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Acute stress differentially alters reward-related decision making and inhibitory control under threat of punishment

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ABSTRACT

Acute stress has various effects on cognition, executive function and certain forms of cost/benefit decision making. Recent studies in rodents indicate that acute stress differentially alters reward-related decisions involving particular types of costs and slows choice latencies. Yet, how stress alters decisions where rewards are linked to punishment is less clear. We examined how 1 h restraint stress, followed by behavioral testing 10 min later altered action-selection on two tasks involving reward-seeking under threat of punishment in well-trained male and female rats. One study used a risky decision-making task involving choice between a small/safe reward and a large/risky one that could coincide with shock, delivered with a probability that increased over blocks of trials. Stress increased risk aversion and punishment sensitivity, reducing preference for the larger/risky reward, while increasing decision latencies and trial omissions in both sexes, when rats were teste. A second study used a "behavioral control" task, requiring inhibition of approach towards a readily available reward associated with punishment. Here, food pellets were delivered over discrete trials, half of which coincided with a 12 s audiovisual cue, signalling that reward retrieval prior to cue termination would deliver shock. Stress exerted sex- and timing-dependent effects on inhibitory control. Males became more impulsive and received more shocks on the stress test, whereas females were unaffected on the stress test, and were actually less impulsive when tested 24 h later. None of the effects of restraint stress were recapitulated by systemic treatment with physiological doses of corticosterone. These findings suggest acute stress induces qualitatively distinct and sometimes sex-dependent effects on punished reward-seeking that are critically dependent on whether animals must either choose between different actions or withhold them to obtain rewards and avoid punishment.

1. Introduction

Acute stress induces a variety of behavioral and neurochemical alterations that facilitate adaptations changing environmental conditions and restore homeostasis, primarily via the hypothalamic-pituitary-adrenocortical axis (HPA), which regulates the release of neuropeptide corticotropin-releasing factor (CRF) and the subsequent secretion of corticosterone (CORT) or cortisol (Herman et al., 2020). Canonically, the stress response reorganizes energy metabolism, reduces inflammation and affects other peripheral physiological processes. However, acute stress also has complex effects on numerous neural processes related to emotionality, motivation, learning and memory and higher-order executive functions such attention and cognitive flexibility. For example, in humans, stress alters learning from positive but not negative reward-prediction errors (Carvalheiro et al., 2021). In rodents, acute stress impairs different forms of spatial memory (Cazakoff et al., 2010; Li et al., 2012; Stillman et al., 1998), working memory (Diamond et al., 1996; Shansky et al., 2006) and can have various effects on different forms of cognitive flexibility (Butts et al., 2013; Hurtubise and Howland, 2017). Importantly, the manner in which stress may affect these behaviors depend on a variety of different factors such as type of stress, duration, timing and sex.

Acute stress can also alter evaluative process related to certain forms of reward-related cost/benefit decision-making, mediated by distributed circuits linking different regions of the prefrontal cortex, basolateral amygdala, and the nucleus accumbens (NAc; Floresco et al., 2008; Orsini et al., 2015a; Winstanley and Floresco, 2016). In rodents, these processes have been modelled using assays entailing choice between smaller, easily obtainable rewards and larger ones associated with some form of cost. Stress appears to have a variable influence on decisions depending on the cost associated with larger or more preferred rewards. For example, previous work by our group has reported that 1 h acute

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restraint decreases preference for larger rewards associated with a greater effort cost on an effort-discounting task (Bryce and Floresco, 2016; Shafiei et al., 2012). Notably, these effects were recapitulated by ventricular infusions of CRF, but not systemic treatment with CORT (Bryce and Floresco, 2016). In contrast, acute restraint did not alter decision biases when rat chose between smaller, immediate rewards and larger, delayed ones (i.e.; delay discounting (Shafiei et al., 2012)), suggesting the effects of this form of stress on effort-related choice are not related to reduced tolerance for delays to reward intertwined with effort costs. In a similar vein, neither restraint nor CRF treatment altered preference for larger, uncertain rewards on a probabilistic discounting task (Bryce et al., 2020). However, even though restraint stress did not alter choice biases during either delay or probabilistic discounting, this manipulation did cause more generalized disruptions in task engagement and indecisiveness, as indexed by increased omissions and choice latencies. Importantly, acute stress did not alter preference for larger *vs* smaller rewards of equal costs (Bryce and Floresco, 2016; Shafiei et al., 2012). Collectively, these findings suggest that acute stress biases choice away from larger rewards linked to greater physically effortful costs, and also caused a generalized reduction in motivation to pursue reward and increased deliberation times, irrespective of the effects of stress on choice. However, acute stress does not appear to exert as great an influence over decision biases in situation where the costs linked to larger rewards are more subjective (e.g., delays or uncertainty).

Punishment, in the form of noxious stimuli such as electric shocks, is another type of cost that can influence the direction of choice between different rewards. In this regard, the manner in which acute stress may modulate action selection and inhibitory control in conflict situations where rewards may be linked to punishment remains to be explored in detail. This is of notable importance given stress-related disorders such as depression have been associated with increased sensitivity to punishment (Eshel and Roiser, 2010; Hevey et al., 2017; Kim et al., 2021). A number of rodent tasks have been developed to assess decision making and reward-seeking involving punishment. For example, a risky decision-making task (RDT) developed by Setlow and colleagues is structured similar to other discounting tasks, in which rats choose between smaller *vs* larger rewards (Simon et al., 2009). However, choice of the larger reward option may result in foot shock, occurring with increasing probability over a session. Another more recently developed assay probes aspects of response inhibition and impulsive action within the context of punished reward-seeking. In this comparatively simpler "behavioral control task" (Verharen et al., 2019), trials begin with delivery of a food pellet, and on half of the trials, rats can retrieve it freely with no consequences. However, on the other trials, food delivery coincides with presentation of a 12 s tone/light stimulus that informs the rat it must withhold reward retrieval until termination of the stimulus, or receive a foot shock. Performance of both of these tasks are regulated by different regions of the frontal lobes, the amygdala and mesoaccumbens dopamine activity (Mitchell et al., 2014; Orsini et al., 2015b, 2018; Simon et al., 2011; Verharen et al., 2019, 2020), systems that are all affected by acute stress.

With this in mind, the present study was undertaken to help clarify how different forms of acute stress modulate the effects of punishment on distinct forms of risky choice and inhibitory impulse control in both male and female Long-Evans rats. The use of females was of particular interest given 1) the majority of clinical and pre-clinical studies available in the literature that discuss this type of behavior have predominantly relied on male subjects, and 2) stress-related disorders such as depression disproportionally affects women (Albert, 2015). To this extent, we assessed whether acute restraint stress altered risky choice and inhibitory impulse control in situations involving rewards and punishment. We also administered systemic challenges of CORT to assess whether this treatment could recapitulate the effects of restraint stress.

2. Method

2.1. Animals

Separate cohorts of male and female Long-Evans rats, purchased from Charles River Laboratory were used, initially weighing 250–280 g. Upon arrival, they were acclimatised to the animal facility for one week prior to being pair-housed and food-restricted to 85–90% their free-feeding weight. Rats were given *ad libitum* access to water for the entire experiment. Prior to training, rats were familiarized to the sweetened reward pellets used in these studies. Weights were monitored daily, and all testing was performed in accordance with the Canadian Council for Animal Care and the University of British Columbia Animal Care Committee.

2.2. Apparatus

All behavioral data were collected using automated operant chambers that precluded any potential observed biases. The chambers (30.5 x 24 × 21 cm; Med Associates, Fairfax, VT, USA), fitted with fan for ventilation and attenuation of external sounds. Sweetened reward pellets (45 mg) were delivered via an external dispenser connected to a central food receptacle port. Two retractable levers were located were located on either side of the food port, and a house light (100 mA) was located in the top-centre of the chamber wall opposite the magazine. Two stimulus lights resided above each lever. An infrared sensor located within the food receptacle registered when rats made a nosepoke in the magazine. The floor of the chambers consisted of a grid of 19 parallel stainless-steel rods spaced 1.5 cm apart. These were connected to a shock generator and solid-state grid scrambler. 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 task (RDT)

Rats were trained at least 5 d/week, and first went through 4 phases of pre-training. In the first, rats received a magazine training session, where they were placed in a chamber and reward pellets were delivered under a variable-interval 30 schedule. Lever pressing training began on the following day, wherein one of the two levers was extended, 4–5 crushed pellets were placed on top of it and rats were trained on a fixed-ratio 1 (FR1) schedule of reinforcement until they met a criterion of 50 presses in 30 min for one of the two levers (typically 1–2 days). On the following day, rats were trained on an FR1 schedule for the other lever, with the side counterbalanced.

