

Acute Stress Induces Selective Alterations in Cost/Benefit Decision-Making

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Acute stress can exert beneficial or detrimental effects on different forms of cognition. In the present study, we assessed the effects of acute restraint stress on different forms of cost/benefit decision-making, and some of the hormonal and neurochemical mechanisms that may underlie these effects. Effort-based decision-making was assessed where rats chose between a low effort/reward (1 press = 2 pellets) or high effort/reward option (4 pellets), with the effort requirement increasing over 4 blocks of trials (2, 5, 10, and 20 lever presses). Restraint stress for 1 h decreased preference for the more costly reward and induced longer choice latencies. Control experiments revealed that the effects on decision-making were not mediated by general reductions in motivation or preference for larger rewards. In contrast, acute stress did not affect delay-discounting, when rats chose between a small/immediate vs larger/delayed reward. The effects of stress on decision-making were not mimicked by treatment with physiological doses of corticosterone (1–3 mg/kg). Blockade of dopamine receptors with flupenthixol (0.25 mg/kg) before restraint did not attenuate stress-induced effects on effort-related choice, but abolished effects on choice latencies. These data suggest that acute stress interferes somewhat selectively with cost/benefit evaluations concerning effort costs. These effects do not appear to be mediated solely by enhanced glucocorticoid activity, whereas dopaminergic activation may contribute to increased deliberation times induced by stress. These findings may provide insight into impairments in decision-making and anergia associated with stress-related disorders, such as depression.

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INTRODUCTION

Substantial evidence suggests that acute stress alters different forms of cognitive functioning, in a manner dependent on a variety of factors, including the type of task, the specific brain circuitry recruited by these tasks, and the severity of the stressor (Shors *et al*, 1992; Stillman *et al*, 1998; Cordero *et al*, 2003; Joëls *et al*, 2006; Shansky *et al*, 2006; Luethi *et al*, 2008). Cost/benefit decision-making is a complex form of cognition mediated in part by the prefrontal cortex (PFC) that may be sensitive to acute stress. Some of the pioneering research on the topic stemmed from the observations that highly skilled pilots fell victim to battle-induced mental errors and poor decision-making during stressful circumstances (Broadbent, 1971). It is now understood that under conditions of acute stress, flexible decision-making dependent on normal PFC functioning is

impaired (Arnsten, 1998), causing an organism to rely more on habitual behavior (Elliott and Packard, 2008). Recent laboratory studies in humans have begun to investigate how acute stress may alter decision-making using tasks designed to simulate real-life decisions, with respect to uncertainty, reward, and punishment. These studies have revealed that either acute cold stress or the administration of exogenous glucocorticoids impair decision-making by either decreasing the net gain of the subject over the session, or biasing the choice towards the riskier option when the chances of losing are higher (Miu *et al*, 2008; Porcelli and Delgado, 2009; Putman *et al*, 2010). Thus, acute stress may interfere with decision-making by altering the manner in which individuals evaluate the relative costs and benefits associated with different options, sometimes leading them to make more disadvantageous choices.

There has been a growing interest in modeling certain aspects of decision-making in experimental animals. One component process that can be assessed in rodents is the evaluation of costs associated with different candidate actions, relative to the potential rewards that may be obtained by those actions. Some response costs that are effective in biasing choice include making the delivery of the reward uncertain or risky, delaying its delivery, or requiring animals

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to exert greater physical effort to obtain a larger reward. Regarding this latter form of decision-making, it is notable that stress-related neuropsychiatric disorders, such as depression, are associated with anergia and other motivational deficits. This suggests that cost/benefit evaluations regarding effort requirements may be particularly susceptible to modulation by stress.

Effort-based decision is mediated by an interconnected neural network that includes the temporal, frontal, forebrain, and midbrain regions. Preference for larger, but costlier rewards is reduced by lesions/inactivation of the dorsomedial PFC (anterior cingulate; Walton *et al*, 2002, 2003; Schweimer and Hauber, 2005), basolateral amygdala (Floresco and Ghods-Sharifi, 2007; Ghods-Sharifi *et al*, 2009), and nucleus accumbens (NAc) (Hauber and Sommer, 2009; Ghods-Sharifi and Floresco, 2010). Dopamine (DA) also has a role in overcoming effort-related costs, as reducing DA activity either systemically or directly in the NAc or PFC reduces preference for higher effort/reward (HR) options (Cousins and Salamone, 1994; Salamone *et al*, 1991; Schweimer and Hauber, 2006). Interestingly, increasing DA activity with drugs such as amphetamine exerts differential dose-dependent effects. Lower doses increase preference for the HR option, whereas higher doses reduce preference for the HR (Floresco *et al*, 2008b).

It is notable that neural circuitry implicated in facilitating effort-based decision-making is particularly sensitive to acute stress. Prefrontal and limbic regions recruited by these processes show dense glucocorticoid receptor expression (Putman *et al*, 2010). Furthermore, there is considerable evidence that acute stress increases extracellular DA concentrations within the PFC, Nac, and amygdala (Roth *et al*, 1988; Abercrombie *et al*, 1989; Imperato *et al*, 1989, 1991; Davis *et al*, 1994; Finlay *et al*, 1995; Inglis and Moghaddam, 1999), which may in turn contribute to stress-induced alterations in cognitive functioning mediated by these systems. Thus, acute stress can cause enhancement in amygdala-dependent associative learning, classical conditioning, and contextual fear conditioning (Shors *et al*, 1992; Cordero *et al*, 2003; Joëls *et al*, 2006), but can impair PFC-dependent working memory (Stillman *et al*, 1998; Shansky *et al*, 2006; Luethi *et al*, 2008). Therefore, the possibility remains that acute stress may interfere with effort-related judgments as it does with various types of cognition in animals and other forms of decision-making in humans.

To investigate this in more detail, we assessed the effects of acute restraint stress on effort-based decision-making, using an operant-based discounting task (Floresco *et al*, 2008b). Restraint is a well-characterized method of inducing stress in laboratory rodents, as it reliably increases circulating levels of stress-associated hormones, including adrenocorticotrophic hormone and corticosterone (CORT; Kant *et al*, 1983, 1987; Williamson *et al*, 2005; Arnsten, 2009). Restraint stress disrupts cognition in rats (Shansky *et al*, 2006; Cordero *et al*, 2003) and induces reliable increases in DA release within the PFC and NAc (Roth *et al*, 1988; Abercrombie *et al*, 1989; Imperato *et al*, 1989, 1991; Finlay *et al*, 1995; Davis *et al*, 1994). The fact that this form of acute stress has been well-characterized in terms of its hormonal, dopaminergic, and cognitive effects made it an ideal approach for studying how acute stress may alter cognitive processes mediated by corticostriatal and dopaminergic

circuitry. An initial study revealed that restraint stress altered effort-discounting. Subsequent experiments clarified the specific cognitive/motivational processes that were affected by stress, which may have contributed to the alterations in decision-making. Furthermore, we also investigated some of the hormonal and neurochemical mechanisms that may underlie these effects, specifically looking at the contribution of CORT and DA to the effects of stress on this form of decision-making.

MATERIALS AND METHODS

Animals

Different cohorts of male Long Evans rats (Charles River Laboratories, Montreal, QC, Canada) weighing 275–300 g, at the beginning of training, were used. Upon arrival, rats were given 1 week to acclimatize to the colony conditions, and were subsequently individually housed and food restricted to 85–90% of their free-feeding weight 1 week before behavioral training. Water was provided *ad libitum* for the duration of the experiment. Body weights were monitored daily, and food was provided in the animal's home cage at the end of each experimental day. All testing was done in accordance with the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia. The number of animals used for each experiment ranged from 12 to 14, with the exception of the reward magnitude discrimination and delay-discounting studies. In our experience, rats performing these latter tasks tend to display less intra-group variability, thereby requiring fewer animals to detect significantly reliable differences across treatment conditions.

Apparatus

All behavioral testing was conducted in operant chambers (30.5 × 24 × 21 cm; Med-Associates, St Alban, VT) enclosed in a sound-attenuating box. Boxes were equipped with a fan that provided ventilation and masked extraneous noise. Each chamber was fitted with two retractable levers, one located on each side of a central food receptacle, where food reinforcement (45 mg; Bioserv, Frenchtown, NJ) was delivered by a pellet dispenser. The chambers were illuminated by a single 100-mA house light located in the top center of the wall opposite the levers. All experimental data were recorded by a personal computer connected to the chambers through an interface.

