to associate a tone (or, more specifically, the introduction to *Eye of the Tiger*) to delivery of banana pellets into the food-cup. Initially, we attempted to skip this conditioning, but rodents were slow to establish the association between interaction with the rope and reward delivery, in particular, because the food-cup was on the other side of the box. During weight-lifting, the reward tone served to alert the animals the moment that they successfully lifted the weight sufficiently off the ground. During the first day of training (or a few days if the animals were slow to acquire), animals only have to lift a 40g weight 2.5in. This requirement was then changed to 5in, and the weight attached to the rope progressed to 80g, 120g, and 160g over consecutive days. Animals performed similarly as different weight requirements, not lowering their activity as the weight increased. Finally, a "progressive weight" pre-test was performed on the cohort of animals in order to determine the rats' maximum weights that could be lifted. This maximum weight lifted (160-220g) was about equal to half the animals' body weights (300-400g). During the post-test, 2 days after stress, the animals' maximum weights were measured again.

Prior work suggested that IS animals will experience changes in the function of specific neural circuits that may result in so-called "learned helplessness" behavioral changes (Maier & Watkins, 2005). The data collected during the present study indicated that IS animals, who did not experience the benefits of controllability of their stressor, had negative consequences leading to a decrease in the maximum amount of weight lifted. The ES and HC animals performed with no significant changes in the pre-test maximum weights and the post-test maximum weights. Stressor controllability, therefore, protected against a decrease in motivation to exert effort in the weight lifting task, and the group that did not experience control did not have the same protective effect of performing in the effort task.

This indicates that the experience of behavioral control imparts benefits on later effortful behaviors. To clarify whether these weight-lifting results were indeed related to changes in motivation and demand for sucrose, rather than changes in hedonic value, we additionally conducted a two-bottle choice test in order to determine whether stress altered sucrose preference. There has been a multitude of studies that indicate that, after chronic stress, rodents may experience anhedonia and reduce sucrose preference in comparison to water (Liu, 2018). Prior work with the ES/IS model (Christianson et al., 2008; Frank et al., 2020) has demonstrated that ES does not protect against stress-induced anhedonia, as measured by decreased sucrose preference (not consumption). Therefore, we anticipated that perhaps both ES and IS would reduce sucrose preference, an effect that should have dissipated over 48-72 hours. Interestingly, neither group showed significantly reduced sucrose preference over water. In contrast to earlier work, rats in this experiment were food-restricted and given access to 5% (as opposed to 2%) sucrose solution. Additionally, rats were repeatedly handled prior to experimentation, potentially buffering against the negative effects of stress. On the other hand, while preference was

unmodified by stress, both ES and IS consumed overall less sucrose 48 hours after stress. Others have interpreted sucrose preference as an indication of how much animals "like" sucrose and consumption as a measure of how much animals "want" sucrose (Meyerolbersleben et al., 2020). Therefore, stress did not make animals like sucrose less than water, but it may have lowered the demand for sucrose. For both ES- and IS- experienced animals, the effects of stress dissipated by one-week post-stress, supporting prior work.

Given these two-bottle choice results suggestive of stress-induced lowered demand for sucrose, we predicted that ES animals would also show reduced weight-lifting, but that was not the case. This suggests that something is unique to the effort task that increases reward value and motivation beyond that produced by the two-bottle choice test. The simplest answer is that the 80mg of banana-flavored sucrose pellets were of greater value to the rats than the 5% sucrose solution. This could be explored empirically by giving animals a choice between different concentrations of sucrose, rather than between sucrose and water. Another possibility is that the extra pre-training and handling required for the weight-lifting task (on average, 2-3 weeks) may have buffered against the anhedonia induced by stress, as prior and early life handling has been shown to provide protection against aversive experiences and even drug-seeking (Cloutier et al., 2014; Lacagnina et al., 2017). Finally, it is also possible that the rats get additional reward value from the weight-lifting task itself. For example, the reward tone likely took on motivational power, or incentive salience, itself (Berridge & Robinson, 1998). To test this, we can run extinction training in which rats are no longer rewarded for weight-lifting and examine whether they continue lifting for the reward tone itself. A combination of these effects may have accounted for the discrepancy between the anhedonia displayed during the two-bottle choice task in the ES group and their HC-like performance during the weight-lifting task.