During the next phase, 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 3 s 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 2–3 days and criterion required them to omit fewer than five times by the last day. Rats then moved on the final phase of pre-training, consisting of two days performing a reward-magnitude discrimination, such that a press on one lever always delivered one pellet, whereas the other lever always delivered a larger three-pellet reward. The specific lever-reward magnitude contingencies were counterbalanced across rats and maintained for each animal for the remainder of the experiment. Each session consisted of four blocks of 12 trials, with the first two being forced-choice and the latter ten free-choice trials.

Rats were then trained on the main, risky decision-making task (RDT) developed by Setlow and colleagues (Simon et al., 2009, Fig. 1A). Sessions consisted of 90 trials grouped into five blocks of 18 trials each. Trials started every 35 s with illumination of the houselight, and 3 s

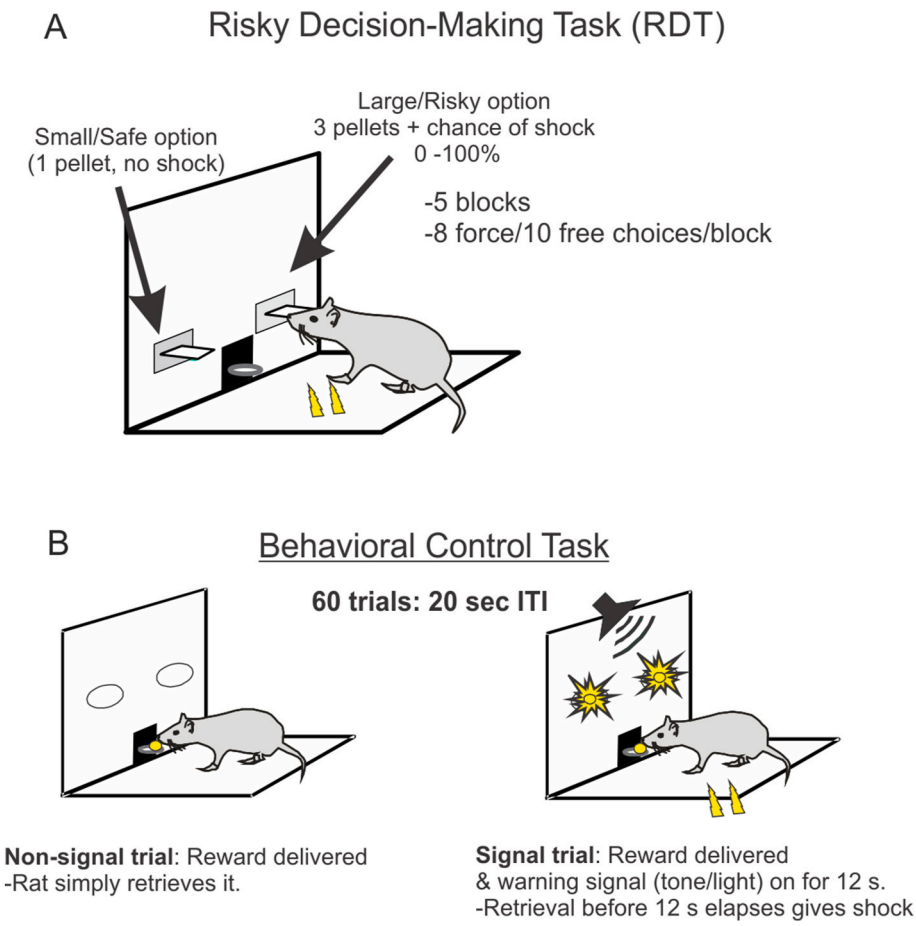

Fig. 1. *Depiction of the two assays used to examine the effects of stress on punished reward seeking.* **A:** In the Risky Decision-Making Task (RDT), rats chose between a 1-pellet reward delivered with no consequences, or a 3-pellet reward that may also come with shock, the probability of which increases over 5 blocks of trials. **B:** In the Behavioral Control task, a reward pellet is delivered every 20 s, and on non-signal trials, the rat must merely retrieve it from the food cup. On signal trials, a 12 s tone/light cue informs the rat that if retrieval is not delayed, shock will be delivered.

later, one or both levers extend. Following lever extension, rats were required to make a response within 15 s, or the trial was scored as an omission, the lever retracted, no reward given, and house light extinguished. After a press, the house light, remained illuminated for 4 s. A press on the "safe" lever always delivered one pellet, whereas "risky" lever presses always delivered three pellets, but could also deliver a 1 s foot shock, with a probability increasing across blocks (0% in 1st block, then 25%, 50%, 75%, and 100%). Each block consisted of eight forced-choice trials in which only one lever was extended (randomized in pairs), followed by 10 free-choice trials, where both levers were extended. Shock probabilities were fixed during a given block of forced-choice trials (e.g., in the 50% block, rats were guaranteed to receive a shock following two out of the four risky-lever presses). 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 preceding trials).

Each rat began training at a shock intensity of 0.25 mA (males) or 0.20 mA (females). A slightly lower initial shock intensity was used for female rats because of some evidence that females have lower current thresholds for evoking certain aversive behaviors compared to males (flinching, shuttling, jumping (Beatty and Holzer, 1978);). Following at least two days of training, the current was increased by 0.05 mA increments if the rats did not show prominent discounting behavior (i.e.; >70% risky choices in the latter blocks). Conversely, if the rats showed a high number of omissions in latter blocks, the current was reduced by 0.05 mA increments. Shock currents were titrated over the course of training for each individual rat until as a group, they showed stable patterns of choice for three consecutive days. Stability was analysed using a two-way repeated-measures analysis of variance (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 × block interaction at $\alpha = 0.05$.

2.4. Behavioral control task

Pre-training on this task did not entail any lever training. Rats were subjected to two days of magazine training, and then trained a task described by Verharen et al. (2019), that entailed reward delivery and retrieval that, in some instances, could be punished (Fig. 1B). Each session consisted of 60 trials where the house light remained illuminated throughout and trials started ever 20 s with delivery of a reward pellet into the food port. The session was comprised of two trial types, presented in a fixed pseudo-randomised sequence, such that 30/60 trials were "non-signal" trials, and the other half were "signal trials." The sequence of trials was the same for all rats, permitting simultaneous testing of multiple rats in the same room without interference by the cues between each box.

On non-signal trials, a reward pellet was delivered and the rat was simply required to nosepoke into the food port to retrieve it, which was detected by an infrared photobeam located in the alcove. On signal trials, however, delivery of the reward pellet coincided with presentation of a 12-s audiovisual warning cue (illumination of the stimulus lights and a 5 kHz tone) that informed the rats that food retrieval would also deliver foot shock until termination of signal. If a rat waited 12 s for

the warning signal to terminate, it could retrieve the pellet without consequences, and the trial was marked a "success." On the other hand, a nosepoke and reward retrieval before cue termination resulted in foot shock, the warning cue was turned off, and the trial was marked a "shock" trial. Thus, on signal trials, rats were required to withhold approach to food until cue termination to avoid punishment. When a rat did not nosepoke and consume the reward during a trial, this was marked an omission, and this prevented delivery of another pellet into the port until a rat retrieved the last one. Similar to rats trained on the RDT, shock currents were titrated for each individual rat and were increased if a rat was not withholding appropriately (>50% of signal 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 group stabilized performance, so that it displayed a success rate of ~60% on stimulus trials (i.e.; responding prematurely and getting shocked on ~ 40% of trials). Rats were trained for ~14 days, when behavioral 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

Some experiments examined how 1 h of acute restraint stress affected choice and related behaviors on the RDT and the behavioral control tasks (*n*s = 16 males and 16 females for each, separate groups for each experiment). The restraint duration was based on previous experiments showing that this was sufficient to induce alteration in effort discounting (Bryce and Floresco, 2016; Shafiei et al., 2012). Once rats displayed stable patterns of choice/inhibitory control, they received a stress test, consisting 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. There were no significant differences in performance on this baseline day compared to the preceding training day. The next day, rats were placed inside Plexiglass restraint tubes in the same neutral room as the previous baseline day. Different sized restraint tubes were used, depending on the size of the rat (124–171 × 60 mm for up to 350–400 g and 152–216 × 73 mm for up to 600 g). A large fan was placed directly in front of the restraint tubes for the duration of the test to offset hyperthermia. At the end of the restraint period, rats were returned to their home cages and left undisturbed for 10 min prior to the beginning of testing.