Restraint Stress

Acute stress was induced by restraining rats in a plexiglas semi-cylindrical tube (83 × 133 × 197 mm; Harvard Apparatus, Massachusetts) in a quiet, lit, and ventilated room. Rats were placed in the tubes and a restrainer length was adjusted to keep the rat immobilized without causing pain. A desktop fan circulated air over the restraint tubes to minimize hyperthermia. Upon being removed from the restraint tube, animals were returned to their home cages and left undisturbed for 10 min before being placed in the operant chambers. In the first experiment, rats received two counterbalanced restraint stress sessions of differing durations (20 min or

1 h) on separate test days. In all other experiments, only the 1-h duration of restraint was used. For all experiments, 1–2 days before a stress test session, rats (in their home cages) were placed in the same room where stress manipulations would take place on the following day. They were left in the room for the amount of time corresponding to the duration of the stressor they would receive on the following day (20 min or, for most experiments 1 h). There were no statistically significant differences in behavior across experiments between training sessions, where animals were placed in the stress-procedure room when compared with the regular training sessions. Therefore, baseline control data for all behavioral measures were obtained by computing an average of the particular measure recorded over the last 2 days before stress, which included data taken from a regular training session, and a session preceded by being placed in the stress-procedure room without restraint. These baseline values served as the key control data for which within-subjects statistical comparisons were made. For experiments where animals received multiple stressors, these tests were separated by at least 7 days.

Initial Lever-Pressing Training

Our initial training protocol has been described previously (Floresco *et al*, 2008a; Ghods-Sharifi *et al*, 2009). On the day before the first exposure to the operant chamber, rats were given approximately 20 reward pellets in their home cage. Before the animal was placed in the chamber for the first training session, two to three crushed pellets were placed inside of the food receptacle and on the active lever. Rats were trained under a fixed-ratio (FR)-1 schedule to a criterion of 60 presses in 30 min, first for one lever, then the other (counterbalanced left/right between subjects). For rats that were to be trained on discounting tasks, subsequent sessions consisted of training on a simplified version of the full task, in which they had to act upon a retractable lever within 10 s of insertion. These sessions consisted of 90 training trials, and began with both levers retracted and the chamber in darkness. Every 40 s, a trial was initiated with the illumination of the houselight and the extension of one of the two levers. If the rat failed to respond within 10 s after lever insertion, the lever was retracted, the chamber returned to darkness, and the trial was recorded as an omission, and the chamber was reset to the intertrial state. A response on the lever within 10 s of its insertion delivered

one reward pellet. Rats were trained for approximately 5 days to a criterion of 80 successful trials (ie, <10 omissions), after which they were trained on the decision-making task.

Behavioral Tasks

We assessed the effects of acute restraint stress on a number of different behavioral tasks to obtain a comprehensive understanding of the manner in which stress may affect the effort-based decision-making. Separate groups of rats were trained and tested on a particular behavioral task before receiving stress and/or drug manipulations.

Effort discounting. The primary task used in these studies was an effort-discounting task we have described previously (Floresco *et al*, 2008b; Ghods-Sharifi *et al*, 2009; Ghods-Sharifi and Floresco, 2010). Each training day, animals received a 32-min session that consisted of 48 discrete trials, separated into 4 blocks. The format of a single trial is presented in Figure 1. A session began with the chamber in darkness and both levers retracted (the intertrial state). At 40-s intervals, the houselight was illuminated, followed by the extension of one or both levers 3 s later. Each of the 4 trial blocks began with two forced-choice trials, where only one of the two levers was randomly presented. During the next 10 trials, both levers were presented. One lever was designated as the low-reward (LR) lever, and the other lever was designated as the HR lever. These levers were counterbalanced (left/right) between animals and remained constant for each animal for the duration of the experiment. Upon lever insertion, rats were required to respond within 25 s; failure to do so (omission) caused the chamber to reset to the intertrial state. A single press of the LR lever resulted in the retraction of both levers and immediate delivery of two pellets. However, after the first response on the HR lever, the LR lever was retracted, and the HR lever remained inserted until the required ratio of presses was completed. The ratio requirement for the HR lever increased within the session. Upon completion of the required effort ratio on the HR lever, the lever retracted and four pellets were delivered 0.5 s apart. The houselight remained on for 4 s after food delivery.

The fixed effort ratio of lever presses required to obtain the HR increased over the 4 blocks of trials, beginning with 2 presses, then 5, 10, and 20 presses. On the rare occurrence when a rat failed to complete the required number of

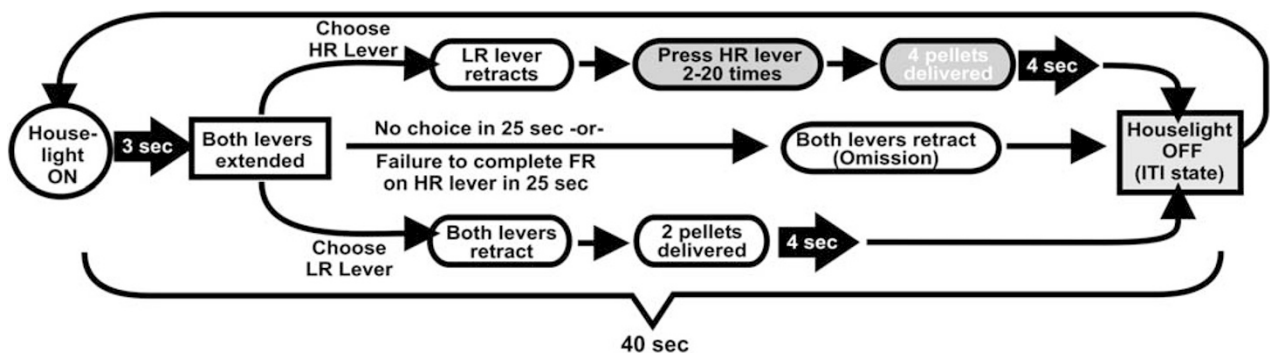


Figure 1 The format of a single free-choice trial on the effort-discounting task.

presses on the HR lever within 25 s after insertion, the lever retracted without food delivery. However, the animal's choice was still incorporated into the data analysis. In addition, latencies to initiate a lever press and complete the required number of presses on the HR lever were recorded.

Rats were trained on the effort-discounting task until as a group, they (1) chose the HR lever during the first trial block on at least 75% of successful trials, and (2) demonstrated stable baseline levels of discounting for 3 consecutive days. Stability was assessed using statistical procedures similar to that described by Floresco *et al* (2008b) and St Onge and Floresco (2009). Data from three consecutive sessions were analyzed with a repeated-measures ANOVA with two within-subjects factors (training day and trial block). If the effect of block was significant at the $p < 0.05$ level but there was no main effect of day or day \times trial-block interaction (at $p > 0.1$ level), animals were judged to have achieved stable baseline levels of choice behavior.

Reward magnitude discrimination. This task was similar to the effort-discounting task (ie, 48 trials, 4 trial blocks, 2 forced choice/10 free choice trials per block, 40 s intertrial interval), with the key exception that a single press of either the LR or HR lever caused the immediate delivery of either 2 or 4 pellets. Thus, animals merely had to choose between a smaller or larger reward, with no additional cost associated with the HR option. Rats were trained for 9 days, after which they displayed a strong preference for the HR lever ($\sim 90\%$). They were subsequently subjected to a baseline session, followed by a 1-h restraint stress challenge on the next day.

Effort-discounting with equivalent delays. This modified version of the effort-discounting task assessed the contribution that delays to reward delivery intertwined with effort requirements made to decision-making. Rats were trained for 16 days on the standard effort-discounting task, and were then trained on a modified version of the original task. Here, a single press on the LR lever caused the immediate retraction of both levers and delivered two pellets after a delay equivalent to that required for rats to complete the ratio of presses on the HR, using the standard effort-discounting procedure (0.7–12.5 s). Thus, for each block of trials, the delay to reward delivery after an initial choice of either lever was equalized. The delay to receive two pellets after a single press on the LR lever increased across trial blocks and was calculated on the basis of the average time it took the rats to press the HR lever 2, 5, 10, and 20 times during the last 3 days of training on the effort-discounting task. Thus, if rats required 12.5 s to press the HR lever 20 times during the last trial block, a single press on the LR lever during this block would deliver two pellets after a 12.5 s delay. During these trials, the houselight remained illuminated throughout the delay. Rats received 8 training sessions with this task (in addition to the 16 sessions with the standard effort-discounting task), at which point they displayed stable levels of choice for 3 consecutive days. On the following day, they were subjected to the baseline control test followed by a restraint stress challenge on the next day.

Delay-discounting. In this experiment, rats received initial lever training as described previously. They then received 1 day of reward magnitude discrimination (four *vs* one pellet). Subsequently, they received daily training sessions

on a delay-discounting task (Zeeb *et al*, 2010). Like the effort-discounting procedure, this task consisted of 48 trials, divided into 4 trial blocks (first 2 forced, next 10 free-choice). The intertrial interval was 70 s (56-min session). On each trial, a single press on the LR lever retracted both levers and delivered one pellet immediately. Selection of the HR lever also retracted both levers and delivered four pellets after a delay that increased across the four trial blocks (0, 15, 30, and 45 s). During the delay, the houselight was extinguished and re-illuminated during food delivery at the end of the delay.

