ACUTE STRESS MODULATION OF RISK/REWARD DECISION-MAKING

by

Giulio Laino Chiavegatti

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

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submitted by Giulio Laino Chiavegatti in partial fulfilment of the requirements for the degree of Master of Science in Neuroscience

Examining Committee:

Dr Stan B. Floresco, Professor, Department of Psychology, UBC
Supervisor

Dr Luke Clark, Professor, Department of Psychology, UBC
Supervisory Committee Member

Dr Victor Viau, Professor, Department of Cellular and Physiological Sciences, UBC
Supervisory Committee Member
Abstract

Discerning which choices are advantageous amongst many based on reward cost or on making and withholding responses is essential for survival, and salient cues may be perceived as threatening via several neurochemical and behavioural changes. This type of decision-making where different actions may yield rewards associated with costs or punishment can be differentially altered depending on the type of costs being evaluated. In previous work from our group, we have shown acute restraint stress does not alter preference for larger/uncertain rewards vs. smaller/certain ones, but markedly shifts preference away from more physically effortful rewards. However, how stress may modulate decisions where rewards are linked to punishment has yet to be fully explored. Here we examined how different forms of acute stress influenced action-selection on two tasks involving punished reward-seeking in male and female rats.

To that extent, we adopted a risky decision-making task involving choice between a small/safe lever always delivering one reward pellet and a large/risky option delivering three pellets but that could also deliver foot-shock with an increasing probability across blocks of trials (0, 25, 50, 75, 100%). In well-trained rats, one-hour restraint increased risk aversion and punishment sensitivity, markedly reducing preference for the shock-associated reward comparably between sexes. In contrast, these effects were not mimicked by either the α-2 noradrenergic antagonist yohimbine or corticosterone.

In a second study a go/no-go task assessed ability to inhibit approach towards a readily available reward associated with punishment. Here, a food pellet was delivered in a cup, and on 30/60 trials the rat merely had to approach and retrieve reward. On the other 30 trials, a 12-s visual/auditory cue signalled food retrieval must be withheld to avoid foot-shock. Restraint
induced an increase in impulsive action on males on test day, but females markedly less the day after, an effect that was recapitulated by yohimbine but not corticosterone.

These findings suggest acute restraint enhances the effects of punishment on choice between different rewards while differentially affecting inhibitory impulse control in situation involving rewards and punishment. The mechanisms underlying this effect may relate to the increased risk aversion observed in individuals with depression.
Lay Summary

Figuring out which decisions are more advantageous for us at any time can be challenging. Our brains can pick up all sorts of signals from the environment and interpret them as a threat, and how stressful this is for us can change depending on the cost that comes with our choice or action. To understand how stress affects these decisions, we adapted a risky decision-making task, where rats can play it safe and receive a sugar reward or risk receiving a mild shock for more sugar. We also adapted a behavioural control task to observe how stress can alter impulsivity, where rats need to learn to restrain collecting free sugar if certain signals are on; otherwise, they get a mild electric shock. By stressing rats in different ways, we show that animals become less-risky players and that this changes impulsivity differently in males and females.
Preface

All experiments were conducted at the University of British Columbia (Vancouver campus) and carried out by Giulio Laino Chiavegatti. In addition, Giulio Laino Chiavegatti performed all restraint tests, injections, statistical analyses, and wrote the current document. Experimental concept and design, as well as the majority of editing, especially on data analysis, was done by Dr Stan B. Floresco, the supervisory author on this investigation. Research for this thesis was approved by the Animal Care Committee of the University of British Columbia, application number A018-042.

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“... we ourselves bring into the appearances that order and regularity that we call nature, and moreover we would not be able to find it there if we, or the nature of our mind, had not originally put it there... The understanding is thus not merely a faculty for making rules through the comparison of the appearances: it is itself the legislation for nature, i.e., without understanding there would not be any nature at all.”

[Critique of Pure Reason | 1781]
For Carolina, without whose unending and unwavering support this would not have happened, 
and who has always been lovingly at my side.
Chapter 1: Introduction

The acute stress response is instrumental for survival, as it directs several behavioural and neuro-chemical alterations to adapt to changing environmental stimuli and restore homeostasis (Bryce & Floresco, 2016; Shafiei et al., 2012). Stressors, in particular, initiate parallel series of neuro-endocrine responses in the hypothalamus, namely the faster and more immediate sympato-medullary system and the slower hypothalamic-pituitary-adrenocortical (HPA) axis (Herman et al., 2020; Won & Kim, 2016). The latter is activated upon stimulation of parvo-cellular neuro-secretory neurones within the paraventricular hypothalamic nucleus to release the neuropeptide corticotropin-releasing factor (CRF) into a system of portal veins. CRF subsequently acts on corticotrophic cells within the adenohypophysis to stimulate production of adreno-corticotropic hormone regulating cortisol production from the cortex of the adrenal glands. A parallel pathway is mediated by the lateral hypothalamus, which sends a direct mono-synaptic connection to the adrenal medulla. At the medullary level, specialised nerve endings are stimulated to release adrenaline into the blood stream, which regulates glucose metabolism by enhancing blood-flow to skeletal myofibers and glucose levels in the blood.

Thus, on the one hand, acute stress increases cortisol production, or corticosterone in rodents (Erickson et al., 2003), while on the other hand, it enhances adrenergic transmission. Of notable importance, factors such as sustained environmental stressors as well as traumatic experience during childhood may lead to hyperactivation of the HPA axis later in life (Kelishadi et al., 2009; Tafet & Nemeroff, 2016). The enhanced CRF transmission and ensuing hyper-cortisolemia indeed characterise stress-related disorders, such as schizophrenia and major depression (MD) (Hauger et al., 2009; Risbrough & Stein, 2006; Smoller, 2016).
1.1 Cognitive and motivational alterations induced by acute stress

Learning and memory can be impaired by acute stress even at a simple level. For example, it has been observed that this type of stress impairs learning about reward and punishment in humans using a reinforcement-learning task (Carvalheiro et al., 2021). Indeed, in the presence of stress (uncontrollable auditory cue) choice was selectively impaired so that participants’ behaviour was altered in regard to gains but not losses, likely due to slower learning from a positive reward-prediction error. Thus, when the outcomes were better than expected, human subjects under stress were significantly less able to learn from those advantageous choices as compared to controls.

In a similar study it was observed that acute stress also impairs retrieval in extinction learning paradigms (Raio et al., 2014). In this context, an image was paired with electric shock in a probabilistic manner while a second image was not, and a day later subjects were either subjected to stress (cold press) or a control task before the extinction test. Notably, participants that received the stress test also displayed poorer extinction retrieval (i.e., a greater conditioned response.

Furthermore, several studies have revealed how acute stressors and stress-related peptides can affect memory processes substantially. For example, in rodents, one-hour acute restraint significantly interfered with memory retrieval in both object-recognition and object-location tasks regardless of inter-trial interval (between acquisition and retrieval) (Li et al., 2012). Additionally, these data revealed post-memory-acquisition restraint prevented consolidation of short-term memory into long-term memory, and it appears these impairments in the retrieval of spatial memories may plausibly be mediated by long-term depression in the hippocampus (Ge et al., 2010; Wong et al., 2007). Generally, there seems to be consensus on the fact that non-
contextual stressors preceding learning or memory retrieval significantly impair performance based on the Morris water maze or object recognition (Baker & Kim, 2002; de Quervain et al., 1998; Wong et al., 2007).

On the other hand, the effects of acute stress on working memory functions appear to be less straightforward and more complex. For example, in one study, rats learnt to retrieve food within a radial-arm maze and, after successful foraging in some of the arms, they were exposed to a novel environment (stressor) for a delayed time and subsequently returned to the maze to locate food in the remaining arms. Importantly, acute stress significantly increased the number of working memory errors (i.e., rats were unable to make use of previously stored spatial information) but did not induce anterograde amnesia, suggesting stress can temporarily inhibit hippocampal function, thereby impairing spatial working memory (Diamond et al., 1996).

Furthermore, acute stress was shown to induce selective performance impairments on rodent cognitive tasks that are mediated by the medial prefrontal cortex (mPFC) as opposed to the hippocampus (Holmes & Wellman, 2009; McAlonan & Brown, 2003; Yuen et al., 2009). For example, exposing rats to an acute stressor (15-minute mild tail-pinching) significantly impacted set-shifting but not reversal learning: stressed animals required a higher number of trials and perseverative errors in the operant chamber, suggesting acute stress selectively impairs changing cognitive strategies when the previously learnt one is no longer advantageous (Butts et al., 2013). Collectively, these findings suggest working memory processes are influenced by acute stress in varying degrees of complexity also potentially dependent on different factors such as type of stress, duration, and timing.

Acute stress has been shown to induce detrimental effects on selective attention in humans and rodents (Elling et al., 2011; Sänger et al., 2014; Shields et al., 2019). Even a mild acute
stressor (cold pressor test) significantly decreased response time in two types of Flanker tasks, plausibly driven by enhanced motor activity related to the behavioural actions. Of note, the higher cortisol levels reported from the subjects was not related to any of the observed changes in the metrics of interest (Shields et al., 2019). Similarly, stress was also found to impair rat performance on the sustained attention task (Eck et al., 2020). Of importance, the study also showed stress induced a morphological change of cholinergic neurones in the substantia innominata (part of the attention circuit of the brain) as well as sex-specific alterations of acetylcholine release onto the mPFC.

It should also be noted that biological sex has in some instances been reported to be a key moderator in the connection between acute stress and cognition, although there has been some inconsistency in terms of rodent-human translatability. For example, while some rodent studies suggest cognitive flexibility is more strongly impaired by stress in females, clinical data reveal males to be potentially more susceptible (Grafe et al., 2017; Kalia et al., 2018; Shields et al., 2016).

1.1.1 Cost/benefit decision-making in the context of acute stress

Decision-making processes usually entail the weighing of costs and benefits associated with each possible choice outcome, and this process can be particularly taxing on the individual (Bryce et al., 2020). Effective decisions are thus paramount for the animal to make adaptive goal-directed responses to benefit from. Comprehending the neural mechanisms by which this type of decision-making is possible is a fundamental question for all aspects of society. These behaviours are complex forms of higher-order cognition relying in part on the pre-frontal cortex and, importantly, they can be modelled in pre-clinical research featuring rodents, as they show the same type of biases that characterise human behaviour in similar circumstances (Winstanley
& Floresco, 2016). Usually, modelling cost/benefit decision-making in rodents entails rats learning an operant paradigm that exposes the animal to two food-associated levers. Choosing one option tends to guarantee a small food reward, whereas the other option is associated with a more complex contingency typically paired with a larger food reward. Thus, animals usually choose between rewards of different magnitude, with the fruition of the larger reward depending on some cost the animal must expend. Some of these assays are devised such that the decision-making of the animal relies on internally-generated cues of action-outcome contingencies (e.g., the rat makes a subjective prediction of what is more likely to happen depending on the outcome of the preceding trial), whereas others employ external cues rats need to pay attention to, as they inform the animal about the likelihood of an action-outcome contingency (e.g., the rat learns to make an informed decision based on what it observes in space) (Schumacher et al., 2021; Wassum, 2022; Yang et al., 2022).