2.6. CORT challenge

In other experiments, we tested the effects of CORT challenge on performance of the two tasks, using within-subjects designs (*n*s = 8 males and 8 females for each, separate groups for each experiment). Following training, drug tests were conducted using a two-day sequence, and after each injection, rats were returned undisturbed into their home cage for 60 min. On the first day, rats were split into two groups and matched for performance to receive a subcutaneous vehicle injection (50/50 propylene glycol/0.9% saline) at a volume corresponding to that they would receive on the next day, where, they were given injections of either 1 mg/kg or 3 mg/kg CORT (Sigma Aldrich) at a concentration of 1 mg/ml. Rats were then re-trained until they again displayed stable levels of performance (3–4 days) and then received a second two-day test sequence, with the dose and injection volume counterbalanced from the first test day. The doses and time-course of the CORT injection were derived from previous studies by our group showing they increase plasma CORT levels in a manner comparable to that caused by 1 h restraint stress (Shafiei et al., 2012).

2.7. Data analysis

The main dependent variable for the RDT was the percent choice of the large/risky option as a function of punishment probability across the five trial blocks, factoring out trial omissions. To accommodate for a disproportionate number of trial omissions that tended to occur in the latter blocks, risky choice was also assessed by comparing the percentage of choices of the large reward in the first, 0% shock probability block with the average number of risky choices in the subsequent blocks, where these responses might be shocked, as described in the Results. Additional variables of interest included sensitivity to reward and punishment, as measured by win-stay and lose-shift behavior. Win-stay ratios were computed as a proportion of trials that a rat repeated a risky choice following a risky win (i.e., large reward without punishment), divided by all non-punished risky choices. Lose-shift ratios were computed by dividing the total number of trials that a rat shifted to the safe option following a risky loss (a shocked choice) with all punished risky choices. Additional motivational parameters analysed included choice latency. These values were a partitioned as a function of punishment probability (first block vs average latency in the punished blocks) or split across choice type (latency to press safe or risky lever, averaged across all blocks). The number of trial omissions were also analysed. Parameters by block were assessed by a three-way repeated-measures ANOVAs, using treatment (baseline *vs* stress or vehicle *vs* CORT) and block as within-subjects factors and sex as a between-subjects factor. Parameters that did not require assessment by block were analysed using a two-way repeated-measures ANOVA using treatment and sex as factors. Furthermore, we also analysed behavioral performance to assess any carry-over effects of restraint stress 24 h later. In this case, we performed separate two- and three-way ANOVA's to compare the appropriate parameters between data obtained on the baseline day and 24 h after the stress test day.

For the behavioral control task, data from signal and non-signal trials were analysed separately. The key variable of interest for signal trials were the percent of 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 ("premature") or waited ("post-signal"). To control for the omissions on signal trials, our main index of performance was a "shock index", which was computed by dividing the number of signal trials where rats responded prematurely and were shocked by the total number of shock + success trials where rats made a response (i.e; factoring out omissions on signal trials). Thus, a value of 100% indicates that for every time a rat retrieved food on a signal trial, it did so prematurely and was shocked, whereas a value of 0% indicates that the rat withheld responding for at least 12 s on every signal trial it retrieved food. For non-signal trials, we analysed the percentage of trials food was retrieved (wherein values lower than 100% indicated that some trials were omitted) and retrieval latencies. Unless otherwise specified, all parameters were assessed using a two-way repeated-measures ANOVA with treatment and sex as factors. In this experiment, changes the behavioral measures of interest were also assessed 24 h after each test session, by adding a third treatment level of to the two-way repeated-measures ANOVAs (ie: baseline, stress test and 24 h).

The ANOVA models used to analyse data across all four experiments are typically robust to violations of assumptions of normality (Glass and Hopkins, 2008; Howell, 2010). Nevertheless, across all data sets, we confirmed that data were normally distributed within each group (Shapiro-Wilk Tests, all *p*s>0.148). With respect to assumptions of homogeneity of variance that are pertinent for the between-subjects comparisons of male and female data, these analyses are also generally robust to violations of the assumption when comparing two groups of equal sizes (Glass and Hopkins, 2008). For analyses that included a different number of males and females, homogeneity of variance was assessed using an F test. Lastly, the repeated-measures ANOVA used for our main comparisons of effects of stress or CORT *vs* control conditions assume sphericity (constant variance across time points/test conditions (Muhammad, 2023)). To control for any potential violations of this assumption, *p* values were corrected using the Huynh-Feldt method. All

statistically significant *p* values from repeated-measures ANOVAs reported here reflect this correction.