Progressive ratio tests. In this experiment, only the left lever was inserted into the chamber and remained in place for the duration of each training session. During the initial 4 days of training, rats learned over 30-min sessions to press the lever for a single reward pellet, using FR-1, FR-2, and then FR-5 schedule. They then received daily training sessions on a progressive ratio schedule, in which the ratio of presses required to obtain a single pellet increased after each pellet delivery. The ratio increased in the following manner: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 693, 737, and 901 presses (Brown *et al*, 1998). Rats had a maximum of 20 min to complete each ratio; failure to do so ended the session. The primary variables of interest were: (1) the total number of lever presses made over the course of a session and (2) the total number of rewards obtained before a session terminates (breakpoint).

CORT Challenge

The effects of acute CORT treatment on effort-based decision-making were assessed using a within-subjects design. Rats were trained on the standard effort-discounting task until they displayed stable levels of choice. They were then subjected to the first of two injection test days. Each test day was part of a 2-day sequence. After injections, rats were returned to their home cage and left undisturbed for 90 min, after which they received a training session. On the first day, all rats received a vehicle injection (50/50 propylene glycol/0.9% saline, *s.c.*). On the subsequent day, rats were split into two groups and matched for choice behavior after vehicle injections, and then received their first counterbalanced injection of CORT (1 or 3 mg/kg, Sigma Aldrich). After this first test-day sequence, rats were retrained for 4 days until they again displayed stable baseline levels of choice. They then received a second injection test-day sequence (ie, vehicle injection on the first day, and a counterbalanced injection of either 1 or 3 mg/kg CORT on the second day). The doses of CORT used and the time course of administration were originally derived from studies showing that they approximate increases in CORT release occurring during comparable durations of restraint stress (Meaney *et al*, 1988; Imperato *et al*, 1991).

Plasma CORT Assay

To compare CORT levels induced by injections (vehicle, 1.0 and 3.0 mg/kg) and 1 h of restraint exposure, tail vein blood samples were repeatedly collected in separate groups of animals not used for behavioral testing, immediately following removal from home cage (0 min), and at 30, 60, and 90 min

after initial s.c. injections, or from restraint onset. Plasma levels of CORT were measured in duplicate using the RIA kit from MP Biomedicals (Solon, OH) as previously described (Gray *et al*, 2010), undertaken in a single analysis to avoid inter-assay variability, with an intra-assay coefficient of variation of 6%.

DA Antagonism

The contribution of DA transmission to the stress-induced alterations of effort-discounting was assessed with a within-subjects design. Rats were trained on the effort-discounting task until they displayed stable baseline levels of choice behavior at 3 consecutive days. They were then subjected to the first of 4 test days: (1) saline/no stress, (2) saline/stress, (3) flupenthixol/no stress, or (4) flupenthixol/stress. Rats were split into four groups of three, with each group receiving the four tests in a counterbalanced order, using a quasi-Latin Square design. This was done to control for any potential habituation to the stressor that may have occurred following repeated exposure to restraint. With this design, none of the rats in any of the groups were subjected to restraint stress on consecutive test days. For example, rats first assigned to the saline/no stress condition subsequently received saline/stress on the second test day, followed by the flupenthixol/no stress condition on the third test day and so on.

On the first test day, all animals were injected with either saline or a low dose of flupenthixol (0.25 mg/kg, i.p., Sigma Aldrich) and then returned to their home cage for 10 min. They were then subjected to either 1 h of restraint stress, or left undisturbed in their home cage in a room similar to the stress procedure room for 1 h. Behavioral testing for each commenced 10 min after being removed from the restraint tube/control room. Each subsequent test day was administered when choice behavior of rats within a particular subgroup was stable for 2 consecutive days. For this particular experiment, rats required a minimum of 5 and a maximum of 8 days of retraining between test days.

RESULTS

Effects of Different Durations of Acute Restraint Stress on Effort-Discounting

The primary aim of this experiment was to determine whether acute restraint stress alters different aspects of effort-based decision-making. A secondary aim was to determine if there is a 'threshold' of stress duration that would affect decision-making. Rats ($n = 14$) were trained for 22 days on the effort-discounting task, after which they displayed stable levels of choice behavior. They subsequently received the first of two counterbalanced stress tests of either 20 min or 1 h in duration. After the first session of restraint, rats were retrained for 7 days before receiving their second stress test. Baseline choice of the HR lever before 20 min or 1 h stress test days did not differ, and were thus averaged and used for the data analysis.

Restraint stress induced a marked decrease in the preference to work harder for a larger reward. Choice data were analyzed with a three-way between/within subjects ANOVA, with the order of stress duration (20 min or 1 h restraint) as a between-subjects factor, and test day (baseline, 20 min or

1 h stress) and trial block as two within-subjects factors. This analysis revealed a significant effect of test day ($F_{2,24} = 7.94$, $p < 0.005$), but not a significant test day \times trial block interaction ($F_{6,72} = 1.15$, n.s.). Multiple comparisons confirmed that 1-h restraint stress significantly ($p < 0.05$) decreased choice of the HR lever relative to baseline (Figure 2a). This effect was apparent in the first trial block,

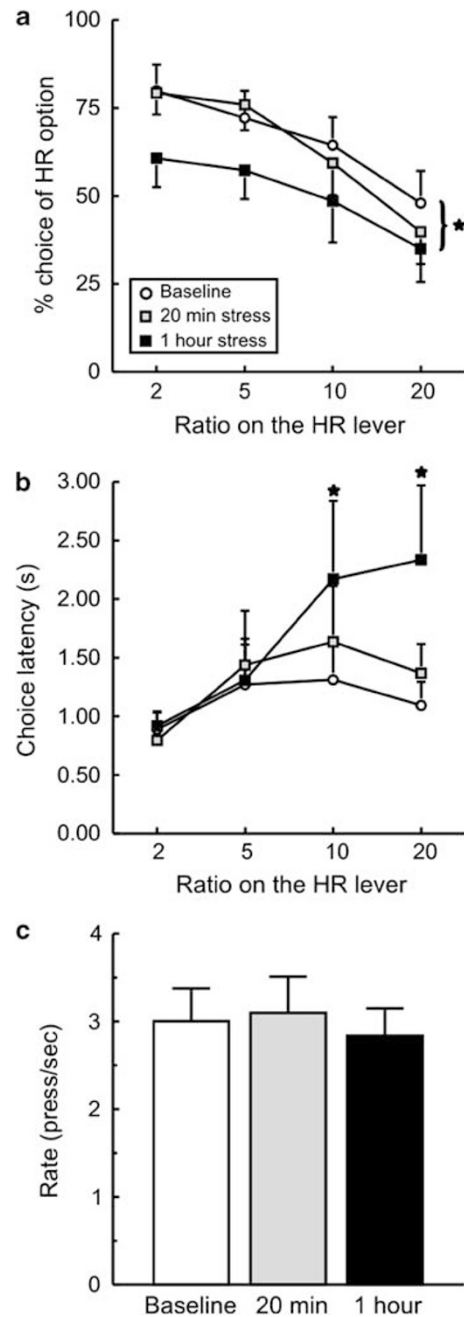


Figure 2 Effects of acute restraint stress (1 h and 20 min) on effort-discounting. (a) The ordinate shows the percent choice of the HR lever across the four trial blocks and the abscissa indicates the four trial blocks with increasing effort ratio; 1 h and not 20 min restraint decreased the selection of the HR option across all trial blocks. (b) The response latency across trial blocks; 1 h restraint increased the latency to respond in the third and fourth trial block. (c) Acute restraint stress did not affect the rates of pressing on the HR lever. Stars denote significant main effect of treatment at $p < 0.05$.

and persisted over the duration of the session. In comparison, 20 min of restraint induced a smaller decrease in preference for the HR lever in the last trial block (20 presses), but this effect did not achieve statistical significance. Notably, there was no main effect of the order of stress duration, or any interactions with the within-subjects factors (all $F_s < 2.27$, n.s.), indicating that rats initially exposed to 20 min stress showed alterations in behavior after 1 h stress comparable to rats that received 1 h stress on the first test day.

Acute stress also led to longer latencies to make a choice. These data were analyzed in a manner similar to the choice data, and revealed a significant test day \times trial block interaction ($F_{6,72} = 2.67$, $p < 0.05$; Figure 2b). Simple main effects analysis confirmed that 1 h (but not 20 min) restraint significantly increased choice latencies during the last two trial blocks when the effort requirements on the HR lever were 10 and 20 presses, respectively. Again, there was no main effect of the order, or any interactions with the within-subjects factors (all $F_s < 1.17$, n.s.).