Now it is relatively well established that acute stress impairs optimal decision-making relying on normal mPFC function, which causes a behavioural shift toward more habitual, non-goal-directed actions (Dias-Ferreira et al., 2009; Elliot and Packard, 2008; Schwabe et al., 2012). Furthermore, stress can have a variable influence on cost/benefit decisions depending on the cost of the food reward involved in the operant assay (Bryce & Floresco, 2016; Bryce et al., 2020; Shafiei et al., 2012), and several tasks have been developed to look at physical effort, probability to receive reward, delays in receiving reward, probability of reward, and positive punishment (Bryce & Floresco, 2016).

The fact that stress-related disorders are associated with a reduction in incentive motivation (e.g., motivational anhedonia), suggests that cost/benefit decisions involving evaluations of physical effort may be particularly sensitive to stress (Shafiei et al., 2012). These types of
decisions are mediated by several neural circuits that include, but are not limited to, the mPFC, the basolateral amygdala, and the nucleus accumbens as revealed by classical pharmacological inactivation studies (Floresco et al., 2018; Hosking et al., 2014; Hosking et al., 2016), and this circuitry is also sensitive to the effects of acute stress (Holmes & Wellman, 2009; Kielbinski et al., 2023; Williams et al., 2022). In this context, previous research from our laboratory investigated the effects of acute restraint stress on an effort-discounting paradigm (Shafiei et al., 2012), where rats were required to choose between two levers: one option always delivered one sucrose pellet after a single press, whereas the second lever delivered four pellets, but it required a higher number of presses (2-20). Upon training on this task, rats become progressively less willing to press the high-reward lever as the number of required presses increases. Notably, following one-hour restraint, the animals become significantly more averse to choosing the more effortful option and slower at making a lever response (i.e., choice latency). However, the same manipulation did not have any major effects on a delay-discounting task, where the lever associated with the larger reward delivers the pellets after a time delay, suggesting the observed effects of acute restraint on effort-related choice plausibly do not depend on an increase in impulsivity tied to the fruition of the delayed reward. Additionally, it is interesting that these findings collide with well-established data from clinical studies that have emphasised how closely related stress and impulsivity are (Berghorst et al., 2013; Moustafa et al., 2017; Raio et al., 2020; Schaefer et al., 2012).

Subsequent research was aimed at understanding whether CRF could plausibly mediate the effects of acute restraint on effort discounting using intra-cerebro-ventricular (ICV) micro-infusions. Importantly, a high 3-µg dose of CRF recapitulated the observed effects of restraint on both choice behaviour and latency; additionally, administration of antagonist α-helical CRF
blocked the above-mentioned effects, as there was no observable change in performance, suggesting CRF is both necessary and sufficient in modulating the effects of acute stress on effort-related decisions by shifting choice away from more physically costly rewards (Bryce & Floresco, 2016). Of note, CRF did not alter preference between larger vs. smaller rewards of equal cost as assessed on a simpler reward-magnitude discrimination task, pointing to how fundamental neural processes that guide choice about different rewards are unlikely players in the context of stress-induced changes in reward-related decisions.

It remained, however, relatively unexplored how acute stress may modulate cost/benefit decision-making where rewards may or may not be delivered (uncertainty). In this context, rats were trained on a probabilistic discounting task, where a press on the “safe” lever guaranteed the immediate delivery of one sugar pellet, whereas a press on the “risky” lever may or may not yield an immediate four-pellet reward. Importantly, the probability of receiving the larger reward changes across five blocks of 18 trials each either in an ascending way (6.25%, 12.5%, 25%, 50%, 100%), or a descending way (100% to 6.25%). Surprisingly, neither acute restraint nor a high 3-μg dose of CRF (ICV infusion) did have effects on choice, so the rats were not significantly more or less likely to choose the “risky” lever; there were, however, some behavioural alterations as evidenced by the higher omission rate, suggesting rats became less motivated to engage with the task (Bryce et al., 2020).

Collectively, these findings suggest that acute stress and increases in CRF bias choice away from more physically effortful rewards and generally reduce motivation to pursue reward irrespective of choice; however, these two factors do not seem to play a pivotal role in contexts where reward costs are more subjective (e.g., delayed or probabilistic rewards). Of note, while the CRF-induced increases in response omissions may be compared to clinical studies of MD,
which is characterised by a hyperactive CRF response (Holsboer & Ising, 2008), the effects on choice appear less clear. For example, some data suggest that acute stress can actually improve decision-making under uncertainty in situations where long-term gains need to be maximised, as study participants were better able to learn what the advantageous option of the task was following acute stress (Byrne et al., 2019).

1.2 Punishment and acute stress

The studies described above revealed that acute stress can alter decisions involving physical effort but does not seem to alter risk/reward decisions in situations involving reward uncertainty, where the risky is merely not receiving a potential reward. However, how acute stress may modulate risky choice when rewards may be linked to punishment remains poorly understood. This is of notable interest given individuals with MD were shown to have increased sensitivity to punishment (Eshel & Roiser, 2010; Hevey et al., 2017; Kim et al., 2021). For example, a clinical study that used a probabilistic stimulus selection task investigated learning about reward and punishment in the context of stress (threat of electric shock). Participants that had a physiological and affective reaction to stress (e.g., increased cortisol production and anxiety) were observed to have deficits in the processing of rewards (i.e., learning from positive feed-back), suggesting acute stress may lead to these without impairing processing about punishment (Berghorst et al., 2013). Furthermore, these data also align with some neuroimaging studies that acute stress caused a reduction in activation of reward-relate neural centres (Burani et al., 2021; Porcelli et al., 2012). Additionally, acute stress can significantly alter blood-oxygen-level-dependent signals in dorsal striatal regions and the orbitofrontal cortex in response to monetary gains and losses (rewards and punishments) (Porcelli et al., 2012). However, much remains to be understood in the context of punishment and how stress may influence or modulate
its effects in cost/benefit reward-related decision-making, which is the primary focus of this thesis.

1.3 **Behavioural assays to measure risky cost/benefit decisions**

While operant paradigms to model cost/benefit reward-related decision-making have been discussed to various extent in the above sections, they are not designed to assess decisions when rewards are linked to explicit punishment. In this regard, certain operant-based assays have been developed that place animals in conflict situations where rewards may be associated with a foot-shock punisher. One task, termed the risky decision-making task (RDT), resembles other discounting task described above in which rats use internally generated representations of choice-outcome contingencies (Orsini *et al*., 2015; Simon *et al*., 2009). In this assay animals learn to choose between two levers both guaranteeing rewards of different magnitude. Notably, the lever associated with the higher reward may or may not yield a mild foot-shock, with the probability of receiving foot-shock following a risky choice increasing across blocks of trials. Thus, animals need to be able to gauge their risk tolerance with respect to how likely they are to receive a shock if they pursue a larger reward.

Another assay that has been developed to investigate other aspects of punished reward-seeking requiring response inhibition, particularly impulsive action, is the comparatively simpler “behavioural control task” (Verharen *et al*., 2019), where rats are presented with a reward pellet in a food cup in an operant chamber. On half of the trials, they merely have to retrieve reward (“go”). On the other half of the trials, however, rats must withhold their approach response towards the food (“no go”) for 12 seconds during the presentation of specific visual-auditory cues signalling that a mild foot-shock is guaranteed if they collect the food before cues termination.
1.4 Inducing acute stress in laboratory rodents

There are a variety of procedures that are used commonly to evoke an acute stress response. One common means of assessing the effects of stress in rats entails physically immobilising the animal (acute restraint), the effects of which are well characterised to induce stress in the laboratory rodent in a way that can reliably increase relevant hormone levels (e.g., monoamines, corticosterone) (Kant *et al*., 1983; Williamson *et al*., 2005). It is a stressful situation over which the rat has no control and which triggers emotional and autonomic arousal in response to it (Reis *et al*., 2011). The latter include, but are not limited to, an increase in blood pressure, heart rate, and body temperature. Importantly, because rats tend to undergo hyperthermia easily (McGivern *et al*., 2009), it is advised to use a means of mechanical ventilation directly in front of the rats while they are being restrained to prevent the phenomenon (Shafieei *et al*., 2012). Additionally, acute restraint induces several important behavioural changes observable after 24 hours, including a diminished rate of exploration in an open field, higher degree of immobilisation as measured in a forced-swim test, and reduced time spent in the open arm of an elevated plus-maze (Korte & De Boer, 2003; Reis *et al*., 2011). These data thus seem to suggest that while restraint immediately induces autonomic alterations, these may produce anxiety-like effects the following day.

It is well established that different stressors reliably increase monoaminergic signalling in the brain (Flügge *et al*., 2004), especially in the case of noradrenaline (NA), which plays a pivotal role in the regulation of the autonomic response to stress. Notably, the $\alpha$-2 adrenergic receptor antagonist yohimbine hydrochloride has been commonly used to as a pharmacological means to induce stress in the laboratory rodent (Bremner *et al*., 1996; Chen *et al*., 2015). The general effect of yohimbine is to increase NA signalling by blocking the auto-receptors and
channelling the signal post-synaptically via the excitatory α-1 receptors (Andén et al., 1982). Importantly, exogenous administration of yohimbine increases autonomic arousal, blood pressure, heart rate and glucocorticoids in both healthy rats and humans (Herman et al., 2019; Lee et al., 2004). Furthermore, the yohimbine response becomes more heightened in some psychiatric populations by inducing manic stages in patients affected by bipolar depression, triggering symptoms of withdrawal in opioid-use disorder, and more pronounced panic episodes in post-traumatic stress disorder (Price et al., 1984; Southwick et al., 1999; Stine et al., 2002; Sun et al., 2010). Notably, yohimbine administration in rats was found as well to increase anxiety-like phenotypes and induce relapses in drug-seeking behaviours in the context of extinction-reinstatement paradigms.

Finally, stress-like responses in rats may also be induced by exogenous administration of corticosterone (CORT), the critical player in the hormonal cascades of the HPA axis following exposure to a stressor. Exogenous administration of CORT is often used to mimic a major hormonal response to stress not only because of the direct and central role it plays in the homeostatic response to stress, but also given its ease of use, storage, and cost for experimental designs.

1.5 Objectives and hypotheses

The primary objective of this thesis was to understand how acute stress modulates distinct forms of cost/benefit decision-making when rewards are linked to punishment in both male and female Long-Evans rats. The use of females, in particular, is notably important given 1) the majority of studies available in the literature that discuss this type of behaviour have predominantly employed males, and 2) our experiments also aim at modelling behaviours that
characterise MD in humans, in which the disorder is disproportionally more prevalent in females (Albert, 2015).

To test this, I used two types of operant assays that are well suited to modelling discounting behaviour under threat of punishment and resolution of response conflict when reward-seeking is punished. Separate squads of male and female rats were well trained in either task before being subjected to acute stress using a within-subjects design. This primary objective can be divided into two sub-objectives, namely,

1. **To assess the effects of acute restraint and pharmacological stress on risky decision-making** involving punishment. We hypothesised *acute stress would decrease risk-taking behaviour* and other measures of motivation.