3. Results

3.1. Restraint stress and risky decision making

Thirty-two rats (ns = 16 males/16 females) were trained for 28 days

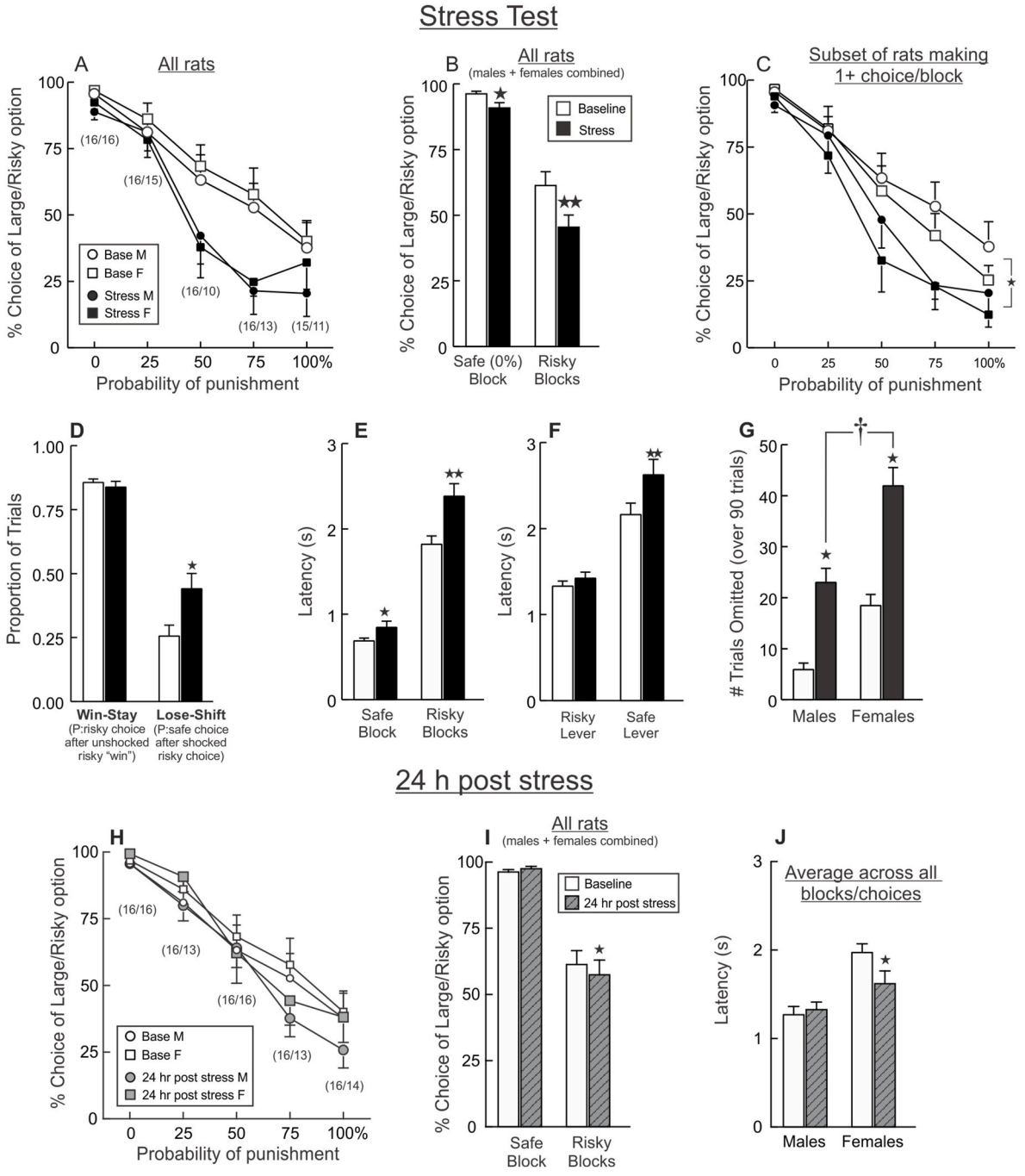

Fig. 2. *Restraint stress induces risk aversion and increased decision latencies on the RDT.* **A:** Mean percentage of risky choices as a function of shock probability under baseline conditions, and after 1 h restraint stress for all male and female rats. Number in parentheses denote the number of males/females that made at least one choice in the particular block. **B:** Percentage of risky choices in the first (0% shock) block and the average of those made in the risky blocks under baseline and stress conditions, for all rats collapsed across sex. Stress caused a proportionally greater reduction in risky choices compared to choice in the first block. **C:** Mean percent risky choice across blocks obtained from a subset of rats making at least one choice in all blocks under baseline and stress conditions. **D:** Win-stay and lose-shift ratios, collapsed across sex. Stress selectively increased punishment ("loss") sensitivity. **E:** Mean choice latency in the first (0% shock) block and the average of those made in the risky blocks under baseline and stress conditions, collapsed across sex. **F:** Mean latency for all rats to choose the risky or safe option (averaged across blocks) under baseline stress conditions. Stress selectively increased decision latencies in risky blocks and prior to small/safe choices. **G:** Number of trial omissions for males and females under baseline and following stress. **H–I:** Percentage of risky choices made 24 h after stress, compared to baseline. **J:** Average choice latencies (collapsed across block and choice type) for males and females, at baseline and 24 h after stress. For this and all other figures, stars and double stars denote $p < 0.05$, 0.001 vs baseline, and dagger denotes $p < 0.05$ main effect of sex, and error bars represent S.E.M.

on the RDT and then received a restraint stress test. By the time choice behavior stabilized, male rats required higher shock intensities (0.43 ±0.02 mA) compared to females (0.25±0.01 mA; $t(30) = 7.33$, $p < 0.0001$). In analysing the choice data, some rats (primarily females) displayed a large number of omissions, and this effect was exacerbated by stress. This complicated the analysis, as 10 rats 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 ANOVA model. To accommodate for this, choice data were analysed with a two-pronged approach. First, we analysed risky choice data obtained during the 0% probability block and the data *averaged* across the other four 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 rats to quantify how some risk of shock altered preference for the larger reward, and how this was affected by stress. The second approach analysed data from a subset of rats that made at least one choice across all blocks under both conditions.

Acute restraint markedly reduced preference for the larger reward associated with probabilistic punishment in a comparable manner across sexes. The discounting curve displayed in Fig. 2A shows the choice data across all blocks under baseline and stress conditions, and also displays the number of rats 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% block and the average risky choices made across the other four blocks (Fig. 2B). This ANOVA revealed a significant main effect of treatment ($F(1,30) = 25.58$, $p < 0.0001$) and treatment × block interaction ($F(1,30) = 9.80$, $p < 0.01$). Partitioning the two-way interaction showed that stress caused a relatively small (~5%) 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 (~16%) reduction in preference for the larger reward in the latter blocks when its delivery might also be punished ($F(1,31) = 23.02$, $p < 0.0001$). Notably, there was no main effect of sex or interaction with the other variables (all *F*s < 1.0, not significant (n.s.)), indicating that stress had comparable effects on choice in both sexes.

To complement this analysis, we examined the choice data across all blocks in the subset of rats that made at least one choice in each block under both baseline and stress conditions. This resulted in 15 males and 7 females included in the analysis (Fig. 2C). Here analysis revealed a main effect of treatment ($F(1,20) = 18.43$, $p < 0.001$), but no treatment × block interaction ($F(4,80) = 2.08$, $p = 0.091$). There was no main effect of sex ($F(1,20) = 0.26$, n. s.) nor a three-way interaction among the factors ($F(4,84) = 0.61$, n. s.), suggesting again that the effects of stress on choice did not differ across sexes. Similar results were obtained when we analysed data from a subset of rats that made at least two choices in each block (12 males/7 females; main effect of treatment: $F(1,17) = 15.24$, $p = 0.001$, data not shown). The variance of the choice data across males and females on baseline and stress test days did not differ between these subsets of rats (both Fs $(7,14)>0.48$, $p < 0.337$).

The decrease in risky choice was accompanied by an increased sensitivity to punishment, as indexed by an increase in lose-shift ratios, revealed by a two-way ANOVA (main effect of treatment; $F(1, 30) = 11.33$, $p < 0.01$); no main effect of sex ($F(1, 30) = 0.08$, n. s.) or interaction ($F(1, 30) = 0.19$, n. s (Fig. 2D).). On the other hand, stress did not affect win-stay behavior (all *F*s < 1.3, n. s.).

Analysis of choice latency revealed restraint stress increased deliberation times. This effect was more apparent in the latter blocks when there was some 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 values from the first, 0% shock probability block and average latencies from the other four blocks (Fig. 2E). A 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 (data not shown). More pertinently, the analysis also revealed a significant main effect of treatment ($F(1,30) = 25.53$, $p < 0.001$), and a treatment × block interaction ($F(1,30) = 15.87$, $p = 0.029$). Stress caused a relatively slight (~150 ms) increase in choice latency in the 1st block ($p < 0.01$), but a much larger increase in deliberation times in the latter blocks ($p < 0.0001$). Analysis of the latency by choice type (Fig. 2F) 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 treatment x choice type interaction ($F(1, 29) = 5.94$, $p = 0.02$; three-way interaction: $F(1,29) = 0.081$, n. s.). The two-way interaction was driven by the fact that stress did not alter 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. We were unable to compare risky and safe choice latencies during the first, 0% shock probability block *vs* the other four blocks because nearly half of the animals did not make any safe choices in the 0% block under baseline or stress conditions.

As noted above, restraint stress also reduced task engagement, indexed by increased trial omissions (Fig. 2G), with the analysis revealing a main effect of treatment ($F(1, 30) = 67.97$, $p < 0.01$) and sex ($F(1, 30) = 41.82$, $p < 0.01$) with females omitting more trials. There was no significant treatment × sex interaction ($F(1, 30) = 1.155$, n. s.), indicating that stress did not induce a disproportionate increase in omissions in males *vs* females.

Acute stress can induce delayed effects on certain aspects of cognition that may persist 24 h after the stressor (Mitra et al., 2005; Rao et al., 2012; Shinba et al., 2010). In light of this, separate analyses compared performance of rats 24 h after restraint to their pre-stress baseline. These data were analysed separately from those obtained in the stress test, as there were fewer rats that omitted all trials in a block on this day compared to the stress test. As displayed in Fig. 2H, both male and female rats appear to return to near baseline levels of choice 24 h after the stress test, although there appeared to be some minor residual reduction in risky choice in the males. Analysis of the choice data from the 0% block and average of the risky blocks yielded a significant treatment × block interaction ($F(1,30) = 4.59$, $p = 0.04$) with no main effect of treatment or interactions with the sex factor (all Fs < 1.9, n. s). This interaction reflected no differences in choice between baseline and 24 h post-stress ($p > 0.50$) but a slight (~4%) reduction in risky choice during the risky blocks ($p = 0.03$; Fig. 2I). In contrast, analysis of the average choice latency (collapsed across block and choice type) yielded a treatment × sex interaction ($F(1,30) = 8.50$, $p = 0.007$), driven by a *reduction* (as opposed to the stress-induced increase) in choice latencies 24 h post-stress relative to baseline in females ($p < 0.004$), but not males ($p > 0.50$). Omissions did not differ 24 hr-post stress relative to baseline in either sex (all Fs < 2.0, n. s., data not shown). Collectively, these data indicate restraint stress decreased preference for larger rewards associated with punishment, increases decision times and reduced task engagement. In addition, females displayed less task engagement, as they had slower deliberation times and more omissions, but stress did not disproportionately affect these measures in females. These effects largely dissipated 24 h after stress.

3.2. Systemic CORT and risky decision making

Another experiment examined whether systemic treatment with physiologically relevant doses of CORT could recapitulate the effects of restraint stress on risky decision-making. A squad of 8 male and 8 female rats (separate from those that received restraint) were trained on the RDT for 28 days prior to administration of two CORT challenges (1 and 3 mg/kg) in a counterbalanced manner. In this cohort, there were no sex differences in the shock intensities needed to evoke stable and prominent discounting (male = 0.39 ±0.02 mA; females = 0.38±0.04 mA p >

0.70.). Choice, latency, and omission data did not differ between the two vehicle treatments given prior to each CORT dose (all *Fs* < 1.2, n. s.), so vehicle data from these two tests were averaged for the analyses. Furthermore, all rats made a sufficient number of choices across all blocks, precluding the need for the two-pronged analysis approach conducted for the restraint stress experiment.