In contrast to the effect on choice latencies, acute stress did not alter the rates of lever pressing (press/sec) on the HR lever ($F_{2,24} = 1.02$, n.s.; Figure 2c). Thus, even though 1 h stress decreased the overall preference for the HR lever, on trials where rats choose this option, they responded as robustly as they did after 20 min of stress or on baseline days. Stress did not increase the number of trial omissions (baseline: 0.48 ± 0.21 , 20 min: 1.21 ± 0.85 , 1 h: 1.86 ± 1.036 ; $F_{1,13} = 0.002$, n.s.). Collectively, these data suggest that 1 h of restraint stress causes a substantial decrease in the preference for animals to work harder to obtain a larger reward, and also led to longer deliberation times when the HR option is associated with a relatively high effort cost. As only the 1 h stressor was effective at altering choice behavior and response latencies on the effort-discounting task, all subsequent experiments only used this duration of restraint.

The Effects of Acute Restraint Stress on Reward Magnitude Discrimination

One potential explanation for the effects of stress on effort-discounting is that it may have caused a decrease in the subjective preference for objectively larger rewards. To address this possibility, we assessed the effects of 1 h acute stress on a reward magnitude discrimination task. A separate group of rats ($n = 8$) were trained for 9 days before

receiving baseline and stress test days. Stress did not decrease the preference for the HR option (main effect of test day ($F_{1,7} = 1.577$, n.s.); test day \times trial block interaction ($F_{3,21} = 1.56$, n.s.; Figure 3a). However, as was observed in the previous experiment, acute stress did increase response latencies (main effect of test day; $F_{1,7} = 16.84$, $p < 0.005$; Figure 3b). Again, trial omissions were unaffected by acute stress (baseline: 0, 1 h: 0). Thus, acute stress did not disrupt the general preference for larger *vs* smaller rewards, but again increased deliberation times to make a selection.

Effect of Acute Restraint Stress on Effort-Discounting with Equivalent Delays

With the effort-discounting task, choice of the high effort option imposes a delay from the time of the initial choice to when the reward is delivered. It is possible that the stress-induced decrease in the preference for the HR option may reflect decreased tolerance for delayed rewards rather than a decreased tolerance for greater effort demands. One manner to evaluate this hypothesis is to employ an equivalent delay procedure in combination with the effort-discounting task. This task has previously been used to dissociate between the effort and delay requirements embedded within the effort-discounting task (Floresco *et al*, 2008b; Ghods-Sharifi *et al*, 2009; Ghods-Sharifi and Floresco, 2010). In this task, the effort requirement to obtain the LR/HR was identical to the standard task, but selection of the LR option incurred a delay to reward delivery comparable to the time required to complete the ratio of presses on the HR lever. Thus, this procedure effectively equalizes the relative delay costs associated with the HR and LR options.

A separate group of rats ($n = 13$) were initially trained on the effort-discounting task for 16 days, followed by another 8 days of training using the equivalent delays procedure. For this experiment, the average delays before delivery of the LR were set at 0.7, 2.6, 6.1, and 12.5 s for each respective block. Restraint stress markedly decreased preference to work harder for the HR when delays to reward delivery were equalized across the two options. A two-way ANOVA revealed a significant effect of test day ($F_{1,12} = 6.93$, $p < 0.05$), but not a significant test day \times trial block interaction ($F_{3,36} = 1.12$, n.s.). This decrease in choice of the HR was apparent in the first trial block and persisted for the duration of the session (Figure 4a).

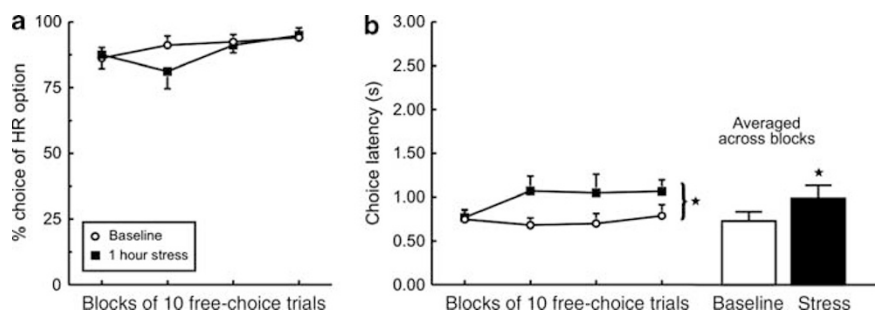


Figure 3 Effects of acute restraint stress on performance in a reward magnitude discrimination task. (a) Acute restraint stress did not alter preference for the HR option when there was no additional cost associated with it. (b) Response latencies across each block of 10 free-choice trials (left) and averaged across the four trial blocks (right). Acute restraint stress increased the latency to make a choice. Stars denote significant main effect where $p < 0.05$.

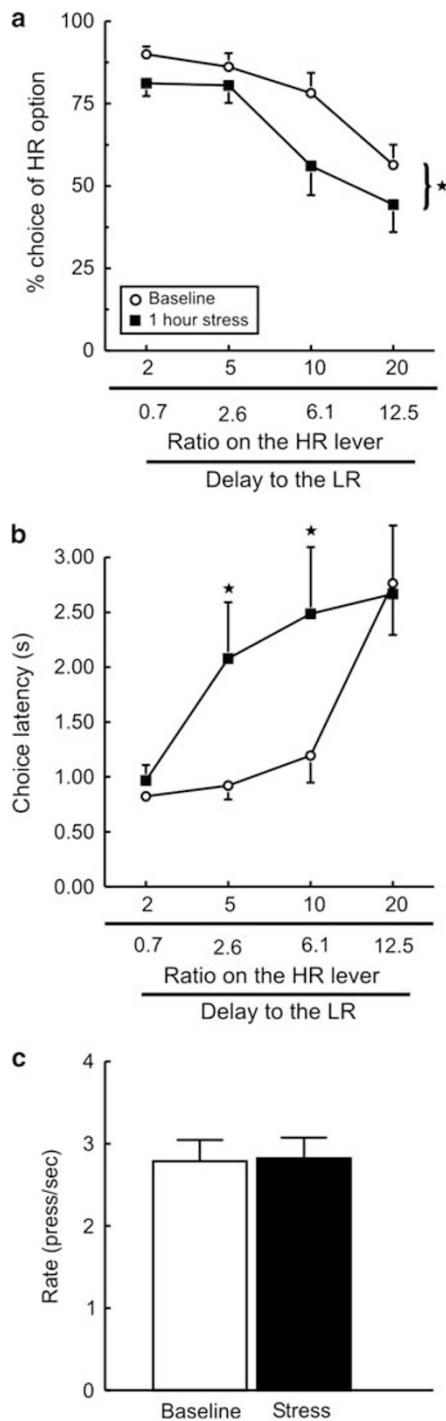


Figure 4 Effects of acute restraint stress on effort-based decision-making with equivalent delays. (a) The ordinate shows the percent choice of the HR and the abscissa indicates the four trial blocks with increasing effort ratio of the HR (top) and increasing delay to reward delivery of the LR (bottom). Acute restraint stress decreased the selection of the HR option even when the delays to the delivery of the LR were similar. (b) Response latencies across the four trial blocks. Acute restraint stress increased the latency to respond in the second and third trial block. (c) Acute restraint stress did not affect the rates of lever pressing. Stars denote significant main effect where $p < 0.05$.

Acute stress also yielded longer response latency in a manner similar to that observed in experiment 1. Analysis of these data revealed a significant test day \times trial block

interaction ($F_{3,36} = 3.37$, $p < 0.05$). Simple main effects analyses confirmed that after 1 h of restraint, the response latency in the second and third trial blocks were significantly longer ($p < 0.05$) than baseline (Figure 4b). However, stress again did not alter the average rates of lever pressing on the HR lever ($F_{1,12} = 0.12$, n.s.; Figure 4c). Additionally, stress did not alter the number of trial omissions (baseline: 2.54 ± 1.24 , 1 h: 4.7 ± 2.30 ; $F_{1,12} = 1.71$, n.s.). Thus, 1 h of restraint stress substantially decreased preference for animals to work harder to obtain a larger reward even when the delays to reward delivery between the HR and the LR options were equalized.

Effects of Acute Restraint Stress on Delay-Discounting

The possibility remained that acute stress may also affect other forms of cost/benefit decision-making related to choice between smaller/immediate and larger/delayed rewards. This was investigated using a delay-discounting task, a well-established method of assessing impulsive choice.