2. **To assess the effects of acute restraint and pharmacological stress on inhibitory impulse control** involving punishment. We hypothesised *acute stressors would impair this type of impulse control and increase impulsive behaviour* in the presence of cues.

Collectively, these experiments aim at elucidating how acute stress may modulate the effects of punishment on risky choice and inhibitory impulse control, thus helping augment our current understanding of decision-making deficits observed in individuals with depression and provide fundamental insight that can inform the clinical setting.
Chapter 2: Methods

2.1 Animals

Separate cohorts of male and female Long-Evans rats purchased from Charles River Laboratory were utilised in all ensuing experiments. Rats had an initial weight of approximately 250-280 g and were acclimatised to the animal facility for one week prior to being pair-housed and food-restricted to 85-90% their free-feeding weight\(^1\). Rats were given *ad libitum* access to water for the entire duration of the experiments as well as chow following each behavioural session (15 g per male and 12 g per female). Animal weight was also monitored daily, and all testing was performed in accordance with the Canadian Council for Animal Care and the Animal Care Committee of the University of British Columbia (protocol no. A18-042).

2.2 Behavioural apparatus

Behavioural experiments were performed in standard operant chambers (30.5 x 24 x 21 cm) provided by Med Associates (Fairfax, VT, USA). Each chamber contained a fan for the purposes of ventilation and attenuation of external sounds. Sugar pellets were delivered via an external dispenser connected to a central food magazine port. On either side of the port two retractable levers were located, and a house light (100 mA) was located in the top-centre of the chamber wall opposite the magazine. Several sensors within the chamber provide indirect measures of the behaviour of the rats: 1) sensors located on the sides of the box can provide locomotion information by recording the number of light beams that are broken; and 2) a similar sensor located within the food receptacle can provide information about the rat nose-poking in

\(^1\) Following initial lever training, rats are maintained to approximately 95% their free-feeding weight.
the magazine. All experimental data from the chamber was transferred onto a desktop computer connected to the chamber via a Med Associates interface.

2.3 Risky decision-making

2.3.1 Magazine training

Initial behavioural training commenced five days following food restriction. Importantly, on the last day of food restriction rats were given several sugar pellets in their home-cage in addition to the chow. The following day each rat was placed in an operant chamber under a variable-interval 30 schedule of reinforcement. This was implemented so the rats could acclimatise to the chamber: no specific behaviour was required on the rats, and a food pellet would simply drop into the magazine after an average 30 seconds, signalling the rats to nose-poke into the food port to retrieve the sugar pellet.

2.3.2 Initial lever training

On the day following magazine training, rats began lever training. To that extent, either lever was extended, and 4-5 sugar pellets were crushed and placed on the top of the extended lever prior to the beginning of the session. Rats were thus trained on a fixed-ratio 1 (FR1) schedule of reinforcement and were required to meet a criterion of 50 presses in 30 minutes for one of the two levers. On the following day, rats were trained on FR1 for the other lever, with the order counterbalanced across animals.

Following this phase of pre-training, rats were trained to press retractable levers. One of the two levers would extend every 35 s and rats were required to make a response within 10 s, or the trial was recorded as an omission and no pellet was dispensed. A response within 10 s retracted the lever and delivered a food pellet. Each trial began with a three-second illumination of the house light followed by random extension of either lever; if a rat omitted any given trial,
the house light would also turn off and the lever retract. Rats were trained on this phase for approximately two to three days and criterion required them to omit fewer than five times by the last day, after which time rats were moved onto the next phase of pre-training.

On the final phase rats were trained for two days on a simple reward-magnitude discrimination, such that one lever always delivered one pellet, whereas the other lever always delivered a larger three-pellet reward, and this latter lever was counterbalanced across rats. Importantly, these lever-reward magnitude contingencies were maintained for the remainder of the experiments. Lever extension and house light illumination were identical to the previous phase, and each session consisted of four blocks of 12 trials, with the first four being forced-choice and the latter eight free-choice trials. This phase permitted rats to discern between an advantageous and disadvantageous option and required a successful criterion of preference for the lever associated with the larger reward of at least 70% of the free-choice trials.

2.3.3 Risky decision-making task

Separate cohorts of rats in experiments were trained for a minimum of five days a week on the RDT first developed by Setlow and colleagues (Simon et al., 2009). Each session consisted of 90 trials grouped into five blocks of 18 trials each. The house light would illuminate and either or both levers extend every 35 s. Following lever extension, rats were required to make a response within 15 s, or the trial was scored as an omission, no reward given, and the house light extinguished. The house light, however, remained illuminated for four seconds after a rewarded press. A press on the “safe” lever always delivered one pellet, whereas a press on the “risky” lever always delivered three pellets; however, in the latter case the animal might or might not also receive a mild one-second foot shock, and the probability of receiving such shock increased across the blocks (0% in 1st block, then 25%, 50%, 75%, and 100%). In order for the rats to learn
the changing of action-outcome contingencies, each block comprised initially of eight forced-choice trials in which only one lever was randomly extended. The following 10 were free-choice trials, where both levers were extended and the animal could press either. Of importance, foot-shock probability during forced-choice trials is fixed within any given block (e.g., in the 50% block, rats are guaranteed to receive a shock following two out of the four risky-lever extensions). In the free-choice trials, however, probabilities were randomised by the software (e.g., in the 50% block, each risky press has a 50% chance of being punished regardless of the outcome of the preceding trial).

Each rat began training at a shock intensity of either 0.20 mA (females) or 0.25 mA (males). Following at least two days, the current was increased by 0.05 mA increments if the rats did not show discounting behaviour (e.g., more than 70% risky presses in the latter blocks). Conversely, if the rats showed high discounting (e.g., high number of omissions in latter blocks), the current was reduced by 0.05 mA increments and at any rate not below initial levels. Rats were trained until they showed stable patterns of discounting behaviour for at least three consecutive days. Stability was analysed using a two-way repeated-measures ANOVA with day and block as within-subjects factor, such that the main effect of block was significant but the there was no main effect of day or a significant day by block interaction at $\alpha = 0.05$.

2.4 Behavioural control task

Pre-training on this task did not entail any type of lever training; instead, rats were subjected to two days of magazine training. Separate cohorts of rats were trained for a minimum of five days a week on a simpler behavioural control task adapted from Verharen et al. (2019) that entailed positively reinforcing active responses intermixed with punishment. Each session consisted of 60 trials where the house light would stay illuminated for the duration. In this task,
each training session consisted of 60 trials in which a food pellet was delivered in the magazine port every 40 s regardless of trial type. The trials were in a fixed pseudo-randomised sequence such that 30/60 trials were “non-signal” trials, whereas the remainder half were “signal trials.” The sequence of trials was the same for all animals trained, which allowed for the simultaneous testing of multiple rats in the same operant room without any sort of interference by the cues between each box.

Thus, at the beginning of every trial a reward pellet was delivered in the food cup. On non-signal trials the rat was simply required to nose-poke into the cup to retrieve reward (“go”), which was detected by an infrared photobeam located in the food alcove. On signal trials, however, delivery of the food pellet coincided with presentation of a 12-s visual/auditory warning cue that informed the animals food retrieval would also deliver foot shock until termination of signal. If a rat waited for 12 s for the warning cue to terminate before approaching the food, it could retrieve the pellet without consequences, and the trial was marked a “success.” On the other hand, nose-poking before cue termination resulted in foot shock, and the trial was marked a “shock” trial. Thus, in this type of trial rats were required to withhold their approach towards the food until cue termination to avoid punishment (“no-go”).

Similarly to the RDT, shock currents were titrated for each individual rat: current was increased if a rat was not withholding appropriately (more than 50% of signalled trials) or decreased if a rat was withholding or omitting too much. Rats were trained until as a group they showed stable patterns of performance for at least three consecutive days. Current intensity was kept constant for the remainder of the experiment after a rat stabilised to approximately 20 success trials out of 30 stimulus trials. Rats were thus trained for approximately 14 days, when
behavioural performance on signal trials was stable for at least three consecutive days, as assessed by one-way ANOVA’s using three levels of “day” as a within-subjects factor.

2.5 Acute restraint stress

One series of experiments examined how acute restraint stress affected choice behaviour and motivational functions in the RDT and the behavioural control task. The restraint time (one hour) was based on previous experiments where said time was sufficient to induce alteration in discounting behaviour (Bryce & Floresco, 2016; Shafiei et al., 2012).

Once animals displayed stable patterns of choice behaviour, they were subjected to stress tests. Each test consisted of a two-day sequence. The first, “baseline” day consisted of placing animals in the room where they would receive restraint stress the following day. If rats responded normally following this procedure (i.e., no significant differences in performance compared to the preceding training day), they would be subjected to a one-hour acute stressor on the following day. Specifically, rats were placed inside Plexiglass tubes depending on the size of each rat that were located in the same neutral room as the previous baseline day (124-171 x 60 mm for up to 350-400 g and 152-216 x 73 mm for up to 600 g). A large fan was placed directly in front of the restrainers for the duration of the test to prevent the risk of hyperthermia. One end of the restrainers was moveable and could be adjusted to keep the rat immobilised. At the end of the restrain period rats were returned to their home cages wherein they were left undisturbed for 10 minutes prior to the beginning of operant testing. Following this two-day testing sequence, rats were re-trained for stable behavioural responding (approximately three-five days) before performing a second stress test in the same manner to assess whether rats habituated to the effects of the acute stressor on risky choice or response inhibition.
2.6 Yohimbine challenge

Following a within-subjects design, the same cohort of rats that received restraint stress tests were retrained on their respective tasks for three-four days and then were used to assess whether a different form of stress could induce effects similar to acute restraint. To this extent, we used the α-2 adrenoceptor antagonist and pharmacological stressor yohimbine hydrochloride at a physiologically relevant dose (1 mg/kg, intra-peritoneal) that has been shown to alter decision making in other studies (Hosking et al., 2015; Montes et al., 2015). Yohimbine was purchased from Tocris Bioscience (Bristol, UK) and dose was calculated in the salt form prior to dissolving in sterile distilled water (dH₂O) at a concentration of 1 mg/mL.

The drug tests consisted of a two-day sequence, whereby on the first day rats were injected vehicle alone and then placed back into their home-cage where they waited for 30 min before commencing operant testing for either task. If performance remained stable, the following day they received an injection of yohimbine 30 minutes prior to assessing operant behaviour.

2.7 Corticosterone challenge

Given the direct role of CORT on the tone of the HPA axis and that its levels increase following acute restraint stress, we tested a CORT challenge on performance of the two tasks in squads of rats that were separate from those receiving restraint and yohimbine challenges. The effects of acute administration of CORT on punished reward-seeking were assessed using a within-subjects design in a manner similar to the yohimbine challenges. Following behavioural training, drug tests were conducted using a two-day sequence and a counterbalanced design, and after each injection the animals were returned undisturbed into their home cage for 60 min. On the first day, rats were split into two groups and matched for choice behaviour to receive a vehicle injection (50/50 propylene glycol/0.9% saline) given sub-cutaneously of either 1 mg/kg
or 3 mg/kg; the next day, rats received CORT (Sigma Aldrich) injections of the same volume they had got the day before and at a concentration of 1 mg/mL.