In contrast to the effects of restraint, systemic treatment with CORT did not affect risky choice in either sex (all *Fs* for effects of treatment and interactions with sex or block <1.61, all ps > 0.20; Fig. 3A and B). In addition, CORT did not recapitulate the effects of restraint on deliberation times or task engagement. Analysis of choice latency partitioned by safe *vs* average of the risky blocks did reveal a significant main effect of treatment ($F(2,28) = 4.01$, $p = 0.02$) and treatment × block interaction ($F(2,28) = 3.38$, $p = 0.048$), with no interactions with the sex factor (all Fs < 1.0, n. s.). However, this effect was actually driven by a reduction in latencies during the risky blocks following treatment with the 1 mg/kg (Dunnett's, $p < 0.05$), but not the 3 mg/kg dose (Fig. 3C). Furthermore, although females in this group again made a higher number of trial omissions compared to males (main effect of sex, $F(1,14) = 6.69$, $p = 0.02$), CORT did not alter these values relative to vehicle (all *Fs* < 2.34, all *ps* > 0.10). Thus, increasing CORT levels alone did not induce any major effects on risky choice, except for a decrease in latency in the risky blocks, an effect opposite to that induced by restraint.

3.3. Restraint stress and behavioral control during punished reward seeking

A separate series of experiments assessed changes in impulsive action induced by restraint stress in conflict situations, where on some trials, rats were required attend to a warning signal informing them to delay reward retrieval to avoid punishment. Thirty-two rats (*ns* = 16 males and females, separate from those trained on the RDT) were trained for 14 days on the behavioral control task and then received a stress test. The ANOVA models used to analyse these data included three levels of treatment (baseline *vs*. restraint *vs*. 24 h) as the within-subjects factor and sex as a between-subjects factor for all measures. The 24 h data were included because we noticed differences in how acute stress affected performance in male *vs*. female rats during this phase of testing, as described below. Shock currents for males (0.45± 0.02 mA) and females (0.44± 0.02 mA) did not differ.

Over the different phases of testing, restraint stress differentially affected impulsive action in males *vs* females on signal trials. The shock index served as our primary measure, which was the proportion of signal trials rats responded prematurely compared to all signal trials rats retrieved food at some point during the trial. Analysis of these data revealed a significant treatment × sex interaction ($F(2, 60) = 4.38$, $p = 0.017$), with no main effects of sex ($F(1, 30) = 2.00$, $p =$ n.s.) or treatment ($F(2, 60) = 1.216$, $p =$ n.s.; Fig. 4A). Partitioning this interaction across sexes revealed that on the stress test, males (but not females) made significantly more premature responses (and received more shocks) relative to baseline (Dunnett's, $p < 0.05$). In stark contrast, comparisons of the shock index 24 h later revealed another an interesting, sex-dependent effect. Here, males showed comparable inhibitory control relative to baseline, whereas females displayed *better* inhibitory control, as evidenced by a reduced shock index *vs* baseline (Dunnett's, $p < 0.05$). Importantly, stress did not alter the percentage of trial

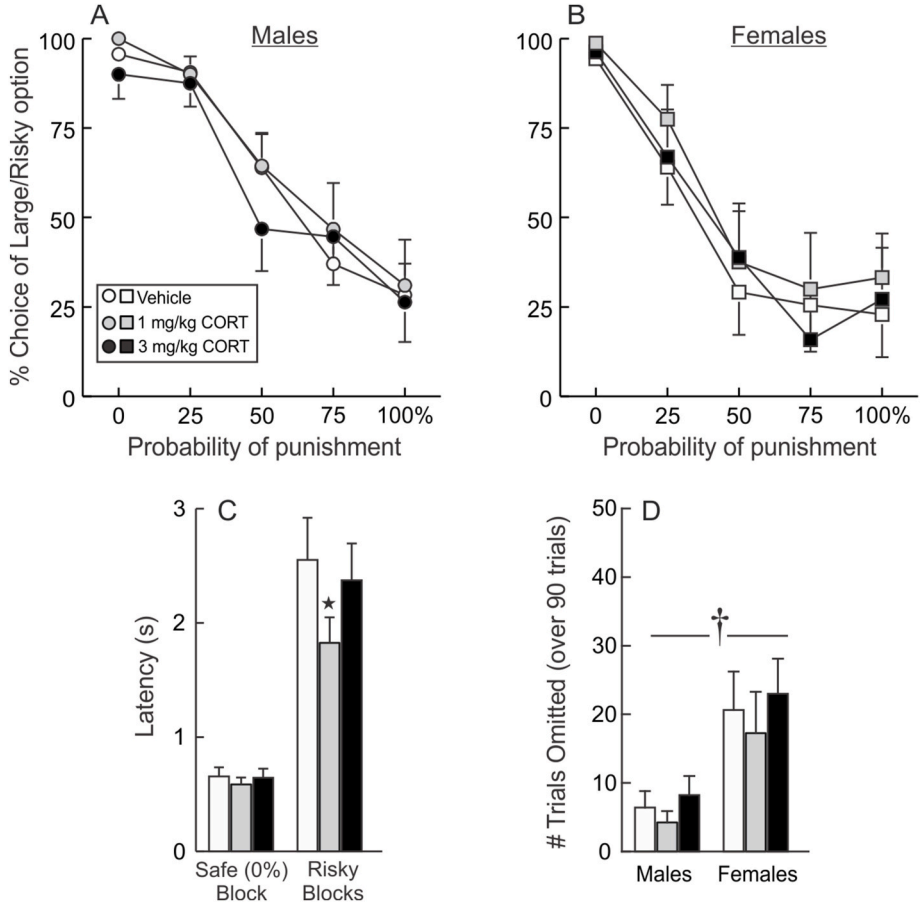

Fig. 3. *Systemic CORT treatment does not recapitulate the effects of restraint stress on risky decision making.* **A, B:** Mean percentage of risky choices following systemic treatment with vehicle, or 1–3 mg/kg CORT, for males (A) and females (B). **C:** Mean choice latency in the first (0% shock) block and the average of those made in the risky blocks, collapsed across sex, following vehicle or CORT treatments. **D:** Number of trial omissions for male and female rats across treatments. CORT treatment did not affect choice or omissions, and the 1 mg/kg dose reduced choice latencies in the risky blocks.