A separate group of rats ($n = 8$) were trained on a delay-discounting task for 25 days before receiving baseline and stress test days. Restraint stress did not alter delay-discounting (main effect of test day ($F_{1,7} = 1.12$, n.s.); test day \times trial block interaction ($F_{3,21} = 1.46$, n.s.; Figure 5a). Interestingly, in this experiment, restraint stress did not alter choice latencies (all $F_s < 3.17$, n.s.; Figure 5b). Furthermore, stress did not alter the number of trial omissions (baseline: 1 ± 0.27 , 1 h: 0.625 ± 0.18 , $F_{1,7} = 1.615$, n.s.). Thus, in contrast to the effects of restraint on effort-related decisions, acute stress does not appear to reduce preference for larger, delayed rewards.

Effect of Acute Restraint Stress on Responding on a Progressive Ratio Schedule of Reinforcement

It is possible that the effects of acute stress on effort-based decision-making may reflect a disruption in certain motivational processes, which may in turn interfere with effort-related judgments. For example, acute stress may render animals either unwilling or unable to respond on a lever repeatedly to obtain a reward. This possibility was assessed using a progressive ratio schedule of reinforcement.

A group of 13 rats were initially trained over 4 days to press a lever on a FR-1, FR-2, and then FR-5 schedule. Rats were then trained using a progressive ratio schedule for 22 days, after which the group displayed stable levels of lever pressing and breakpoints for 3 consecutive days (ie, $< 15\%$ variation within the group). Acute stress did not alter responding on a progressive ratio of reinforcement. Analysis of the total number of lever presses and breakpoints reached confirmed no significant differences on these measures on baseline vs stress test days (total lever presses: $F_{1,12} = 0.85$, n.s.; breakpoints: $F_{1,12} = 0.14$, n.s.; Supplementary Fig 1A, B). Rates of lever pressing on baseline and stress test days were also analyzed. On the stress test days, all rats successfully completed the first eight ratio requirements; however, the final breakpoint varied across rats. We calculated the average rates of lever pressing to obtain the first seven and the final two pellets on baseline and stress test days. Stress did not alter rates of lever pressing relative to baseline, (main effect of test day; $F_{1,12} = 1.905$,

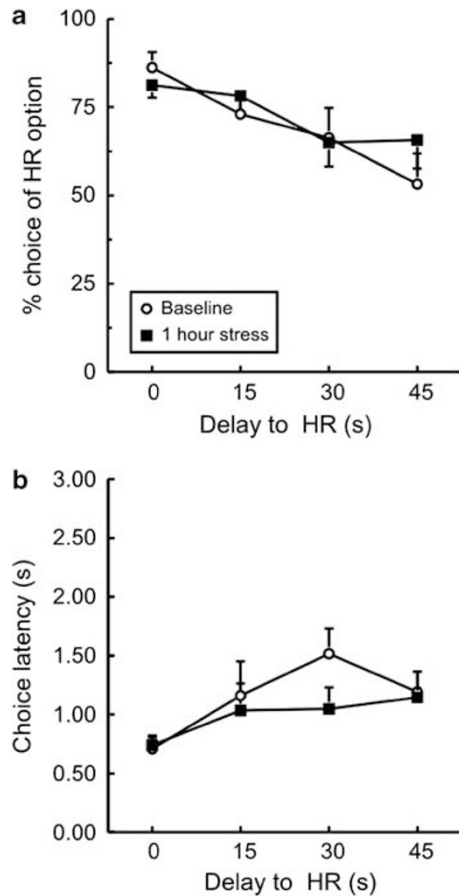


Figure 5 Effects of acute restraint stress on delay-discounting. (a) The ordinate shows the percent choice of the HR lever and the abscissa indicates the delays to HR delivery across the different trial blocks. Acute restraint stress did not alter the choice of the delayed HR. (b) Response latency across the four trial blocks. Acute restraint stress did not alter response latencies when choosing between a delayed HR and an immediate LR.

n.s.; test day \times pellet interaction $F_{8,96} = 1.464$, n.s.; Supplementary Fig 1C). These findings are consistent with the lever pressing and breakpoint data, indicating that acute restraint stress does not appear to cause a general reduction in motivation to work for food.

The Effects of Exogenous CORT on Effort-Based Decision-Making

Restraint stress increases the release of CORT, which in turn mediates numerous physiological, neural and behavioral changes associated with stress. To probe whether the effects of acute restraint on effort-based decision-making were attributable to increases in CORT activity, we investigated whether administration of exogenous CORT could alter effort-discounting in a manner similar to acute restraint stress.

A group of 12 rats were trained on the effort-discounting task for 30 days before receiving injections of vehicle or CORT on separate test days. Choice data from these challenge sessions were analyzed using a three-way between/within-subjects ANOVA, with the order of

treatment (1 or 3 mg/kg CORT first) as a between-subjects factor, and dose (vehicle, 1 and 3 mg/kg CORT) and trial block as two within-subjects factors. The behavioral parameters of interest did not differ between the first and second vehicle injections (all $F_s < 1.9$, n.s.). Thus, the average values of these measures were used for the data analysis. As displayed in Figure 6a, treatment with either dose of CORT did not alter preference for the HR lever relative to vehicle (main effect of dose: $F_{2,20} = 0.004$, n.s.; dose \times block interaction: $F_{2,20} = 1.16$, n.s.). There were also no effects of the order of treatment or interactions with the within-subjects factors (all $F_s < 1.74$, n.s.). Similarly, there were no effects of CORT on choice latencies (Figure 6b), trial omissions (vehicle: 0.17 ± 0.11 , 1 mg/kg: 0.25 ± 0.13 , 3 mg/kg: 0.33 ± 0.14) or lever presses per second (vehicle: 3.6 ± 0.4 , 1 mg/kg: 3.6 ± 0.4 , 3 mg/kg: 3.7 ± 0.5 ; all $p > 0.35$). Thus, administration of exogenous CORT did not mimic the effects of acute restraint stress on effort-discounting or other behavioral measures.

To confirm that increases in CORT plasma levels induced by exogenous treatment with the hormone were comparable to those experienced by rats subjected to 1 h restraint stress, we conducted another experiment in separate groups of rats ($n = 6$ per group). Specifically, we assessed changes in plasma CORT levels relative to baseline after 1 h restraint, or injections of vehicle, 1 or 3 mg/kg CORT. Blood samples were taken before restraint/injections, and then 30, 60 and 90 min after the respective manipulation. For animals in the injection groups, the 90 min time point represented the time after vehicle/CORT treatment when testing would have commenced for rats in the behavioral experiment. For those in the restraint condition, the 60 min time point corresponded to when rats in our behavioral studies were released from restraint, 10 min before when behavioral testing was initiated.

Changes in plasma concentrations of CORT were analyzed using a two-way repeated measures ANOVA, which yielded a significant main effect of treatment ($F_{3,20} = 52.54$, $p < 0.001$) and treatment \times time interaction ($F_{3,60} = 37.73$, $p < 0.001$). As displayed in Figure 6c, restraint stress increased plasma CORT at the 30-min mark, followed by an additional increase by 60 min, and then decreasing at 90 min (ie, 30 min after release from restraint), although levels at this point were still significantly ($p < 0.05$) elevated relative to controls. Injection with 1 or 3 mg/kg CORT caused an increase in plasma levels that was significantly higher (Tukey's, $p < 0.05$) than those induced by restraint at the 30-min mark. By 60 min, the 1 mg/kg dose yielded levels comparable to restraint, whereas rats treated with 3 mg/kg continued to display higher levels of plasma CORT compared with restrained rats. To directly compare relative changes in CORT exposure induced by 1 h after restraint to those displayed 90 min after CORT injections, integrated hormone levels were determined with the trapezoidal rule, and the data expressed over time of sampling (area under the curve/time). As shown in Figure 6d, injections of either dose of CORT yielded areas under the curve after 90 min that were actually higher than those observed after 1 h restraint (60-min mark, both $t_{s(10)} > 9.70$, both $p_s < 0.001$). When comparing these values at the 90 min time point across all groups, levels induced by the 3 mg/kg dose were still higher ($p < 0.05$) than those observed in the restraint