After the first two-day sequence, rats were re-trained until reaching stable levels of performance (approximately three-four days). Then they received a second testing sequence such that all animals were given the dose they had not received previously. Of note, the doses and time-course that we followed were derived from studies conducted in this laboratory and others, as they are plausibly comparable to post-restraint levels of CORT.

2.8 Data analysis

The main dependent variable for the risky decision-making task was the percent choice of the large reward/“risky” lever as a function of punishment probability (i.e., for each of the five trial blocks where the punishment probability changed), or simply denoted “risky choice.” Choice was also assessed by averaging all risky presses across all blocks with a non-zero punishment probability and analysing risky choice in the 1st block (un-punished) as well as risky presses on subsequent blocks (probable punishment). Additional parameters of interest included sensitivity to reward and punishment as measured by Win-Stay and Lose-Shift behaviour: the former denotes the probability that a rat repeats a risky lever choice following a risky win (i.e., large reward without punishment), whereas the latter the probability that a rat shifts responding onto the safe lever following a risky loss (shock). Furthermore, motivational parameters were also recorded and analysed. The first was choice latency, or deliberation time for a rat to make a lever response: this was averaged across all blocks (average latency), and further partitioned as a function of punishment probability (latency by block) or split across choice type (latency to press safe lever and latency to press risky lever). The number of trial omissions, where rats failed to make a lever response within the required time was also analysed. Parameters by block were
assessed by a three-way repeated-measures analysis of variance (ANOVA) using treatment (baseline vs. stress or dH₂O vs. yohimbine) and block as within-subjects factors and sex as the between-subjects factor. Parameters that did not require assessment by block were analysed using a two-way repeated-measures ANOVA using treatment and sex alone as factors. In all ANOVA’s the p-values were compared to an α = 0.05 for significance testing. Furthermore, we also analysed behavioural performance to assess any carry-over effects of restraint stress 24 hours later. In this case, we performed separate two- and three-way ANOVA’s to compare the appropriate parameters between the second day and baseline. Importantly, the restraint test was performed a second time, and the relevant behavioural data were analysed using analogous ANOVA’s to the first test.

For the behavioural control task, data from signal and non-signal trials were analysed separately. The key variable of interest for signal trials were the percent successful trials (no shocked received), the number of shock trials, response omissions, and response latencies, partitioned in terms of whether the animal nose-poked before cue termination (“early”) or waited (“hold”). Importantly, when a rat did not nose-poke and consume the sucrose reward during a trial, this was marked an omission and prevented subsequent pellet delivery until next retrieval. Thus, to control for the omissions, we computed a “shock index”: the number of shock trials divided by the total number of shock and success trials. In other words, this was the proportion of non-omitted signal trials as a fraction of the total number of responses made. Therefore, a score of one means that a given rat was shocked during every signal trial it did not omit, whereas a score of zero means the animal successfully withheld responding for at least 12 s on every signal trial and did not receive a shock for the entire session. For non-signal trials, we analysed the percentage of trials food retrieved and retrieval latencies. All parameters, unless otherwise
specified, were assessed using a two-way repeated-measures ANOVA with treatment and sex as factors and setting $\alpha = 0.05$ for significance testing. Furthermore, because the restraint test was performed twice for the purposes of this task as well, we also analysed behavioural data from this second test with similar two-way ANOVA’s.

Lastly, 1) behavioural measures of interest were also assessed 24 hours after each test session by adding a level of treatment to the above-mentioned two-way repeated-measures ANOVA’s, and 2) all bars on graphs represent the standard error of the mean (S.E.M.).
Chapter 3: Results

3.1 Experiment 1: Acute restraint stress increases risk aversion and decreases motivation during risky decision-making

The main objective of the first experiment was to assess how acute restraint stress alters choice preference and associated motivational functions in animals performing the RDT. To this extent, 32 rats (N = 16 males and 16 females) were trained for 28 days on the task and then received their first restraint stress test. Differences in shock current were analysed with a paired-sample t-test and revealed male discounting behaviour stabilised at a significantly higher level (0.43 mA; S.E.M. =0.021) compared to females (0.25 mA; S.E.M.=0.012) (p<0.0001). Choice data were analysed with two- and three-way repeated-measures ANOVA with treatment (baseline vs. stress; or baseline vs. 24 hours) and block (5 levels, where applicable) as withinsubjects factors and sex (2 levels) as a between-subjects factor. Notably, some animals displayed a large number of omissions, particularly the females in the group, and this effect was exacerbated by stress. This complicated the data analysis because 10 animals did not make any choices in at least one of the shock probability blocks under control and/or stress conditions, leading to missing data in the overall ANOVA model. To accommodate for this, a two-pronged approach was used to analyse the choice data. First, we analysed the data from the 0% probability block and the risky choice data averaged across the four other blocks with a three-way ANOVA using treatment (2 levels), block (2 levels) and sex as factors. This allowed us to include data from all animals and provided one way to quantify how the risk of shock altered preference for the larger reward and how this was affected by stress. The second approach was to analyse choice data from a subset of animals that made at least one choice across all blocks under both conditions.

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Acute restraint markedly reduced preference for the larger-reward lever associated with probabilistic punishment in a manner that was comparable between the two sexes. The discounting curve displayed in Figure 1A shows the choice data across all blocks under baseline and stress conditions and also displays the number of animals that made at least one choice in each particular block. Notably, all male and female rats made some choices in the first block where there was 0% probability of shock, but the number of females not making any choice increases in the latter blocks where choice of the larger reward came with a risk of shock.

Subsequently, we compared risky choice in the 0% probability block and the *average* of risky choices made across the other four blocks (Fig 1B). The results of this ANOVA revealed a significant main effect of treatment (F(1,30)=25.58, p<0.0001) and treatment by block interaction (F(1,30)=9.80, p<0.01). Partitioning the two-way interaction showed that stress caused a relatively small reduction of choice of the larger reward in the first, non-shock block of the task (F(1,31)=8.83, p<0.01). On the other hand, stress caused a comparatively larger reduction for the larger reward in the latter blocks when its delivery may also come with a foot shock (F(1,31)=23.02, p<0.0001). Notably, there was no main effect of sex or interaction with the other variables (all F’s<1.0, n.s.), indicating that stress induced a comparable effect on choice in both males and females.

To complement this analysis, we examined the choice data across all blocks in a subset of rats that made at least one choice in each block under both baseline and stress conditions. This resulted in a final N of 16 males and 7 females being included in the analysis (Fig. 1C). Here the ANOVA’s for risky choice revealed a main effect of treatment (F(1,21)=18.47, p<0.01), but only a trend towards a block by treatment interaction (F(4,84)=2.38, p=0.058). There was no main
effect of sex (F(1,21)=0.27, p=n.s.) nor a three-way interaction among the factors (F(4,84)=0.55, p=n.s.), suggesting again that the effects of stress on choice did not differ across sexes.

The decrease in risky choice was also accompanied by an increase in sensitivity to punishment but not to larger rewards as indexed by changes in Win-Stay (WS) and Lose-Shift (LS) ratios (Fig. 1D). The two-way ANOVA’s here revealed stress significantly increased lose-shift behaviour (main effect of treatment; F(1, 30)=11.329, p<0.01); no main effect of sex (F(1, 30)=0.08, p=n.s.) or interaction (F(1, 30)=0.193, p=n.s.). On the other hand, win-stay behaviour was unaffected by stress (all F’s<1.91, n.s.). Taken together, the results of these analyses suggest acute stress reduces risk-taking behaviour in a sex-independent manner and increases sensitivity to punishments.

Analysis of choice latency revealed restraint stress increased deliberation times. In particular, this effect was seemingly driven by the latter blocks with higher probability of punishment and prior to making a “safe” choice. Latency-by-block data were analysed using a similar design as the choice data, taking data from the first, 0% shock probability block and average latencies from the other four blocks (Fig. 1E). Analysis of these data with a similar three-way ANOVA showed a main effect of sex (F(1,30)=17.33, p<0.001) but no interactions with the treatment factor or three-way interaction (all F’s<1.0, n.s.), indicating that overall, females were slower to make choices compared to males but stress did not exacerbate this disproportionately. More pertinently, the analysis also revealed a main effect of treatment (F(1,30)= 25.53, p<0.001), and a significant block by treatment interaction (F(1,30)=15.87, p=0.029). As was observed with the choice data, stress caused a relatively slight (~150 ms) increase in choice latency in the 1\textsuperscript{st} block (F(1,31)=8.19, p<0.01), but a much larger increase in deliberation times in the latter blocks (F(1,31)=23.85, p<0.0001). Latency data by choice type
were assessed with a similar three-way ANOVA using choice type as a within-subjects factor (Fig. 1F). This analysis again revealed a main effect of sex (F(1, 29)=15.33, p<0.01), treatment (F(1, 29)=16.05, p<0.01), and in particular, a two-way interaction (F(1, 29)=5.94, p=0.02), although the three-way interaction was not significant (F(1,29) =0.081, p=n.s.). The two-way interaction was driven by the fact that stress did not alter the latency to make a risky choice (p>0.10) but did increase deliberation times prior to making a “safe” choice (p<0.01), suggesting the slower deliberation times following acute restraint primarily occurred prior to rats selecting the smaller reward.

As noted above, restraint stress also reduced task engagement, as indexed by an increase in trial omissions (Fig. 1G), with the analysis of these data revealing a significant main effect of treatment (F(1, 30)=67.968, p<0.01). The analysis also revealed a main effect of sex (F(1, 30)=41.82, p<0.01) with females omitting a greater proportion of trials. However there was no significant treatment by sex interaction (F(1, 30)=1.155, p=n.s.), indicating that stress did not induce a disproportionate increase in omissions in males vs. females. Collectively, these data suggest one-hour acute restraint stress significantly decreased preference for risk and decreased motivation to pursue the preferred, larger reward. In addition, females displayed less task engagement, as they had slower deliberation times and higher omissions than was observed in males, but stress did not disproportionately affect these measures in females.

Acute stress has also been reported to induce delayed effects on certain aspects of cognition that may persist 24 hours after the initial stressor (Mitra et al., 2005; Rao et al., 2012; Shinba et al., 2010). In light of this, separate analyses were conducted comparing performance for rats 24 hours after restraint stress to their pre-stress baseline using analogous two- and three-way ANOVA’s. However, we did not observe significant differences compared to baseline.
levels. Choice data did not reveal a main effect of treatment (F(1, 27)=1.265, p=n.s.) nor a block by treatment interaction (F(4, 112)=2.433, p=n.s.; data not shown). Furthermore, analysis of choice latency data also did not show a main effect of treatment (F(1, 30)=1.122, p=n.s.; data not shown), although a main effect of sex remained (F(1, 30)=11.72, p<0.01), suggesting females were overall slower than males. A similar assessment on omissions revealed no main effect of treatment (F(1, 30)=0.838, p=n.s.; data not shown), notwithstanding females overall omitting a greater proportion of trials, as evidenced by the main effect of sex (F(1, 30)=34.1, p<0.01).

Overall, these data reveal how restraint stress does not induce major effects on risky decision-making performance 24 hours after the initial testing.