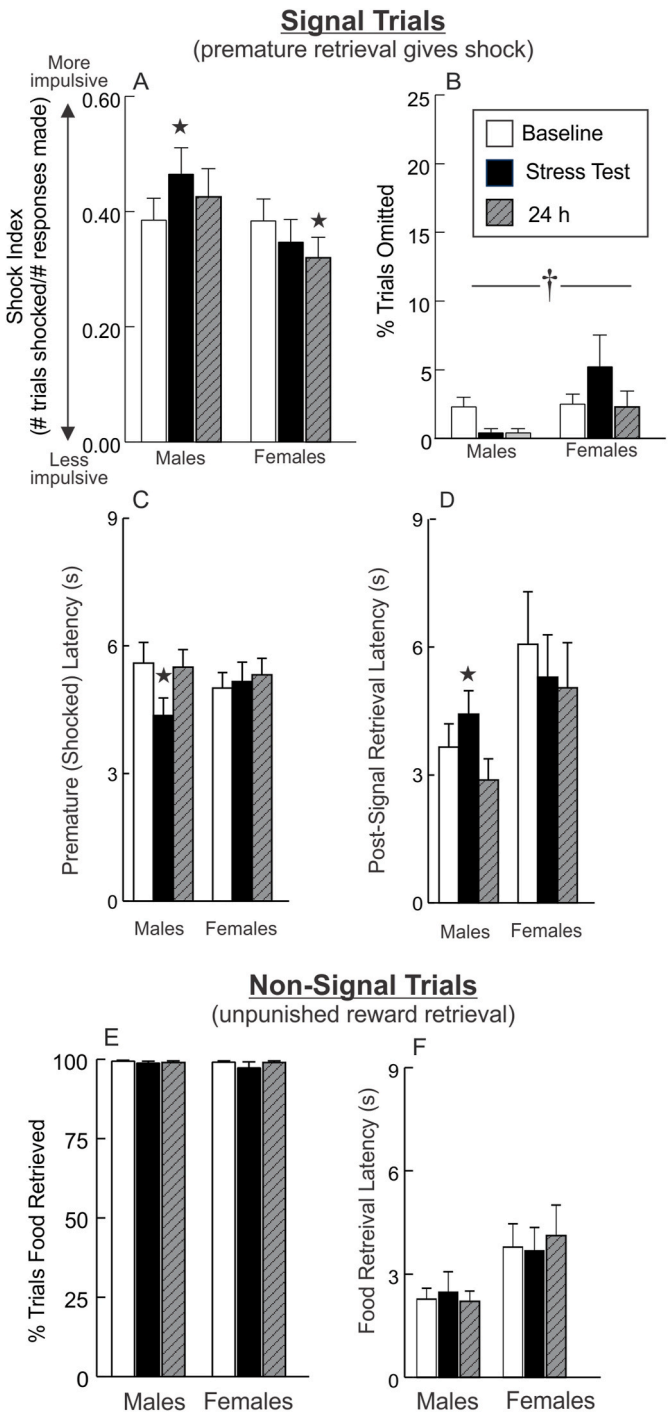

Fig. 4. *Restraint stress induces sex and timing-dependent effects on the behavioral control task.* **A:** Mean shock index (number of "shock" trials divided by total trials where a response was made) on signal trials at baseline, after restraint stress and 24 h later, for males and females. Acute stress increased punished responding on signal trials in males, but decreased it 24 h after stress in females. **B:** Percentage of trial omissions on signal trials across the treatment conditions. **C,D:** Latencies to collect rewards on signal trials when rats responded prematurely before the signal had terminated (C) and when rats waited out the warning signal for at least 12 s to retrieve food (D). **E,F,** On non-signal trials, where rats merely had to retrieve food with no consequences, stress had no effect on the percentage of rewards retrieved (E) or retrieval latency (F).

omissions on signal trials (i.e.; those trials where rats did not retrieve food before the start of the next trial; all F's < 2.12, n. s. Fig. 4B), indicating that changes in shock index were not merely attributable to a reduced number of responses on these trials. There was a main effect of sex ($F(1,30) = 5.561$, $p = 0.025$), indicating that females omitted a greater proportion of trials.

Stress also affected reward retrieval latencies on signal trials in a sex-dependent manner. We conducted separate analyses of latencies on shock trials when rats responded prematurely, and on successful trials, where they waited 12+ s for the cue to terminate before retrieving reward. Analysis of latencies on premature punished trials revealed a main effect of treatment ($F(2,60) = 3.172$, $p = 0.05$) and more pertinently, a treatment × sex interaction ($F(2, 60) = 3.355$, $p = 0.043$). In keeping with increased impulsive action in males, these rats were quicker to retrieve reward prematurely on the stress test day (Dunnett's, $p < 0.05$; Fig. 4C). Females did not differ from baseline on the stress test day or 24 h later on this measure. In contrast, stress made males more hesitant to retrieve rewards on trials where they waited for the 12 s signal to terminate, displaying longer post-signal retrieval latencies on the stress test vs baseline whereas females values were unchanged by stress (treatment × sex interaction ($F(2,60) = 3.43$, $p = 0.038$, and Dunnett's, $p < 0.05$; Fig. 4D).

In comparison, stress did not alter behavior on non-signal trials, where rats merely retrieved food without the threat of shock. No main effects of stress effects or interaction with the sex factors were observed for the percentage of successful trials (Fig. 4E) or response latencies (Fig. 4F; all F's < 1.0, n. s.). For the latency data, the main effect of sex approached statistical significance ($F(1, 30) = 3.96$, $p = 0.056$), reflecting that females were slightly slower than males to retrieve freely-available rewards. Thus, stress did not alter motivation to retrieve freely-available food on the test day or 24 h later. Collectively, these data indicate acute stress differently affects inhibitory control in conflict situations involving punished reward-seeking in a sex dependent manner, making males more impulsive shortly after stress, whereas females show reduced impulsive tendencies 24 h after a stressor.

3.4. *Systemic CORT and behavioral control*

In a separate squad of 16 rats (8 males/females), we examined whether systemic treatment with CORT might mimic the effects of restraint stress on inhibitory control. After 14 days of training, rats received two, two-day test sequences of vehicle then CORT challenges (1 mg/kg and 3 mg/kg) in a counterbalanced manner. In this cohort, we again saw no difference in shock currents required for males (0.56±0.3 mA) and females (0.55±0.3 mA). Notably, we observed a difference in the shock index across the two vehicle tests that preceded challenge with 1 and 3 mg/kg CORT ($F(1,15) = 11.24$, $p < 0.01$), precluding us from averaging the vehicle challenge data for analysis of this measure. To accommodate for this, shock index data were analysed with a three-way ANOVA using sex as the between-subjects factor, and treatment day (vehicle *vs*. CORT injection *vs* 24 h) and test dose (1 *vs* 3 mg/kg) as within-subjects factors. However, for all other measures, there were no differences across the two vehicle test days (all *F*s < 1.7, n. s.). Thus, for these other analyses, we used the average values of the two vehicle tests and compared them to those obtained on each challenge days and 24 h later using two-way ANOVAs, sex as a between-subjects factor and 5 levels of treatment (average vehicle, 1 mg/kg CORT, 24 h after 1 mg/kg CORT, 3 mg/kg CORT and 24 h after 3 mg/kg CORT) as a within-subjects factor.

In contrast to the sex-dependent effects of restraint stress on signal trials, systemic CORT treatments had no effect on impulsive action on the challenge day or 24 h later. Analysis of the shock index did not yield main effects of treatment or any interactions with the other factors (all F's < 2.67 n. s.; Fig. 5A). There was a main effect of dose ($F(1,14) = 4.741$, $p < 0.05$) that appeared to be driven by a higher baseline shock index prior to the 3 mg/kg challenge. Likewise, signal trial omissions