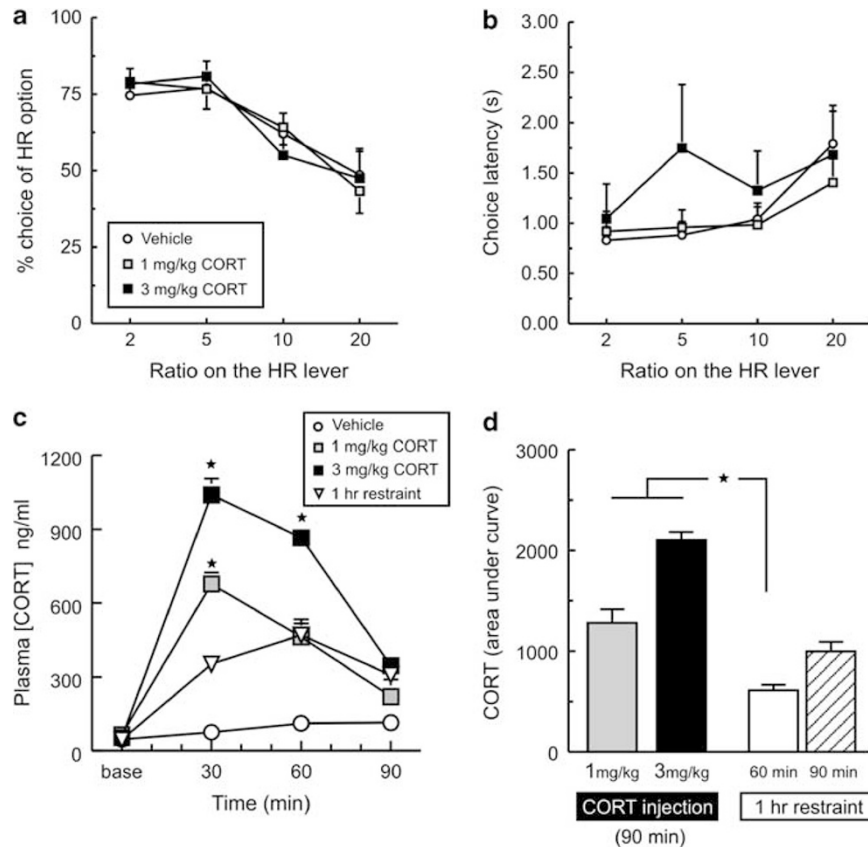


Figure 6 Effects of exogenous corticosterone (CORT) administration (1 and 3 mg/kg) on effort-based decision-making. (a) Choice behavior following the administration of either dose of CORT was comparable to choice after vehicle injection. (b) Similarly, response latencies were not affected by exogenous CORT relative to vehicle treatment. (c) Mean \pm SEM plasma CORT concentrations taken at baseline, and 30, 60, and 90 min after 1 h restraint and s.c. injections of vehicle, 1 and 3 mg/kg CORT. Stars denote significant difference vs restraint at $p < 0.05$. (d) Integrated (area under curve/time) plasma hormone responses to 1 and 3 mg/kg CORT injections over the entire 90-min period, compared with values obtained from restrained rats subjected to restraint rats taken from baseline 60 min (white bar) and baseline 90 min (hatched bar). Injections of either dose of CORT resulted in higher integrated plasma levels over 90 min (stars denote $p < 0.05$) compared with those observed following 1 h of restraint.

group. Thus, these data confirm that injections of 1–3 mg/kg of exogenous CORT increases plasma CORT to levels that are comparable or even higher than those experienced following 1 h restraint stress. As such, the lack of effect of CORT injections on effort-discounting (when compared with restraint) is unlikely to be due to insufficient dosing with exogenous CORT.

Dopaminergic Contribution to the Effects of Acute Stress on Effort-Based Decision-Making

It is well-established that acute stress (including restraint) increases DA release in forebrain regions such as the PFC and NAc, and that these increases may underlie disruption in more complex cognitive functioning induced by stress (Roth *et al*, 1988; Abercrombie *et al*, 1989; Imperato *et al*, 1989, 1991; Davis *et al*, 1994; Finlay *et al*, 1995). Notably, increasing endogenous release of DA with higher doses of drugs such as amphetamine alters effort-based decision-making in a manner similar to that induced by acute restraint stress, decreasing preference for a high effort/HR option on an effort-discounting task (Floresco *et al*, 2008b; Floresco and Whelan, 2009). Therefore, it is possible that the effects of acute stress observed in our previous experiments

may be mediated by excessive increases in endogenous DA. To test this hypothesis directly, we assessed how treatment with a low dose of the broad-spectrum DA antagonist flupenthixol (0.25 mg/kg) altered the effects of acute 1 h restraint stress on effort-related choice and response latencies. In this experiment, flupenthixol was administered 10 min before the start of restraint stress. As this compound has a particularly long half life (> 19 h, Jorgensen *et al*, 1971), this procedure would be expected to block DA receptors during the initial increase in DA release that occurs during restraint, and during increased efflux occurring after release from restraint (Imperato *et al*, 1991). With respect to dosage, previous work in our laboratory has shown that a higher, 0.5 mg/kg dose of flupenthixol causes a robust and reliable decrease in preference for the HR lever on this task, whereas 0.125 mg/kg does not significantly alter preference (Floresco *et al*, 2008b). As both acute stress and higher doses of this DA antagonist reduces choice of the high effort/HR option on this task, we used 0.25 mg/kg dose in an attempt to block stress-induced alterations in decision-making without occluding these effects by the direct action of the drug itself.

A group of 12 rats were trained on the effort-discounting task for 40 days, after which they were subjected to the first

of four counterbalanced test days: (1) saline/no stress, (2) saline/stress, (3) flupenthixol (0.25/mg)/no stress, or (4) flupenthixol/stress (see Figure 7f). Choice data were analyzed with a three-way between/within-subjects ANOVA, with stress condition (no stress and stress), drug treatment (saline and flupenthixol), and trial block as three within-subjects factors. This analysis revealed a significant main effect of stress ($F_{1,11} = 6.76, p < 0.05$), but no stress \times trial block interaction ($F_{3,33} = 2.29, n.s.$ Figure 7a). Thus, across drug/saline treatments, restraint stress decreased the overall preference to work harder for a larger reward without differentially altering the rate of discounting across blocks, replicating our effects observed in prior experiments.

Importantly, there was no main effect of drug on choice ($F_{1,11} = 0.44, n.s.$), indicating that this dose of flupenthixol, administered 80 min before behavioral testing was insufficient to alter decision-making. Of particular note, the analysis did not yield significant stress \times drug or stress \times drug \times trial block interaction (all $F_s < 0.87, n.s.$). Thus, there were no differential effects of acute stress on choice after saline or flupenthixol treatment. This is demonstrated in Figure 7b and c, which show that although 1 h of restraint stress decreases selection of the HR option, administration of flupenthixol before the stressor did not alter these effects when compared with the saline/stress treatment.