After the first stress test, rats were retrained on the task until they again showed stable patterns of discounting. They then received a second stress test to assess whether the effects of restraint on choice would habituate with repeated exposure. We analysed the choice data in a similar manner to the first test, taking that from the 1st, 0% shock probability block and the average of the latter 4 blocks (Fig. 2A). These ANOVA’s revealed a main effect of treatment that did not achieve statistical significant (F(1, 30)=3.61, p=0.067), or significant interactions (all F’s<3.36, p=n.s.), suggesting the effects of restraint on choice diminished following the first test. However, a complementary analysis was conducted on the choice data across all blocks in which a subset of rats made at least one choice in each block under both baseline and stress conditions. This resulted in a final N of 14 males and 6 females being included in the analysis (data not shown). Here the ANOVA’s for risky choice revealed did reveal a main effect of treatment (F(1, 19)=8.58, p<0.01), but not main effect of sex or a treatment by sex interaction (all F’s<1.383, p=n.s.).
Analysis of the choice latency data revealed the second restraint test did not alter deliberation times, although females were generally slower than males. Indeed, the ANOVA’s on latency from all rats averaged across all blocks and choice type (Fig. 2B) only revealed a main effect of sex (F(1, 30)=18.47, p<0.001), but not of treatment nor an interaction (all F’s<1.897, p=n.s.).

Despite the lack of effect on the second stress test on choice latency, this manipulation did reduce task engagement, as indexed by an increase in trial omissions (Fig. 2C), with the analysis of these data revealing a significant main effect of treatment (F(2, 60)=44.51, p<0.001). The analysis also revealed a main effect of sex (F(1, 30)=86.55, p<0.001) and a treatment by sex interaction (F(1, 30)=6.58, p<0.001), indicating stress induced a disproportionate increase in omissions in females. Collectively, these data suggest the effects of restraint on choice persist following the second stress, albeit in a less pronounced form.
Figure 1. Discounting behaviour and motivation following one-hour acute restraint. A) Percent choice of the “risky” option as a function of punishment probability following one-hour no-stress baseline compared to restraint stress for all rats tested (N=32). Numbers below each data point denote the number of animals (male/females) that made at least one choice during that block. B) Percent choice of the risky lever in the safe block and the average number of choices across the 4 risky blocks. Stress affected risky choice only when risky choice could be punished (p<0.01). C) Percent choice of the “risky” option as a function of punishment probability following one-hour no-stress baseline compared to restraint stress for all rats that made at least one choice in each block. Restraint decreased risky choice (no sex difference; p<0.01). D) Probability of either making a risky press after a non-punished risky choice (WS) or making a safe press after a punished risky choice (LS). Restraint had no effect on WS but increased LS (p<0.01). E) Latency to make a lever response in the safe block and the four risky blocks (averaged). Stress affected decision latency only when risky choice could be punished (p<0.01). F) Latency by type of choice. Restraint had no effect on risky-lever latencies but increased safe-lever latencies (p<0.01). G) Overall omissions of lever responses. Acute restraint increased response omissions (p<0.01) with females overall omitting a greater proportion of trials.
Figure 2. Choice behaviour following the second restraint test. A) Percent choice of the risky lever in the safe block and the average number of choices across the 4 risky blocks for all rats tested (N=32). Restraint stress did not affect choice. B) Average latency to make a lever response across all blocks and choice type. Females were overall slower, but stress did not affect decision latencies. C) Overall omissions of lever responses. Acute restraint increased response omissions (p<0.001) with females overall being disproportionally more affected.
3.2 Experiment 2: Yohimbine does not affect risky choice but increases motivation

The aim of the second experiment was to determine whether the pharmacological stressor yohimbine, which increases noradrenergic release, altered risky decision-making in a manner similar to one-hour acute restraint stress. To this extent, the same 32 rats (N = 16 males and 16 females) used in the restraint stress experiment were retrained and then tested on the RDT four days after the second restraint test, when behavioural performance was stable. Here, choice data were similarly analysed with three-way repeated-measures ANOVA’s using block, treatment, and sex as factors. Likewise, we also utilised our two-pronged approach in the choice analyses due to six females that omitted at least one block in its entirety in either the vehicle or drug conditions, which led to missing data in the overall ANOVA model. Hence, we first analysed data from the 0% probability block and the averaged risky choice data across the remaining four blocks with a three-way ANOVA using treatment (2 levels), block (2 levels) and sex as factors.

Yohimbine did not induce any major effects on risky choice in either males or females. The discounting curve displayed in Figure 3A shows the choice data for all blocks under vehicle and drug conditions, and it includes data from all rats, including those that did not make any choices in at least one block. Similarly to experiment one, all male and female rats made at least one choice in the first block where punishment probability was 0%, whereas the number of females that did not make any choice increased in the latter blocks where there was at least a 25% probability of foot-shock delivery associated with the larger reward. Notably, the higher number of omissions by female rats occurring in the risky blocks was not exacerbated by yohimbine, thus it appeared to be limited to the vehicle condition. The results of this ANOVA did not reveal a main effect of sex, treatment, or significant interactions (all F’s<3.174), suggesting yohimbine did not generally affect choice behaviour. We then compared risky choice
in the 0% probability block and the average of risky choices made across the other four blocks (Fig. 3B). The results of this ANOVA revealed a main effect of sex (F(1, 30)=7.814, p<0.005), reflecting that by this phase of training, female rats had become more risk averse than males. However, there were no other significant effects nor interactions (all F’s<2.163), indicating that yohimbine did not alter preference between the safe and risky options.

Despite the lack of effects on choice, yohimbine did alter several motivational measures that contrasted with the effects of restraint. Analysis of the choice latency data revealed yohimbine decreased deliberation times (Fig. 3C), an effect that, unlike following physical restraint, was not seemingly driven by performance in the riskier blocks. Latency-by-block data were analysed using a similar design as the choice data, comparing data from the first, 0% probability block to the average latencies from the remaining four blocks. These ANOVA’s revealed a main effect of sex (F(1, 30)=25, p<0.0001) and a sex by block interaction (F(1, 30)=23.38, p<0.0001) but not an interaction with the treatment factor or a three-way interaction (all F’s<2.924), suggesting overall females were slower to make a lever response compared to males, but that yohimbine did not exacerbate this disproportionally. More pertinently, the analysis only revealed a trend toward a main effect of treatment (F(1, 30)=2.924, p=0.0976). However, exploratory comparisons showed that yohimbine caused a larger decrease in response latency in the first, non-shock block of the task (F(1, 30)=16.877, p<0.0005) but not when delivery of larger reward may also be accompanied by foot-shock (F(1, 30)=0.999, p=n.s.). On the other hand, when we analysed the effects of yohimbine on choice latency partitioned by choice type (risky vs. safe; Fig. 3D), we did observe a significant main effect of treatment (F(1, 30)=8.672, p<0.01) in the absence of any interaction with the treatment or sex factors (all
F’s<0.857, p=n.s.). Taken together, these data indicate that, in contrast to the effects of restraint stress, yohimbine reduced decision latencies.

In keeping with the reduced choice latencies, yohimbine also increased task engagement as evidenced by the reduced omission rates (Fig. 3E), with the pertinent analysis revealing a main effect of treatment (F(1, 30)=23.73, p<0.0001). The ANOVA also showed a main effect of sex (F(1, 30)=39.3, p<0.0001), with females omitting a greater proportional of trials, and a significant treatment by sex interaction (F(1, 30)=13.22, p<0.005), indicating a disproportionally higher decrease in females following treatment with yohimbine. Collectively, these data suggest a 1 mg/kg dose of yohimbine, while it may not affect risky choice, increases task engagement.
Figure 3. Discounting behaviour and motivation following acute administration of yohimbine. A) Percent choice of the “risky” option as a function of punishment probability following intra-peritoneal administration of dH$_2$O (vehicle) compared to yohimbine (1 mg/kg) for all rats (N=32). Yohimbine had no effect on choice. B) Percent choice of the risky lever in the safe block and the average number of choices across the four risky blocks. Yohimbine did not affect choice. C) Latency to make a lever response in the safe block and the four risky blocks (averaged). Yohimbine affected decision latencies only when risky choice was not punished (p<0.0001). D) Latency by type of choice. Yohimbine decreased choice latencies (p<0.01). E) Overall omissions of lever responses. Yohimbine significantly decreased response omissions (p<0.01) with females overall being disproportionally affected.
3.3 Experiment 3: Corticosterone does not affect risky choice but increases focus

The aim of the third experiment was to examine whether systemic treatment with physiologically relevant doses of CORT could recapitulate the effects of restraint stress on risky decision-making, especially since CORT is a major player in the rat stress response. To this extent, a different squad of rats (N = 8 males and 8 females) were trained on the RDT for 28 days prior to administration of two CORT challenges (1 mg/kg and 3 mg/kg) in a counterbalanced manner. Differences in shock currents were analysed with a paired-sample t-test and did not reveal a difference in current level between males (0.39 mA; S.E.M.=0.022) compared to females (0.38 mA; S.E.M.=0.035) (p=n.s.). Here, choice and motivational data were analysed with two- and three-way ANOVA’s analogous the first and second experiments, using three levels of treatment (average vehicle vs. 1 mg/kg dose vs. 3 mg/kg dose) and, where appropriate, two levels of block. First, we compared the choice, latency, and omission data from the two vehicle treatments given on the day prior to different doses of CORT: there were no differences on any of these measures across the vehicle tests (all F’s < 5.403, n.s.), so the data from these two vehicle tests were averaged for the final analysis. Furthermore, because only one female in each drug condition omitted at least one block entirely, we analysed choice data without the need for the previous two-pronged approach.

Figure 4A and B reveal the effect of CORT on risky choice. The relevant ANOVA’s did not show any main effects or interactions for both doses (all F’s <1.295, n.s.), suggesting CORT does not affect risky choice.

Analysis of choice latency data revealed both doses of CORT decreased deliberation times. Here, we compared latency for the 1st, non-shock block and the averaged latency for all four blocks where risky choice may also deliver foot-shock (Fig. 4C and D). These ANOVA’s
showed a main effect of treatment ($F(2, 28)=4.027$, $p<0.05$), of block ($F(1, 14)=39.542$, $p<0.0001$), but not a main effect of sex ($F(1, 14)=0.462$, $p=n.s.$), suggesting the decrease in deliberation times was not different between males and females. Notably, there was a significant block by treatment interaction ($F(2, 28)=3.398$, $p<0.05$), driven by the fact that CORT did not affect decision latencies in the 1st block, when a risky press was always safe, but it decreased deliberation times in the riskier blocks, when punishment probability was at least 25% ($p<0.05$), suggesting the gluco-corticoid primarily affected choice latency when a risky press could also be punished. Further post-hoc analyses using Dunnett’s test revealed both doses of CORT were equally effective at inducing the observed decrease in latency (all $p’s=n.s.$), suggesting this effect was not dose dependent.