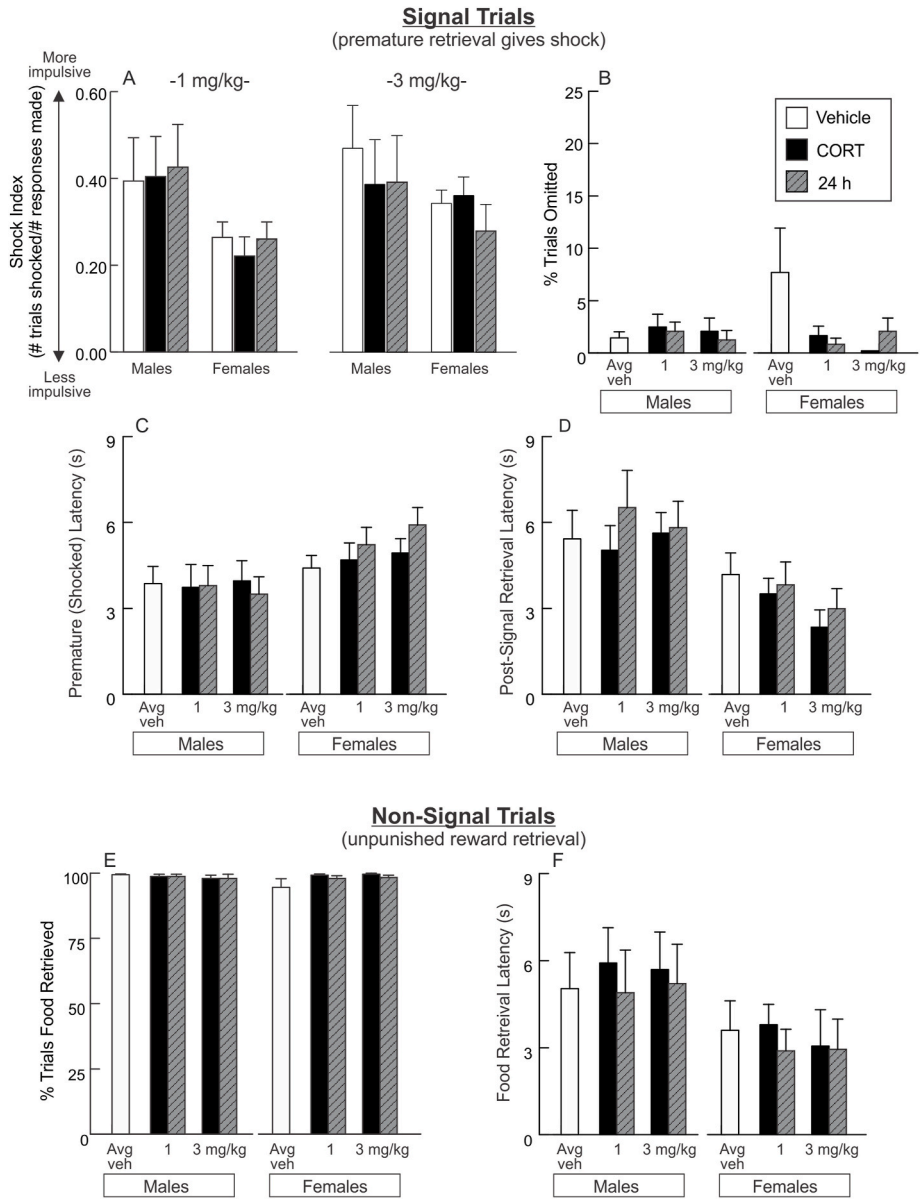

Fig. 5. *Systemic CORT treatment does not recapitulate the effects of restraint on impulsivity and inhibitory control.* **A:** Mean shock index on signal trials following systemic treatment systemic treatment with vehicle, 1 (left) or 3 mg/kg (right) CORT. **B:** Percentage of trial omissions on signal trials across the treatment conditions. **C,D:** Latencies to collect rewards on signal trials when rats responded prematurely (C) and when rats waited out the signal to retrieve food unpunished. **E:** Percentage of rewards retrieved on non-signal trials. **F:** Reward retrieval latency on non-signal trials. CORT did not alter any measure in either sex.

(Fig. 5B), premature (Fig. 5C) or post-signal (Fig. 5D) reward retrieval latencies were unaffected by CORT challenge in either sex (all *F*s < 2.0, n. s.). A sex difference was observed in terms of post-signal reward retrieval latencies ($F(1,14) = 5.61$, $p = 0.03$), with females responding more rapidly than males across all treatment conditions.

On non-signal trials, neither dose of CORT altered unpunished reward retrieval or induced carry-over effects 24 h later, as evidenced by the lack of effects or interactions on all ANOVAs for successful trials or retrieval latency (Fi.g 5 E,F) (all F's < 1.8, n. s.). Collectively, these data suggest increased CORT activity alone has no major effects on inhibitory impulse control in conflict situations where reward retrieval may be punished.

4. Discussion

The present findings provide novel insight into how acute stress exerts complex and differential effects on reward-seeking in conflict situations involving punishment. These effects can in some instances vary by sex, and depend on whether these situations entail choice between actions linked to different risks and rewards, or require inhibitory control over reward retrieval to avoid noxious outcomes. Acute restraint stress caused a marked, sex-independent reduction in choice of larger rewards associated with probabilistic punishment, that was accompanied increased deliberation times and reduced task engagement. In comparison, the same stressor induced sex-dependent alterations in impulsive action on a behavioral control task, increasing punished reward retrieval shortly after stress in males, while reducing this behavior in females 24 h after stress. Notably, none of these effects on punished reward seeking were mimicked by systemic treatment with CORT, suggesting that other neural/neurochemical changes induced by this form of stress drives these differential effects on risky choice and impulsive action.

4.1. Stress and cost/benefit decisions involving punishment

In examining how acute restraint stress affected risky decision making involving punishment, we employed a well-established task that pitted biases for larger rewards against increasing likelihood of shock (Orsini et al., 2015b; Simon et al., 2009). Stress reduced preference for larger, risky rewards by increasing punishment sensitivity, as rats were more likely to shift responding towards the safe lever after a punished risky choice. On the other hand, stress had no effect on reward sensitivity, indexed by win-stay ratios, as rats were just as likely to follow a non-punished risky choice with another such choice under baseline *vs* stress conditions. In this regard, previous research showed that neither acute restraint nor increased CRF activity alters choice for larger *vs* smaller rewards of equal costs (Bryce and Floresco, 2016; Shafiei et al., 2012). Thus, it is unlikely that stress-induced reductions in risky choice described here were due to a generalized disruption in preference for larger *vs* smaller rewards. With respect to sex differences, although females have been reported to be more risk averse on this task (Orsini et al., 2016), in this study, we were able to titrate shock currents across sexes so that males and females showed comparable baseline levels of risky choice. Notably, stress-induced reductions in risky choice were sex-independent, as males and females showed comparable reductions on this measure, although the analysis of the choice data was complicated by the higher omission rates observed in females. Furthermore, unlike the effects of stress on other behaviors (e.g. exploration; Curzon, 1989; Korte and De Boer, 2003; Reis et al., 2011), these effects on cost/benefit decision making largely dissipated 24 h after restraint, suggesting that they were driven by acute neural/neurochemical changes occurring around the time of the stressor.

Restraint also altered decision latencies and task engagement. In particular, stress slowed deliberation times in the latter blocks of the task, where there was some chance of receiving shock, but notably, had minimal effects in the initial, 0% probability block. Furthermore, partitioning latencies by choice type revealed that rats took considerably longer to choose the small/safe option compared to the large/risky one under baseline conditions. Similar latency effects have been reported larger *vs* smaller rewards of equal costs (Jenni et al., 2022; St Onge et al., 2012; Stopper and Floresco, 2014), likely reflecting greater incentive salience of the larger reward option, despite its potential for also delivering shock in the present study. Interestingly, stress did not alter latencies to make risky choices, but instead, increased them prior small/safe choices. This combination of findings suggests that the increased choice latencies does not necessarily reflect a broader reduction in motivation, but rather, prolonged decision times and greater hesitation to select less preferred rewards when larger ones might be punished. Similarly, stress also increased omissions. In this regard, we did observed sex differences on these measures, with females showing longer choice latencies and more omissions *vs* males under baseline conditions. However, even though stress increased these measures, it did not have a disproportionately greater effect in females. These impairments are in keeping with previous work showing that restraint stress (or increased CRF activity) generally slows reward-related decision latencies, irrespective of whether these stressors alter the direction of choice on different forms of cost/benefit decision making (Bryce et al., 2020; Bryce and Floresco, 2016, 2021; Shafiei et al., 2012).

It is of particular interest to compare the effects of restraint stress on risky decision making involving physical punishment to other types of decisions involving choice between rewards of different magnitudes and costs. For example, the effects of stress on choice reported here are remarkably similar to those on effort discounting in male rats, entailing choice between a smaller, two-pellet reward delivered after one lever press or a larger, four-pellet one that could be obtained following 2–20 presses. Here, stress reduced preference for the larger reward associated with greater physical cost (Bryce and Floresco, 2016; Shafiei et al., 2012). Yet, the same stressor did not affect choice on decision assays with a similar task structure, but where the costs may be viewed as more subjective. Thus, 1 h restraint did not affect delay discounting, entailing choice a one reward pellet delivered immediately vs four pellets delivered 15–45 s after a choice (Shafiei et al., 2012). Similarly, neither this form of stress nor increased CRF activity affected probabilistic discounting, where rats choose between a smaller, certain reward and a larger one that may or may not be delivered (Bryce et al., 2020). Thus, it appears that acute stress preferentially biases choice away from rewards linked to physical costs (i.e., caloric costs linked to work or aversive stimuli like shocks), but has less of an effect on action selection when costs are more subjective in nature, related to disappointment/frustration associated with reward delays or omissions. However, stress has a more ubiquitous effect on increasing decision latencies and reducing task engagement on all of these forms of cost/benefit decision making (Shafiei et al., 2012; Bryce et al., 2020, present study). This is in keeping with the finding that restraint stress increases vicarious trial-and-error evaluation of different options on a T-maze choice task, which was interpreted to reflect over-thinking, and indecisiveness (Amemiya et al., 2020). A holistic integration of these findings suggests that in situations involving choice between actions associated with different rewards and costs, stress uniformly prolongs deliberation times to make a decision, independent of whether or not stress may influence the direction of action selection. In comparison, stress more selectively reduces pursuit of rewards occluded by physical *vs* subjective costs (i.e., when a decision maker has “skin in the game”), so that these hurdles are perceived as less surmountable, thereby biasing choice towards rewards that are less preferred yet more easily obtained.