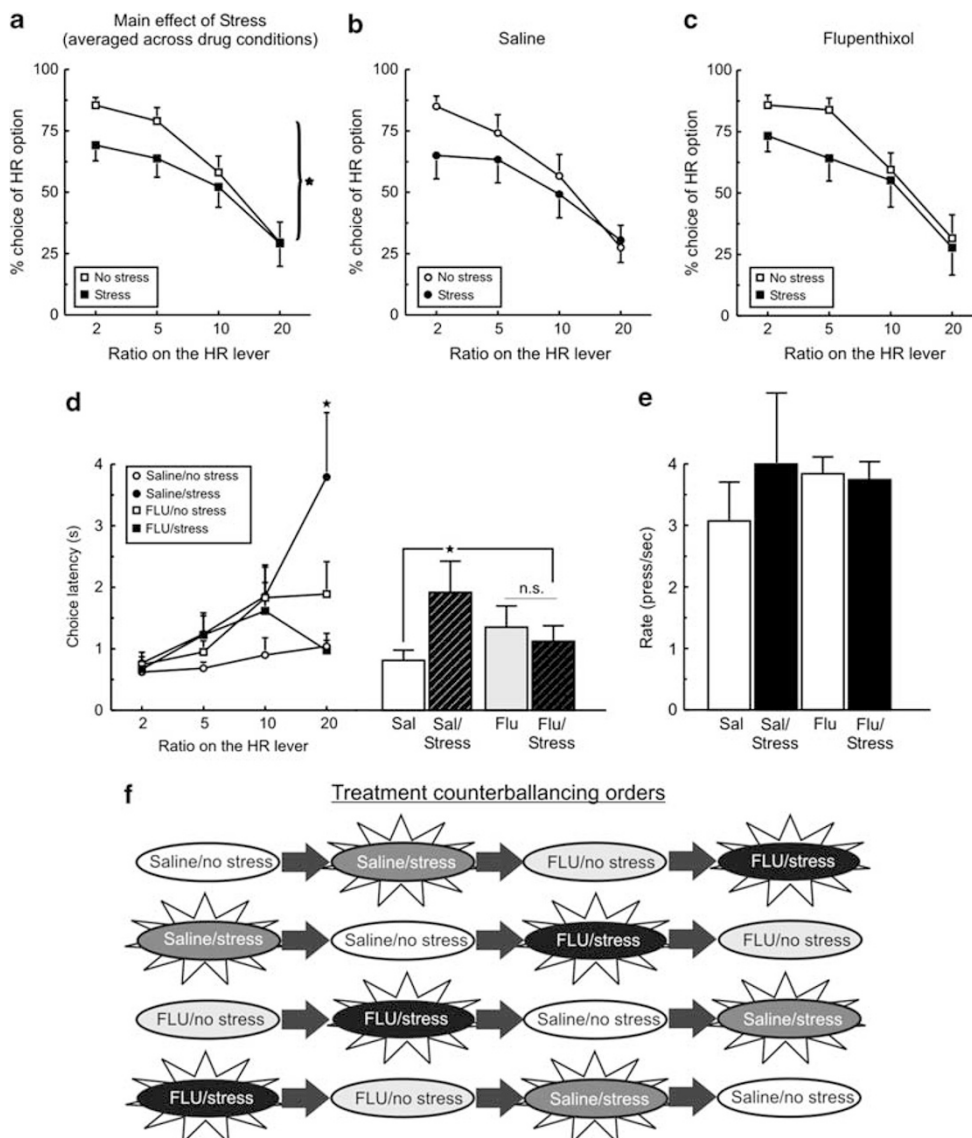


Figure 7 The effects of dopamine antagonism on stress-induced alterations in effort-based decision-making. (a) Percent choice of the HR lever after baseline or restraint stress displaying the main effect of stress, averaged across saline (Sal) and flupenthixol (Flu; 0.25 mg/kg). Stress significantly decreased the selection of the HR options. The effect of (b) saline and (c) flupenthixol treatment, before restraint stress on effort-discounting. Rats displayed a comparable stress-induced decrease in choice of the HR lever after saline and flupenthixol treatment, relative to injection days without stress. (d) Response latencies across the four trial blocks (left) and averaged across blocks (right). Restraint stress preceded by saline injection caused a significant increase in choice latencies compared with saline alone. However, administration of flupenthixol before the stressor abolished this stress-induced increase in response latency. (e) There was no difference in the rate of lever pressing across the different experimental conditions. Stars identify significant main effect at $p < 0.05$. (f) Counterbalancing orders used for this experiment.

In contrast to the lack of effect of DA receptor blockade on choice, flupenthixol did attenuate the effects of restraint on response latencies. Specifically, although there were no significant main effects of drug or stress (all $F_s < 3.68$, n.s.), the analysis did reveal a significant drug \times stress ($F_{1,11} = 5.29$, $p < 0.05$) as well as a drug \times stress \times trial block interaction ($F_{3,33} = 4.52$, $p < 0.01$). As shown in Figure 7d (right), acute stress + saline significantly ($p < 0.05$) increased latencies to make a choice relative to saline alone. However, the stress-induced increase in response latency was abolished by pretreatment with flupenthixol, in that choice latencies after saline/stress treatment were significantly longer ($p < 0.05$) than following flupenthixol/stress. Moreover, response latencies following flupenthixol/stress did not differ from those after flupenthixol alone (which also did not differ from saline alone). Subsequent simple main effects analysis of the three-way interaction further revealed that the attenuation of stress-induced increases in response latencies by flupenthixol occurred most prominently in the last trial block (Figure 7d, left panel). Rate of lever pressing did not differ across the four treatment conditions (all $F_s < 1.42$, n.s.; Figure 7e). Trial omissions did not differ across treatment conditions (saline/no stress: 0.08 ± 0.08 ; saline/stress: 0.33 ± 0.2 ; flupenthixol/no stress: 1.08 ± 0.6 ; flupenthixol/stress: 1.75 ± 1.1 ; all $F_s < 0.29$, n.s.). Collectively, the results of this experiment show that broad-spectrum blockade of DA receptors with flupenthixol before acute restraint stress (1) does not interfere with the ability of stress to disrupt effort-related choice, but (2) can mitigate the longer decision latencies induced by stress.

DISCUSSION

The main findings of this study are that decision-making involving evaluation of effort costs associated with different reward magnitudes is sensitive to acute stress. Restraint stress of 1 h reliably and robustly decreased preference for rats to work harder to obtain a larger reward. Acute stress also led to longer latencies to initiate a decision. These effects are not easily attributable to a reduced tolerance for delays to reward delivery, decreases in preference for larger vs smaller rewards or more general disruption in motivational processes. Furthermore, stress-induced alterations in decision-making were not mimicked by the administration of exogenous CORT, suggesting that these effects do not appear to be mediated solely by increases in endogenous glucocorticoid activity. However, some (but not all) of the effects of stress appear to be mediated by increased DA activity. Administration of a DA antagonist reversed the increased choice latencies induced by restraint stress, but did not affect the decreased preference to work harder for a larger reward.

Effects of Acute Stress on Choice

In our initial experiment, we observed that 1 h restraint reduced preference from the high effort/HR option. In comparison, 20 min of restraint induced a slight, non-significant reduction in this preference. Other studies have shown that certain forms of memory retrieval are impaired by shorter durations of restraint (eg, 10 min; Rashidy-Pour *et al*, 2004).

However, the fact that we observed alterations in choice only with a longer duration of restraint suggests that dissociable forms of cognition may be differentially sensitive to the duration of an acute stressor, with decision-making processes being somewhat less sensitive to shorter duration stressors than simpler forms of learning and memory. Moreover, our results imply that the neural substrates activated by restraint stress, which may interfere with normal decision-making, may differ as a function of stress duration and corresponding neurochemical/hormonal changes.

There are a number of cognitive/motivational processes that may be disrupted by acute stress, which would in turn shift the preference away from the high effort/HR option. For example, acute stress alters metabolic processes, suppresses appetite and digestion, but also increases intake of highly palatable food (Adam and Epel, 2007). Furthermore, stress can impair spatial memory (Diamond and Rose, 1994; de Quervain *et al*, 1998; Kim *et al*, 2007), which could disrupt discrimination between the levers. Yet, it is unlikely that these explanations account for the present findings, because acute restraint stress did not affect choice on a reward magnitude discrimination task, suggesting that the effects of acute stress on effort-discounting are not due to a decrease in the subjective value of objectively larger rewards or an impairment in spatial discrimination abilities.

Choosing a higher effort option invariably delays receipt of a particular reward. Therefore, it was unclear if the effects of acute stress on effort-discounting were attributable, specifically to a reduced preference, to wait longer or work harder for the HR (or both). We assessed this possibility in two ways, the first being the use of an effort-discounting with equivalent delays procedure, which we have used previously, to dissociate between the effort and the delay components embedded within the effort-discounting task (Floresco *et al*, 2008b). In this procedure, delivery of the LR is comparable to the average time necessary to complete the ratio of presses on the HR lever. Thus, the relative delay cost associated with each reward is effectively equalized across options. If the effects of stress on effort-discounting were attributable primarily to a reduced tolerance for delayed rewards, then this manipulation would be expected to diminish the stress-induced effects on effort-discounting. However, this was not the case, as acute stress again shifted preference away from the high effort/HR option under these conditions.

We then investigated whether acute stress affects other forms of decision-making related to choice between smaller immediate rewards and larger delayed rewards using a delay-discounting task, a well-established means for assessing impulsive choice in rodents. In stark contrast to the effects of acute stress on effort-related decisions, similar treatments did not decrease preference for larger delayed rewards, providing further evidence that stress-induced effects on effort-based decision-making are not because of increased 'impatience' for delayed rewards. This is a key finding in that it demonstrates that acute stress does not uniformly interfere with all forms of cost/benefit decision-making in the same manner, and instead exerts a more selective effect on evaluations related to effort expenditures. Note that this lack of effect of acute stress on delay-discounting contrasts with some studies with humans that have pointed to a link between stress and impulsivity

(Giora, 1987; Wittman and Paulus, 2008; Diller *et al*, 2011). Differences between temporal discounting tasks used in human and animal studies may account partially for this discrepancy. Studies with humans typically give choices between hypothetical rewards delivered days–weeks after the test session, whereas rat studies require choice between rewards obtainable during the same training session. Thus, it may be that the ability of stressful events to alter sensitivity of temporal discounting may be more contingent on long-term time perception and not a general intolerance toward delays to reward.

We also investigated the possibility that acute stress may have caused a more general deficit in the motivation to work for food, or somehow interfered with the rats' ability to lever press at high ratios, using a progressive ratio schedule of reinforcement. In well-trained rats, acute stress did not alter the total number of presses, breakpoints, or rates of pressing. Similar lack of effects on progressive ratio responding have been observed after chronic mild stress (Barr and Phillips, 1998). The fact that restraint stress did not interfere with the ability to maintain responding at considerably higher ratios (as high as 77 presses) than in the effort-discounting task indicates that this manipulation does not render animals incapable of completing the required number of presses, nor it is decreasing their motivation to work for food. Thus, in the absence of a choice between different options, acute stress does not simply decrease the effort 'currency' an animal is willing to spend for a set reward. Furthermore, as restraint did not alter on progressive ratio responding or delay-discounting, this suggests that acute stress does not uniformly disrupt encoding changes of response costs. Rather, an acute stressor causes rats to choose options that require less work in exchange for less food. As such, it appears that acute stress shifts the decision criteria, whereby the relative, rather than absolute effort costs associated with larger rewards become less tolerated.