Furthermore, analysis of the omission data revealed CORT did not affect task engagement (data not shown). Indeed, while the ANOVA’s showed a main effect sex ($F(1, 14)=6.692$, $p<0.05$), indicating females overall omitted a greater proportion of trials than males, there were no effects of treatment nor interactions for both doses (all $F’s<2.353$, n.s.). Collectively, these data revealed CORT did not induce any major effects on risky choice, except for a decrease in latency in the risky blocks, suggesting that increased CORT release on its own is not the primary mediator of the effects of acute restraint on risky decision-making.
Figure 4. Discounting behaviour and motivation following acute administration of corticosterone. A, B) Percent choice of the “risky” option as a function of punishment probability following administration of CORT (A: 1 mg/kg, B: 3 mg/kg) compared to vehicle (50%-50% Propylene glycol-NaCl) for all rats (N=16). There were no effects on choice. C, D) Latency to make a lever response in the safe block and the four risky blocks (averaged) (C: 1 mg/kg, D: 3 mg/kg). CORT decreased decision latencies in the risky blocks (p<0.05).
3.4 Experiment 4: Acute restraint stress alters inhibitory impulse control differently between males and females

A separate series of experiments assessed changes in impulsive behaviour and associated motivational functions following acute restraint in situations presenting a response conflict wherein rats were required to delay responding for reward to avoid punishment. To this end, 32 rats (N = 16 males and 16 females) were trained for 14 days on the behavioural control task and then received two stress tests, which were analysed with two-way ANOVA’s using three levels of treatment (baseline vs. restraint vs. 24 hours) as the within-subjects factor and sex as a between-subjects factor for all measures. Post-hoc analyses on group means were conducted on Dunnett’s tests that compared the restraint and 24 hours conditions means against the baseline means. These analyses included data from the 24-hour condition because we noticed differences in how acute stress affected performance in male vs. female rats during this phase of testing, as described below. Average shock currents for males and females were compared with a paired-sample t-test and did not reveal a difference between males (0.45 mA; S.E.M.=0.021) and female (0.44 mA; S.E.M.=0.024) (p=n.s.).

With respect to non-signal trials, where animals merely retrieved food without the threat of shock, acute restraint did not alter un-punished food retrieval. The ANOVA’s did not reveal any effects or an interaction for the number of successful trials (Fig. 5A) or response latencies (Fig. 5B) (all F’s < 3.96, n.s.), suggesting stress did not alter the basic motivation to retrieve freely-available food rewards, nor did it affect performance on these trials 24 hours later. We did observe a trending main effect of sex (F(1, 30)=3.96, p=0.056), which points to females being overall slower than males.
In contrast, analysis of the effects of stress on signal trials revealed impulsive action in males and females was affected differently, as males showed a significant increase in premature response from baseline, but female behaviour was seemingly not altered. In particular, the ANOVA on the shock index (Fig. 5C) showed a treatment by sex interaction (F(2, 60)=4.380, p<0.05), but not a main effect of sex (F(1, 30)=2.003, p=n.s.) or of treatment (F(2, 60)=1.216, p=n.s.). Multiple comparisons confirmed that the treatment by sex interaction was driven in part by the fact that on the test day, stress increased impulsivity in males (p<0.05) but not females. Of interest, stress did not affect the proportion of successful signal trials, (all F’s < 2.092, p=n.s.; data now shown), indicating only premature responding was altered. In stark contrast, analysis of the shock index 24 hours later reveals an interesting, sex-dependent effect. Multiple comparisons with Dunnett’s tests showed that 24 hours after the stress test day, females showed a decrease in impulsive action (p<0.05), whereas male behaviour returned to baseline levels.

Furthermore, stress also affected the animals’ motivation to pursue reward. Analysis of latency measures revealed males but not females showed faster response latencies on trials where they made a premature response before the warning signal was turned off and received a shock (Fig. 5D). The ANOVA’s showed a main effect of treatment (F(2, 60)=3.172, p<0.05) and a treatment by sex interaction (F(2, 60)=3.355, p<0.01) but not a main effect of sex (F(2, 60)=0, p=n.s.), suggesting the increase in male but not female impulsive behaviour was accompanied by faster approach latencies. However, males also became slower to retrieve food when they were able to withhold nose-poking in the presence of cues (Fig. 5E), as the relevant ANOVA’s revealed a main effect of treatment (F(2, 60)=5.348, p<0.01), but not of sex (F(1, 30)=2.435, p=n.s.) and a treatment by sex interaction (F(2, 60)=5.348, p<0.005). Partitioning the two-way interaction further revealed that it was driven by the fact that on test day males (p<0.05) but not
Figure 5. Impulsive action and motivation following one-hour acute restraint. A-F) First restraint stress test (N=32). A, B) Non-signal trials. A) Percent trials food successfully retrieved following one-hour no-stress baseline compared to one-hour acute restraint stress. B) Latency to nose-poke to retrieve food. Females were overall slower, but acute restraint had no effect on any of these measures. C-F) Signal trials. C) Shock Index: Proportion of trials that were punished divided by total number of responses made following one-hour no-stress baseline compared to one-hour acute restraint stress. Acute restraint increased the index in males only (p<0.05); however, twenty-four hours later the shock index is reduced in females only (p<0.05). D, E) Latency either to make an early nose-poke (punished trial) or to nose-poke after cue termination (non-punished trial). Acute restraint altered “early” deliberation times (p<0.05) as well as “hold” latencies (p<0.05) in males. F) Number of trials omitted following one-hour acute restraint stress. Females overall omitted more trials, but acute restraint did not alter response omissions. G-J) Second restraint stress test (N=32). G) Shock Index. Acute restraint did not affect males but continued to decrease impulsive action in females 24 hours post-stress (p<0.05). H, I) “Early” and “hold” decision latencies. Restraint did not alter either measure. J) Response omissions. Acute restraint had no effect on omitted signal trials.
females (p=n.s.) were faster at retrieving reward after cues, indicating males may have been hesitant to pursue reward when cues signalled foot-shock. In contrast to this effect, no other differences were observed for any of the other motivational measures obtained 24 hrs after the stress test (all p’s=n.s.).

Finally, stress did not seemingly affect task engagement on signal trials, as evidenced by the analysis of the omission data (Fig. 5F). The relevant ANOVA’s indeed did not show a main effect of treatment or an interaction (all F’s<2.124, n.s.), suggesting restraint primarily affects deliberation times, but we observed also a main effect of sex (F(1, 30)=5.561, p<0.05), showing females generally omitted a greater proportion of trials. The lack of effect of stress on omitted trials indicates that the increase in shock index observed in males was not attributable merely to a reduced number of trials where they made a response.

After the first stress test, rats were re-trained on the task until they again displayed stable patterns of operant performance. They then received a second stress test to assess whether the effects of restraint on inhibitory impulse control would habituate with repeated exposure, analysing these data in a manner similar to the first test. The ANOVA’s did not reveal any effects on the non-signal trials (all F’s 1.953, n.s.; data not shown), except again females were generally slower than males (F(1, 30)=4.856, p<0.05). On the other hand, analysis of the shock index (Fig. 5G) revealed once more an overall treatment by sex interaction (F(2, 60)=5.780, p<0.01), but not a main effect of sex or of treatment (all F’s<1.061, n.s.). In this instance, multiple comparisons with Dunnett’s tests revealed that the second acute stress test no long affected impulsive action in males, whereas this treatment continued to reduce premature responses in females 24 hrs after the stress test (p<0.05). Lastly, no differences on motivational
measures were observed following the second restraint test (Fig. 5H, I, and J; all F’s<1.760, p=n.s.).

Collectively, these data indicate acute stress differently alters the effects of punishment in conflict situations involving reward-seeking between males and females. In addition, in contrast to the effects of restraint on the RDT, we observed that 1) there were delayed effects of stress on inhibitory impulse control on females alone, suggesting the two forms of punished reward-seeking may be differentially influenced by stress, and 2) that male rats habituated to the effects of stress on response inhibition, whereas the differential effect observed in females remained following a second stress exposure.
3.5 Experiment 5: Yohimbine mimics the effects of acute restraint on inhibitory impulse control

The aim of the fifth experiment was to determine whether yohimbine would alter inhibitory impulse control in a similar manner to acute restraint, akin to experiment two in the context of the RDT. To this extent, the same 32 rats (N = 16 males and 16 females) used in experiment four were re-trained and then tested on the behavioural control task 3-4 days after the second restraint test, when behaviour reached stable levels. Similarly to experiment four, these data were all analysed using two-way ANOVA’s using treatment (3 levels; vehicle vs. drug vs. 24 hours) as a within-subjects factor and sex (2 levels) as a between-subjects factor.

Regarding the non-signal trials, yohimbine did not alter un-punished reward retrieval, nor did we observe delayed effects the next day, as evidenced by the absence of main effects or interactions for successful trials (Fig. 6A) (all F’s < 2.000, n.s.) or response latencies (Fig. 6B), as the ANOVA’s revealed a main effect of sex (F(1, 30)=6.186, p<0.05) but not of treatment or a significant interaction (all F’s<1.557), suggesting females were generally slower at retrieving food.

Analysis of the shock index (Fig. 6C), on the other hand, revealed yohimbine mimicked the effects of restraint on impulsive action. The ANOVA’s showed a main effect of treatment (F(2, 60)=27.87, p<0.0001) but not a main effect of sex or a significant interaction (all F’s<2.02, n.s.), suggesting impulsive action was increased for all rats. Further post-hoc comparisons revealed 24 hours later rats returned to baseline levels of performance (p=n.s.), indicating that when averaged across all rats, the observed increase in impulsive action decreases back to baseline levels the day after. However, in light of the delayed and sex-dependent effects on shock index observed following acute restraint, additional exploratory comparisons were
conducted to assess how males and females performed on drug test day vs. 24 hours. To this extent, shock index values were compared using two levels of treatment (vehicle vs. drug; or vehicle vs. 24 hours) to compute separate ANOVA’s for the 24 hours data. On the day of injection, these additional ANOVA’s revealed a main effect of treatment (F(1, 30)=10.650, p<0.01) and also a treatment by sex interaction (F(1, 30)=4.076, p=0.05), but not a main effect of sex (F(1, 30)=0.984, p=n.s.), which was driven by an increase in male impulsive action (p<0.01). On the following day, the ANOVA’s also revealed a main effect of treatment (F(1, 30)=15.343, p<0.0005) but not of sex nor a significant interaction (all F’s<1.822, n.s.), and that this effect was driven by a decrease in female impulsive action (p<0.005). The results of these additional comparison suggest that the main effect of treatment effect observed in the overall ANOVA with three treatment levels was driven by males on the day of injection, but by females 24 hours later.

Lastly, there were seemingly no major effects on motivation to pursue reward either on test day or 24 hours later. In this context, none of the ANOVA’s on latency (Fig. 6D and E) or omission (Fig. 6F) data were significant (all F’s<2.535, n.s.), suggesting the effects of yohimbine on inhibitory impulse control may not alter task engagement or willingness to retrieve reward under threat of shock.

Collectively, these results suggest that yohimbine can alter inhibitory impulse control differently between males and females and, at least in part, some of the effects observed following restraint stress may plausibly be driven by increased noradrenergic transmission.
Figure 6. Impulsive action and motivation following acute administration of yohimbine.