Finally, we aimed to understand whether the ES benefits were imparted by the PL and whether PL activation could provide these benefits for IS rats. We assessed the role of the prelimbic cortex by stimulating this region and observing if the protective ES effects were seen in the IS rats. Previous work suggested that various areas of the prefrontal cortex, specifically the prelimbic cortex and infralimbic cortex, are involved in an animals' willingness to expend effort. Inactivating the prelimbic cortex impaired the animals' abilities to perform effort expending tasks (Hosking, 2016). Due to the PL's role in modulating behavioral responses in relation to effort and stress, we hypothesized that stimulating the prelimbic region of the brain would reinstate the protective effects similar to the ES rats in the IS rats that previously had a decrease in weight-lifting expenditure. We suspected that stimulation of the prelimbic region would be sufficient in being able to establish protective effects and rescue the IS group's performance in weight-lifting. Through the use of DREADDS, the prelimbic cortex was chemogenetically stimulated by the injection of 1 mg/kg CNO. Our preliminary results do not support our hypothesis because it shows that the CNO injection did not rescue the IS rats in their weight-lifting behavior. The IS rats still had a decrease in performance compared to the pre-stress levels, while the ES and HC groups did not have a significant decrease in the maximum weight-lifting task.

Although the expected results did not occur, there are a few alternative explanations for why we did not see the IS group's reinstatement of weightlifting behavior. Some are technical. This experiment is still in progress, and another cohort will begin soon. For the first cohort, histological verification of both DREADD placement and DREADD activation of PL neurons has not yet occurred. Confirming with the histology that the DREADDS were correctly placed in the prelimbic cortex is important for the continuation of the project. If the DREADDS did not

chemogenetically activate the prelimbic cortex and it missed this region, then we would need to repeat the experiment in order to correctly activate the targeted region.

Assuming, however, that our surgeries were successful, and the CNO was capable of activating the PL, there are additional explanations for why PL stimulation is insufficient to produce ES-like effects. It may be that the effort and reward modulation by stress might work in concert with another brain region. As previously discussed, extensive work has indicated that the ACC plays a role in effort-based behavior (Porter et al., 2019). In another study, it was seen that the prelimbic and infralimbic lesions are not sufficient to damage effortful behaviors on a T-maze, and ACC lesions (though not specific to Cg1) instead changed the rodent's behavior in choice regarding reward (Walton et al., 2003). The PL may therefore be a part of a larger circuit in stressor-controllability related resiliency (Jing et al, 2021). While PL activation is sufficient to rescue future stress-related behaviors (Christianson et al., 2009) these effects are likely modulated by PL-DRN activity. It could be that the IS deficits in weight-lifting occur independently of the PL-DRN circuit. The prelimbic cortex may require communication with ACC to fully modulate the decision to expend effort after stress and create protective effects similar to the ES rats. In other words, stress-mediated changes in reward-related behaviors may require simultaneous PL and ACC activity, or PL and nucleus accumbens (NAc) activity. Stimulation of the prelimbic cortex may protect against some of the behavioral changes associated with the IS group, however, these deficits that occur may also work in concert with a separate stress-induced mechanism which makes the prelimbic activation not sufficient to fully recover the effort exerting behavior in the IS rats. Work remains to be done to determine what this larger circuit might be.

Given that the data showed that prelimbic activation was not sufficient in reinstating weight-lifting behavior, it suggests that different regions of the brain work in concert with the prelimbic cortex. Prelimbic activation was not sufficient in reinstating weight-lifting behavior, suggesting that while PL activity may be insufficient, this does not rule out that PL activity is still necessary. Therefore, an important follow-up experiment would be to inhibit the prelimbic cortex in the ES animals. By chemogenetically silencing this region of the brain, we can more closely understand the regions that are necessary for the ES protective effects and compare them to the results of the IS group. If PL inhibition also has no effect, this would diminish the likelihood that ES modulates motivated behaviors through a PL-dependent circuit. Moreover, this would confirm the hypothesis that other parallel circuits are operating in the brain to induce ES resilience, such as ACC-NAc communication.

Finally, all of the current work has been done on male rats. In the future, assessing the effects of stress on expending effort in female rats could be a significant addition to understanding the modulation of this behavior. Understanding the differences in brain changes during effort-related tasks between the two sexes is still widely unknown. However, we do know that females are not receptive to the benefits of stressor controllability using the ES/IS paradigm (Baratta et al., 2018). Other labs are dedicated to understanding this phenomenon, which may be related to physical aspects of the stressor that needs to be modulated for the small female size (e.g., shock intensity, box size). Therefore, in the future, stress-induced changes in motivation will be a viable avenue by which to follow up on this work.

Concluding Remarks

This is the first investigation to demonstrate that stress-induced changes in effort are sensitive to the benefits of stressor controllability. While IS reduced effortful reward-seeking, ES did not. This work additionally brings insight into prior work on stress-induced anhedonia, highlighting the importance of discriminating between aspects of motivated behavior. Moreover, our investigation of the role of the prelimbic cortex in stress-induced behavioral changes in effortful reward-seeking behavior provides insight into the brain regions responsible for modulating this decision-making.

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