4.2. Stress and inhibitory control of punished reward seeking

In contrast to the relatively straightforward effects of restraint on risky decision making, this manipulation had more complex sex and time-dependent effects on punished reward-seeking requiring response inhibition assessed with a behavioral control task. On the stress test day, male rats became more impulsive, retrieved rewards faster and received more shocks on signal trials, where a 12 s cue warned them that premature reward retrieval would be punished. Conversely, males were slightly more hesitant to retrieve reward on signal trials where they were able to wait out the 12 s cue. These effects were no longer apparent 24 h after stress. These impulsogenic effects of acute stress in male rats are comparable to action of other stressors on different assays of impulsive action, such as the 5-choice serial reaction time task (5-CSRTT) and differential-reinforcement-of-low rate responding (DRL). Treatment with the α2 adrenergic antagonist and pharmacological stressor yohimbine impairs “waiting”, as indexed by increased premature responses on the 5-CSRTT (Adams et al., 2017; Baek et al., 2017; Barlow et al., 2018; Broos et al., 2017; Chernoff et al., 2021; Sun et al., 2010) and higher response rates on a DRL task (Sanger, 1988), both of which reduced opportunities to obtain rewards. Similarly, cold stress also increases DRL responding (Thomas et al., 1991), and inescapable shock stress increases premature response on a 1-CSRTT (Girotti et al., 2022). These previous findings, in combination with the present data suggest that in males, acute stress impairs response inhibition in a variety of circumstances, where premature responses may either delay reward delivery or result in more explicit punishments.

Restraint stress induced qualitatively different effects on behavioral control in female rats. Unlike their male counterparts, females were neither more nor less impulsive compared to baseline on the stress test day. Furthermore, they actually showed a delayed effect of stress 24 h later, where they were significantly less impulsive on signal trials, as evidenced by a lower shock index, while males reverted back to baseline levels of responding on this measure. This latter finding resembles that reported by Briggs and McMullen (2020), where 1 h restraint induced a delayed impairment of extinction of passive avoidance of a shock-associated chamber in female rats. These findings highlight a key sex difference in how acute stress may affect inhibitory control of reward seeking, suggesting that females may be more impervious to the

impulsogenic effects of recent stress. Moreover, the carry-over effect of reduced impulsive action observed 24 h after stress suggests that punishments experienced by females on the stress test day may have been amplified in some manner, leading to reduced punished responding the next day. In essence, females appeared more likely to learn from their mistakes following stress.

In contrast to the effects on stress on signal trials, restraint had no effect in either sex on non-signal trials when rewards could be freely retrieved without consequences, as all rats retrieved as many rewards and as quickly following stress (or 24 h later) compared to baseline. This null effect suggests that this form of stress does not cause non-specific disruptions in motivation to retrieve and consume food rewards. Moreover, the lack of effect in this experiment further supports our argument that stress-induced increases in choice latencies on the RDT were not driven by generalized reductions in reward-related motivation, but instead reflected prolonged deliberation and increased indecisiveness. It is also important to highlight that the proportion of premature responses punished by shock were either unaffected (females) or increased (males) following stress. Juxtaposition of these observations to those from the RDT experiment suggest that the stress-induced reductions in risky choice observed in the latter are unlikely to be attributable to an increased reactivity to the painful effects of shock. Viewed collectively, the results of these two studies reveal how acute stress induces qualitatively different effects on punished reward-seeking. These effects can sometimes vary based on sex, timing of the stressor, and are critically dependent on whether animals must either choose between different actions or withhold them to obtain rewards while avoiding punishment.

4.3. Neurochemical Underpinnings of stress effects on punished reward seeking

A primary hormonal/neurochemical effect of stress (including restraint) is to increase CORT secretion. Thus, we also examined whether increases in plasma CORT levels induced behavioral changes similar to restraint stress on these two forms for punished reward-seeking. In so doing, CORT was administered using procedures and doses we have shown previously to mimic changes in plasma CORT levels induced by 1 h restraint (Shafiei et al., 2012). These treatments had no effect whatsoever on action selection or response inhibition on either task, and also did not recapitulate the effects of restraint on response latencies or task engagement. Indeed, the only observed effect of CORT was a reduction in choice latency on the RDT induced by the 1 mg/kg dose. These null effects contrast with other reports that exogenous CORT mimicked the effects of acute restraint on other cognitive functions, such as non-spatial memory and contextual fear conditioning (Cordero et al., 2003; Vargas-López et al., 2015). However, the lack of effect shown here is in keeping with our previous findings that the effects of restraint on effort-discounting were not replicated by similar CORT treatments (Shafiei et al., 2012). Thus, whereas increased glucocorticoid activity may mediate some effects of stress on certain types of learning, it does not appear to be a contributing factor in stress-induced alterations in executive processes used to manage conflict situations pitting rewards against potential punishments.

The question remains as to what neurochemical perturbations may drive the effects of stress reported here. One potential candidate may be increased CRF activity, acting in brain regions more removed from the HPA axis, although comparisons between the effects of acute stress and increased CRF activity on punished reward-seeking remains to be explored. That said, it bears mentioning that the effects of restraint stress on effort-related choice were blocked by a CRF antagonist and reproduced by intracranial infusion of CRF into the ventricles or ventral tegmental dopamine cell body region (Bryce and Floresco, 2016). Speaking of dopamine, restraint or other stressors can increase different parameters of dopamine neuron activity (Anstrom and Woodward, 2005; Valenti et al., 2011), and enhances dopamine efflux prefrontal cortex and NAc terminal regions (Doherty and Gratton, 2007; Imperato et al., 1991; Kalivas and Duffy, 1995; Lillrank et al., 1999). This is notable, as the effects of restraint on decisions involving physical costs (i.e., effort, punishment) can be reproduced by pharmacological increases in dopamine D_2 receptor activity in the NAc (Bryce and Floresco, 2019; Mitchell et al., 2014), whereas neither stress nor increased NAc D_2 activity affects decisions involving subjective costs such as reward uncertainty or delays (Bryce et al., 2020; Shafiei et al., 2012; Stopper et al., 2013; Yates and Bardo, 2017). Thus, increases in NAc D_2 activity could be another mechanism through which stress biases choice away from larger rewards associated with physical costs, but whether this may also relate to effects on inhibitory control is unclear (Moreno et al., 2013; Pezze et al., 2007). Along similar monoaminergic lines, stress increases noradrenergic transmission in numerous CNS regions. As noted previously, the α2 antagonist yohimbine (which increases noradrenaline release) impairs inhibitory control on a variety of assays (Adams et al., 2017; Chernoff et al., 2021; Sanger, 1988), suggesting stress-induced increases in impulsive action may be driven in part by augmented noradrenaline signalling. On the other hand, pharmacological manipulations of noradrenergic transmission have little effect on risky decision making (Blaes et al., 2018), and thus, stress-related noradrenergic signalling may play less of a role in biasing action away from punished rewards. Indeed, given the considerable differences in how stress affected the two forms of punished reward seeking studied here, it is plausible that different constellations of neurochemical changes may contribute to the reduced risky choice and sex-depended alterations in impulsive action that can be induced by acute stress.

4.4. Conclusions

Collectively, the results reported here reveal that acute stress can differentially influence deliberative and inhibitory processes that help a decision maker navigate situations where reward-seeking may yield aversive consequences. The sex-independent reduction in preference for larger rewards associated with potential punishments and increased deliberation times may reflect a broader ability of acute stress to sway choice biases away from physical costs and hamper timely resolution of these response conflicts. In comparison, stress exerts disruptive influence on inhibitory control in males, whereas females are more impervious to these effects. The revelation of these complex actions of acute stress on executive functions related to decision making and impulse control may also provide insight into reward hyposensitivity and punishment hypersensitivity associated with stress-related disorders, such as certain types of depression. Indeed, there is evidence suggesting that some depressive symptoms stem from maladaptive learning about rewards and punishments and a consequential inability to adjust behavior (Alloy et al., 2016; Eshel and Roiser, 2010; Hevey et al., 2017; Pizzagalli, 2014). In this regard, it is clear that the neurochemical/hormonal abnormalities associated with depression are far more complex than those induced by acute stress. Nevertheless, elucidation of the neural/neurochemical perturbations that drive stress-induced changes in reward-related decision making and inhibitory control under threat of punishment may help clarify some of the pathophysiological mechanisms that underlie dysfunction in these processes in stress-related disorders.

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CRediT authorship contribution statement

Giulio Laino Chiavegatti: Writing – review & editing, Methodology, Investigation, Formal analysis. **Stan B. Floresco:** Writing – review & editing, Writing – original draft, Supervision, Project administration,

Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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