A-B) Non-signal trials. A) Percent trials food successfully retrieved after intra-peritoneal administration of dH$_2$O (vehicle) compared to yohimbine (1 mg/kg) for all rats tested (N=32). Yohimbine had no effect on food retrieval. B) Latency to nose-poke to retrieve food following one-hour acute restraint stress. Females were overall slower, but yohimbine did not affect latency. C-F) Signal trials. C) Shock Index. Yohimbine increased the index in males only (p<0.01), but twenty-four hours later it is reduced in females only (p<0.01). D) Number of trials omitted. E, F) Latency either to make an early nose-poke (punished trial) or to nose-poke after cue termination (non-punished trial). Yohimbine did not alter any of these measures.
3.6 Experiment 6: Corticosterone does not recapitulate the effects of acute restraint on inhibitory impulse control

The aim of the sixth experiment was to determine whether systemic treatment with CORT might mimic the effects of restraint stress on inhibitory impulse control. To this extent, a different squad of rats (N = 8 males and 8 females) were trained on the behavioural control task for 14 days prior to administration of two CORT challenges (1 mg/kg and 3 mg/kg) in a counterbalanced manner. Differences in shock currents were analysed with a paired-sample t-test and did not reveal a difference between males (0.56 mA; S.E.M.=0.026) and female (0.55 mA; S.E.M.=0.027) (p=n.s.). In this experiment, there was a difference in the shock index on vehicle test days that preceded tests with the 1 and 3 mg/kg doses of CORT (F(1,15)=11.24, p<0.01), precluding us from averaging the vehicle test days in this analysis. To accommodate for this, each variable was analysed with three-way ANOVA’s using sex as the between-subjects factor, and treatment day (vehicle vs. CORT injection vs. 24 hours) and dose on test (1 vs. 3 mg/kg) as the within-subjects factors.

On the non-signal trials neither dose of CORT altered un-punished reward retrieval or induced carry-over effects 24 hours later, as evidenced by the lack of effects or interactions on all ANOVA’s for successful trials (Fig. 7A and B) and latency (Fig. 7C and D) (all F’s < 2.553, n.s.), suggesting increasing glucocorticoids do not affect pursuit of freely-available reward.

Analysis of the shock index (Fig. 6E and F) revealed both doses of CORT did not affect impulsive action in males or females. In particular, the ANOVA’s only revealed a main effect of dose (F(1, 14)=4.741, p<0.05) as all other factors or interactions did not show significance (all F’s<2.916, n.s.), simply suggesting that the shock index was higher on 3 mg/kg test days overall.
Notably, neither males nor females showed delayed effects on impulsive action on the following day (p=n.s.).

Furthermore, analysis of the latency data reveals CORT did not affect speed of premature responding (Fig. 7G and H), as the ANOVA’s did not show any significant effect or interactions (all F’s<3.127, n.s.). However, CORT altered speed of responding after holding reward retrieval on signal trials (Fig. 7I and J), as the ANOVA’s revealed a main effect of sex (F(1, 13)=5.173, p<0.05) and a dose by day interaction (F(2, 26)=5.840, p<0.01), whereas all other factors or interactions did not yield significant effects (all F’s<2.014, n.s.), suggesting CORT increased reward retrieval latencies after cues on the day of injection. Partitioning the two-way interaction showed this effect was driven by the higher dose (p<0.05) but not the lower dose, indicating that rats were quicker at retrieving food after holding approach after receiving the 3 mg/kg dose.

Finally, CORT did not alter task engagement, as evidenced by the absence of effects or interaction in the appropriate ANOVA’s on omission data (Fig. 7K and L) (all F’s < 2.553, n.s.), and none of these motivational measures showed significant effects 24 hours post-injection (all p’s=n.s.).

Collectively, these data suggest CORT does not induce major effects on inhibitory impulse control, indicating that increases in this glucocorticoid may not be the primary driver the behavioural alterations observed following restraint.
Figure 7. Impulsive action and motivation following acute administration of corticosterone. A-D) No-signal trials (A, C: 1 mg/kg; B, D: 3 mg/kg). A, B) Percent trials food successfully retrieved following vehicle injection (50%-50% Propylene Glycol-NaCl) compared to CORT for all rats tested (N=16). CORT had no effect on food retrieval. C, D) Latency to nose-poke to retrieve food. CORT had no effect on deliberation times. E-L) Punished trials (E, G, I, K: 1 mg/kg; F, H, J, L: 3 mg/kg). E, F) Shock index. CORT had no effect. G-J) Latency either to make an early nose-poke (punished trial) or to nose-poke after cue termination (non-punished trial). CORT affected “hold” latencies at the high dose (p<0.05). K, L) Number of trials omitted following one-hour acute restraint stress. Corticosterone did not alter response omissions.
Chapter 4: Discussion

The goal of this investigation was to elucidate the effects of acute stress on distinct forms of reward-related cost/benefit decision-making under risk of punishment in different reward-seeking paradigms. Firstly, we adapted a risky decision-making task and found that one-hour acute restraint reduces preference for the larger, yet riskier reward option. This was accompanied by an increase in punishment sensitivity and a reduction in motivation to engage in risky decision-making, as revealed by slower response latencies and a greater proportion of trials omitted, and there were no delayed effects 24 hours later. Secondly, we administered yohimbine and CORT challenges to determine whether another form of stress or stress-related hormone would mimic the behavioural and motivational effects of restraint. Importantly, the α-2 adrenergic antagonist did not recapitulate the effects of restraint on punished choice, nor did it mimic restraint-induced motivational alterations, as it increased focus and engagement on task given response latencies were faster and fewer trials were omitted. Similarly, CORT did not appear to mediate any of the observed effects of acute restraint. Next, we investigated how acute stress may modulate reward-seeking processes that require behavioural restraint on a different, simpler operant assay. Thus, we found that restraint increases impulsive action on males alone, whereas females become less impulsive 24 hours later. Lastly, exogenous administration of yohimbine, but not of CORT, is able to mimic these effects, suggesting changes in impulsive action may be mediated by noradrenergic transmission but not glucocorticoids. Collectively, these findings suggest acute stress enhances the effects of punishment on choice between a small/safe reward and a large/risky reward and impairs inhibitory impulse control in conflict situations involving rewards and punishment, and that some of these effects are plausibly mediated by noradrenergic transmission.
4.1 Stress and decision-making involving punishment

The goal of our first experiment was to investigate the effects of acute restraint on risky decision-making involving punishment. Here we observed one-hour restraint significantly reduces preference for the larger, punishment-associated reward. This decrease in risky choice was also accompanied by an increase in punishment but not reward sensitivity, as rats were more likely to shift responding onto the safe lever upon a punished risky press. Previous research from our laboratory also showed that either acute restraint or increases in CRF transmission do not alter choice between smaller and larger rewards of equal costs as assessed in a reward magnitude discrimination (Bryce & Floresco, 2016; Shafiei et al., 2012), suggesting restraint should not have interfered with choice of either type of reward per se. Notably, these stress-induced alterations on choice were not sex-dependent, as we did not observe any difference between male and female behaviour. Furthermore, these effects are similar to what had been previously reported in the context of effort discounting, where the larger reward is associated with greater physical cost (Bryce & Floresco, 2016; Shafiei et al., 2012), but are in stark contrast to previous studies on probabilistic and delay discounting, in which restraint did not alter preference for either uncertain or delayed rewards (Bryce et al., 2020; Shafiei et al., 2012). Thus, these results seem to indicate that acute restraint enhances the effects of punishment on choice between small/safe and large/risky rewards, and that these effects are expected to be more pronounced when reward costs are not subjective, i.e., when animals must expend a caloric cost or potentially receive punishment upon choosing a larger, more preferred reward.

Acute restraint also reduced motivation to engage in risky decision-making, as evidenced by the slower response latencies to choose the risky option on the RDT. This effect, interestingly, seems to be driven by the blocks in which punishment probability was higher (at
least 50%) and to occur prior to a “safe” choice. Indeed, this would suggest animals were motivated to pursue the larger reward and avoid punishment, thus plausibly “hesitating” before settling onto the safer option. Importantly, the level of task focus was high at baseline and females were overall slower than males at responding; however, restraint did not affect females more disproportionally, as the increase in the deliberation times was comparable between the two sexes. Similarly, task engagement was also reduced following restraint, as the omission rates were substantially higher; even in this context, males and females were not disproportionally affected, as the latter were displaying a relatively high omission profile at baseline as well. This effect, nonetheless, does show how male and female behaviour differs during stress, as both males and females were more likely to shift onto the safer option, but females were also more likely to disengage from task performance. These impairments in motivation are also in line with previous work showing how restraint stress (as well as increased CRF transmission) generally reduces motivation to pursue reward irrespective of choice (Bryce et al., 2020; Bryce & Floresco, 2016; Shafiei et al., 2012), even in the face of null effects on the latter. This would suggest acute stress alters the sustained levels of performance in situations that require cost/benefit deliberation between different options to achieve a desired reward, which has also been observed in individuals with depression displaying motivational anhedonia (Horan et al., 2007; Pizzagalli et al., 2007).

As mentioned previously, we also repeated the restraint test a second time to investigate whether rats would habituate to any of its effects, and we observed restraint also reduced preference for the punishment-associated larger reward. Contrary to the first stress test, however, this was much less pronounced, as the effect was only observed when analysing data from rats that made at least one choice in each block, suggesting rats may have habituated to restraint. This
second physical stressor also reduced motivation to pursue the risky reward, albeit in a less pronounced manner; indeed, while the increase in omission rates was similar, we did not observe an increase in deliberation times – except for the generally slower females – suggesting rats showed some levels of habituation in terms of task focus. Notably, we also did not observe any delayed effects 24 hours after either test for all behavioural measures. This would indicate the effects of physical restraint on risky choice did not induce any longer-lasting changes and that optimal performance was not altered beyond the acute response to restraint, suggesting rats may recover quickly from and display some levels of habituation to the effects of repeated stress on some aspects of motivation.

4.1.1 Neurochemical mechanisms of restraint stress on risky decision-making

We investigated potential mechanisms mediating the effects of restraint on risky choice, as the goal of experiment two was to assess whether yohimbine – a pharmacological means of inducing stress – could recapitulate the above-mentioned effects of restraint. Notably, yohimbine did not alter choice on the RDT, since rats did not become more risk-averse as we had observed following restraint or more risk-preferring. Furthermore, the drug also did not mimic any of the effects of physical stress on motivation: in the first experiment we observed a decrease in task focus and engagement, whereas here there was an increase in task engagement, as evidenced by the lower omission rates, suggesting noradrenergic transmission does not play a role in mediating the effects of restraint on risky decision-making. These data are also in line with previous reports on the effects of increased NA on decision-making in rodents treated with yohimbine or the NA uptake blocker atomoxetine (Blaes et al., 2018; Chernoff et al., 2021) and humans treated with yohimbine (Metz et al., 2020). Thus, although restraint stress can increase
noradrenergic transmission, the results of this experiment suggest the effects of this treatment on risky decision-making do not appear to be driven by enhanced noradrenergic signalling.

The goal of the third experiment was similar to that of experiment two, in that we investigated whether CORT would mimic the effects of acute restraint on the RDT. In this context, increased levels of the glucocorticoid did not alter risky choice, an effect that contrasted with what we had observed following restraint. Motivation to engage in risky decision-making also had distinct effects compared to restraint, as there were no alterations on omissions following either challenge but there was a decrease in decision latencies in those blocks where a risky press may also be punished. The CORT data overall suggest that the effects of physical stress on risky decision-making are not plausibly mediated by CORT, and that these effects are perhaps unsurprising given our previous study on effort discounting revealed the reduction in preference for the larger reward associated with greater physical effort costs induced by restraint was not recapitulated by CORT (Shafiei et al., 2012). It may be speculated, however, that the faster response latencies observed here, and which contrast with the lack of motivational alterations therein observed, relate to the potential added cost of punishment: while CORT is not affecting speed of responding when any choice will never be punished (safe block), it may make rats more sensitive to the unpleasant consequences of foot-shock when a (risky) choice may be punished, thus increasing task focus. The null effects, on the other hand, contrast with other studies in which exogenous CORT mimicked the effects of acute restraint on other cognitive functions, such as non-spatial learning and contextual fear conditioning (Cordero et al., 2003; Vargas-López et al., 2015). In humans it is also relatively well established that increased levels of cortisol are associated with cognitive impairments related, e.g., to memory and attention (Bohnen et al., 1990; Newcomer et al., 1999). Therefore, it can be argued that the disruptive
effects of glucocorticoids on cognitive functions are selective and dependent on whether or not the animal must make a decision.

4.2 Stress and response inhibition involving punishment

The aim of the fourth experiment was to ascertain how one-hour restraint stress would affect punished reward-seeking in situations requiring response inhibition as assessed on the behavioural control task. In this context, we observed a significant change in impulsive action as compared to baseline, whereby operant behaviour was modulated by restraint in a sex-dependent manner. However, stress did not alter retrieval of freely available rewards, as we observed no change in the percent successful trials in which the cues were not on, nor did it alter motivation to retrieve reward, as evidenced by the response latencies. Overall, this would suggest that restraint stress does not reduce motivation to pursue rewards generally, as experiment one as well as previously reported data seemed to entail (Bryce &Floresco, 2016; Shafiei et al., 2012), rather that they may become more apparent in situations where animals choose between different types of rewards, which require greater cognitive effort related to evaluating the costs and benefits of different options.

4.2.1 Sex differences in impulsive action

As has been alluded to, in the trials where the cues signalled foot-shock if reward is retrieved early, we observed significant sex differences regarding the effects of stress on impulsive behaviour. As evidenced by the shock index, males alone became more impulsive following restraint: they were more likely to retrieve reward early and receive a foot-shock when they were in a state of stress. Importantly, previous studies have reported discordant findings regarding the effects of different forms of stress on impulsivity. For example, pharmacological
challenges of MK-801² and atomoxetine administered to the offspring of chronically stressed dams during pregnancy (Wilson et al., 2012) increased premature responding on the five-choice serial reaction time task (5-CSRTT), while maternal deprivation showed the same effect on both 5-CSRTT (Kentrop et al., 2016) and differential reinforcement of low rates of responding (DLR; Lovic et al., 2011). On the other hand, while acute restraint did not affect choice between small/immediate vs. large/delayed rewards in a delay discounting paradigm (Shafiei et al., 2012), maternal deprivation actually increased choice of the larger, delayed reward (Lovic et al., 2011). In particular, the latter effect was only observed in males, as females were not affected, suggesting males may be more susceptible, but females more resilient, to the stressor. Collectively, these and our data plausibly suggest 1) that stress generally increases impulsive action (5-CSRTT, DLR, behavioural control task), and 2) that the effects of stress on impulsive choice (delay discounting) seem to be apparent when the stressor is administered earlier in life.

Females, however, displayed a distinct change in behaviour in response to acute restraint as compared to males in the signal trials. On the day of restraint females were not any more or less impulsive than at baseline, whereas 24 hours later they became significantly less impulsive. Females were less likely to retrieve reward early and receive foot-shock, whereas on this measure, males showed no differences from baseline 24 hours later. This suggests that the punishments that were experienced by females on the stress test day may have been amplified in some manner that led to reduced punished responding on the following day; in essence, females were more likely to learn from their “mistakes” while they were in a state of stress. In contrast to what we observed on the RDT, there were not major motivational differences between males and

² A non-competitive inhibitor (channel pore blocker) of the glutamate N-methyl-D-aspartate receptor
females, except that the former 1) were generally faster at retrieving early on test day, and 2) became slower to nose-poke after cues. Furthermore, previous studies reported various delayed effects of acute stress: restraint was shown to induce an anxiety-like phenotype on the elevated-plus maze (Hartman et al., 2019; Mitra et al., 2005) and an increase in dendritic spine density in the basolateral amygdala (Mitra et al., 2005); whereas foot-shock alone induced a NA increase in hypothalamus and cortex seven and ten days post-stress, respectively (Shinba et al., 2010).

Importantly, all rats employed in these previous experiments were exclusively males, while much remains unknown in regard to females. It can be speculated, in our case, that stress did not affect female impulsive action on test day because they generally displayed lower levels of motivation compared to males, hence they were less willing to engage in task behaviour, and because the effects of stress seem to become apparent when animals exert greater inhibitory control. On the other hand, the behavioural and physiological state they experienced while under stress could have enabled them to realise that they had made a greater number of premature approaches than it was otherwise optimal, and that based on this outcome, they were able to adjust their behaviour on the following day and manifest a decrease in impulsivity.

4.2.2 Neurochemical mechanisms of restraint stress on impulsive action

Experiment five examined whether yohimbine could mimic the above-mentioned effects on inhibitory impulse control. Contrary to the effects of this treatment on the RDT, yohimbine recapitulated the effects of restraint on the behavioural control task: the pharmacological stressor did not affect unpunished reward-retrieval; male impulsive action increased on test day; and female impulsive action decreased the day after. On the other hand, we did not observe any major motivational changes, as yohimbine did not alter response latencies or omissions. Furthermore, previous studies also showed that acute yohimbine
administration increases premature responding in both the 5-CSRTT, the cued rat gambling task (Broos et al., 2017; Chernoff et al., 2021; Sun et al., 2010) and a response-inhibition task\(^3\) (Mahoney et al., 2016). Thus, while these and our experiments plausibly confirm the role of NA on impulsive action, only our data, to the best of our knowledge, address sex differences (as opposed to assessing male behaviour exclusively) as well as the role of explicit, positive punishment (i.e., foot-shock) in the context of response inhibition. Taken together, experiments four and five would then suggest that the effects of restraint, at least in part, are driven by NA transmission.

Lastly, the goal of the sixth experiment was to investigate whether CORT would recapitulate the effects of restraint on the behavioural control task. We observed, however, that neither dose of CORT affected impulsive action, and that animals became quicker at nose-poking after cues, suggesting the gluco-corticoid did not mimic the effects of physical restraint stress. In particular, and similarly to the effects of CORT described in the third experiment, the faster “hold” latencies may indeed point to a potential role for CORT in modulating the rats’ sensitivity to the unpleasantness of punishment by increasing task focus, as the animals waited significantly less before reward-retrieval following the end of the punishment signals. Contrary to the RDT, however, the role of CORT in impulsivity in situations involving punishment or behavioural restraint remains relatively unexplored, especially in rodents. For example, adolescent rats chronically treated with CORT did not show major behavioural alterations in adulthood, but became more sensitive to the effects of yohimbine, on the 5-CSRTT; on the other

\(^3\) Rats learn right or left alone lever is always rewarded (discriminative light cue), but must wait a variable time before pressing, otherwise the next trial is initiated without being rewarded.
hand, rats became more impulsive as assessed on the stop-signal reaction time task, suggesting
the effects of CORT may be affecting impulsive choice rather than impulsive action
preferentially (Torregrossa et al., 2012). Collectively, these data suggest the effects of restraint
on impulsive action may not be plausibly mediated by gluco-corticoids.

4.3 Implications

The observed deficits in cost/benefit decision-making can provide fundamental insights
in the context of stress-related disorders, notably MD, which is a complex and heterogeneous
psychiatric disorder, the diagnosis of which requires either of two core symptoms: persistently
low (depressed) mood and motivational anhedonia (Davidson et al., 2010). The latter,
importantly, refers to the lack of interest or pleasure in activities previously found to be
interesting or pleasurable; however, individuals with MD are still able to experience interest and
pleasure in said activities once they participate in them (Rizvi et al., 2016; Treadway et al.,
2012).

There is a growing body of evidence suggesting the more predominant clinical symptoms
may plausibly stem from maladaptive learning about rewards and punishments and a
consequential inability to adjust behaviour (Alloy et al., 2016; Hevey et al., 2017; Tafet et al.,
2016). Indeed, motivational anhedonia (reward hyposensitivity) and negative bias (punishment
hypersensitivity) in the MD population seem to point to depression as a cognitive disorder of
disrupted reinforcement learning characterised by augmented punishment but diminished reward
sensitivity. Consistent with this hypothesis, clinical studies using functional magnetic resonance
imaging have shown a diminished reward-prediction error signal in striatal regions during
learning but normal signals in different contexts (Kumar et al., 2018; Rutledge et al., 2017), and
these abnormalities seem to persist both with and, importantly, in the absence of anti-depressant
medication. Furthermore, while affective dysfunction is a classical feature of the disorder, cognitive impairments remain one of the most debilitating features of MD (Clark et al., 2009; Perini et al., 2019). These include, but are not limited to, deficits in visual memory, attention, processing speed as well as higher-order executive functions such as working memory and behavioural flexibility.

These data thus seem to suggest a broader decision-making deficit that underlies the observed impairments in motivation and reward learning in individuals with MD. In line with several pre-clinical studies, including previously described work from our laboratory, individuals with depression were indeed shown to bias choice away from more preferred but delayed rewards as well as rewards that may or may not be delivered (Felton et al., 2020; Pulcu et al., 2014; Treadway et al., 2012). This shift in decision-making was even greater when the cost of the preferred reward required exerting greater physical effort (Treadway et al., 2012), pointing out abnormal decision-making in MD may be modulated by the type of reward cost. Although the neurochemical and hormonal abnormalities associated with MD are far more complex than those induced by acute stress, the results of the studies described here suggest that deficits observed in MD may be driven in part by mechanisms that are similar to those triggered by acute stress.

4.4 Conclusion

Here we report pre-clinical evidence that acute restraint stress can modulate distinct forms of punished reward-seeking selectively. In particular, we show how restraint enhances the effects of punishment on choice between rewards of different magnitude by decreasing risk-taking without any delayed effects. Yet, we also show that restraint alters inhibitory impulse control differently between males and females in conflict situations involving punishment and
rewards with pronounced delayed effects on female behaviour. Furthermore, modulation by 
noradrenergic transmission differs depending on the behaviour being evaluated, as yohimbine 
mimicked the effects of restraint on impulsive action but not on risky choice. Notably, because 
these stress-induced impairments in behaviour are not mediated exclusively by increased levels 
of CORT, these data also point to a plausible role for the centrally active CRF. These findings 
are as well consistent with studies in humans involving individuals with MD who display an 
enhanced sensitivity to punishment as well as impairments in the execution of aversively 
motivated behaviours, thereby emphasising the importance of uncovering the contribution of 
stress in modulating risk/reward decision-making to inform and guide the clinical context.