Alterations in effort-discounting induced by restraint provide novel insight into how decision-making may be influenced after acute stress. Stress has been reported to alter decision-making by increasing the likelihood of habitual behavior and impairing optimal foraging upon changes in the relative utility of different options in rats (Elliott and Packard, 2008; Graham *et al*, 2010) and promotes riskier, disadvantageous courses of action in humans (Miu *et al*, 2008; Porcelli and Delgado, 2009; Putman *et al*, 2010; Diller *et al*, 2011). The present data expand on these findings, showing that effort judgments following a stressor are biased towards the less strenuous course of action, which reduces the amount of food reward that may be obtained in the long term. However, because this alteration in decision policies would also lead to greater energy conservation, this supposed suboptimal decision-making pattern may not be completely disadvantageous. Rather, it appears that stress affects reward-related decision-making by changing response priorities, favoring those that require less energy expenditure in the pursuit of food rewards.

The Effect of Stress on Decision Latencies

In addition to altering effort-related choice, acute stress also led to longer deliberation time during effort-discounting. Similar effects of stress were observed on both the reward

magnitude discrimination and the effort-discounting with equivalent delay procedure. These effects suggest that the effect of stress on choice does not appear to be the result of increased motor impulsivity, (ie, rats quickly choosing the LR option when faced with a choice). The fact that the latency effects were apparent as early as the second trial block suggest that they do not reflect some form of satiety-induced hesitation. Moreover, the lack of effect of stress on rates of lever pressing argues against the notion that stress induced a general slowing down of behavior. Additionally, the effects of stress on deliberation time and choice selection could also be dissociated, where acute restraint increased response latencies without affecting preference for the HR during the reward magnitude discrimination. Notably, choice latencies during delay-discounting were not affected by stress, although this may be because of procedural differences between the delay-discounting relative to the other tasks (eg, differences in reward magnitudes, longer intertrial intervals). Despite this one result, acute restraint did increase choice latencies in all of the other experiments of this study, suggesting that at least under some circumstances, stress increases hesitation to choose between options that differ in terms of reward magnitude and their relative effort costs.

The effects of restraint on choice latencies observed here are consistent with other reports showing increased response times following acute stress. Thermal stress has been shown to cause increases in response latencies during performance of an avoidance-escape task (Weiss and Glazer, 1975) and attack initiation (Corum and Thurmond, 1977). In these studies, animals were slower to initiate avoidance-seeking behavior, suggesting that the stress-induced increases in latency are not limited to selection-related actions. Collectively, these findings show that in addition to shifting decision biases away from larger rewards associated with a greater effort cost, hormonal/neurochemical changes occurring in response to acute stress also can make animals more 'indecisive', increasing processing times required to make these decisions.

Probing Hormonal and Neurochemical Mechanisms Underlying Stress-Induced Alterations in Decision-Making

Restraint stress triggers the release of CORT, which can contribute to alterations in cognition induced by these manipulations. To mimic this aspect of the stress response, rats were treated with CORT at doses that result in plasma levels comparable to those obtained after similar periods of restraint (Meaney *et al*, 1988; Imperato *et al*, 1991; Zhang *et al*, 2010; present study, Figure 6c and d), as well as to effectively induce corticosteroid receptor occupancy (Meaney *et al*, 1988) and translocation (Sarabdjitsingh *et al*, 2009). Treatment with 1–3 mg/kg CORT did not affect effort-related choice or other behavioral measures (rates of pressing, response latency). This lack of effect contrasts with some other findings, demonstrating that exogenous CORT treatment does alter learning and cognition in a manner similar to acute stress. Administration of CORT in rats (1–5 mg/kg) mimics the effects of acute stress on contextual fear conditioning (Cordero and Sandi, 1998) and spatial memory retrieval (de Quervain *et al*, 1998) by enhancing and impairing performance, respectively. In humans,

cortisol or dexamethasone alters simple and more complex forms of cognition, such as declarative memory (Newcomer *et al*, 1994) and cost/benefit decision-making (Putman *et al*, 2010). However, other studies in rats have shown that CORT treatment does not replicate the effects of stress on place or response discrimination learning (Sadowski *et al*, 2009) or decision-making in response to changes in the relative utility of different reward options (Graham *et al*, 2010). Thus, it may be that increased CORT activity does not have as a disruptive an effect on learning or cognition in situations where animals must discriminate or choose between different options. Note that the present findings do not completely rule out the role of CORT in mediating the effects of stress on effort judgments. Changes in plasma CORT levels may interact with other stress-induced hormonal and neurochemical fluctuations to alter decision-making (eg, noradrenaline; Kukulja *et al*, 2008; Enkel *et al*, 2010). Studies using corticosteroid antagonists before stress would provide further insight into this issue. Nevertheless, these results suggest that in the absence of a physical/psychological stressor, increasing glucocorticoid levels was not sufficient to affect choice or other behavioral measures associated with effort judgments.

Another well-characterized neurochemical effect of acute stress is increased release of DA in the forebrain and mesolimbic structures (Roth *et al*, 1988; Abercrombie *et al*, 1989; Imperato *et al*, 1989, 1991; Davis *et al*, 1994; Finlay *et al*, 1995; Rougé-Pont *et al*, 1998; Tsigos and Chrousos, 2002). It has been proposed that stress-induced impairments in processes such as spatial working memory are because of excessive increases in DA release, as they are reversed by DA receptor blockade (Murphy *et al*, 1996a, 1996b, 1997; Arnsten and Goldman-Rakic, 1998; Arnsten *et al*, 2000). With respect to the present study, effort-based decision-making appears to be sensitive to DA levels, as pharmacological reductions (Salamone *et al*, 1991, 1997, 2003, 2005) or enhancements in DA activity (Floresco *et al*, 2008b) decreases the preference to work harder for a larger reward. Therefore, we investigated whether the effects of acute stress on decision-making and related behaviors could be ameliorated by reducing DA activity.

Treatment with flupenthixol alone did not affect effort-discounting task relative to saline. This confirms that administration of a relatively low dose (0.25 mg/kg) of this DA antagonist 80 min before testing does not alter choice behavior, even though higher doses of the antagonist given shortly before testing (0.5 mg/kg, 20 min before task) does shift choice biases away from the HR option (Floresco *et al*, 2008b). Following restraint, rats again shifted preference away from the HR option, replicating our initial effects. However, the effect of stress on choice was not altered by the DA receptor blockade. This is in contrast with previous work demonstrating that stress-induced impairments of other forms of cognition such as working memory can be ameliorated by systemic treatment with DA antagonists (Murphy *et al*, 1996a, 1996b). Thus, these finding suggests that systemic, broad-spectrum blockade of DA receptors is insufficient to attenuate the effects of stress on effort-based decision biases. A parsimonious conclusion would therefore be that stress-induced increases in DA activity are not an underlying cause of alterations in choice biases during this form of decision-making. However, the possibility remains

that acute stress may perturb decision-making through increases in DA transmissions in specific terminal regions, or acting on a subset of DA receptors. Alternatively, it is possible that changes in DA efflux that occur after the release from restraint may be a contributing factor to the stress-induced alterations in decision-making observed here. Future studies, using local administration of receptor-selective DA antagonists, administered either before or after stress may address these issues more completely.

In contrast to the inability of flupenthixol to reduce the effects of stress on decision-making, this treatment did counteract the effect of restraint on longer choice latencies. Thus, restraint after saline injection led to longer response latencies, replicating findings from our previous experiments. Moreover, flupenthixol by itself did not affect this measure. However, the stress-induced effect on deliberation time was reversed by pretreatment with flupenthixol, confirming that the dose used here was sufficient to reverse some of the behavioral effects of acute stress. Admittedly, this is a somewhat counterintuitive finding, given that DA antagonists typically increase response latencies. Yet, the fact that DA receptor blockade ameliorated the effects of restraint on decision latencies suggest that the ability of acute stress to increase hesitation before response selection may be mediated by increases in the DA activity.

Given that neither CORT nor DA appears to solely mediate the effects of stress on choice, the question remains regarding the hormonal and neurochemical mechanisms underlying these effects. Administration of a noradrenaline antagonist has been reported to reverse-working memory impairments induced by a pharmacological stressor, suggesting that increases in this monoamine may contribute to some of the effects reported here (Birnbbaum *et al*, 1999). Corticotropin-releasing hormone is another transmitter that is released during stress, and post-training intra-amygdala infusions of this neuropeptide has been shown to mimic the effects of acute stress on stress-associated behaviors and avoidance learning (Liang and Lee, 1988). It is therefore possible that the effects of acute stress on effort-based decision-making may be the result of increased corticotropin-releasing hormone and noradrenaline release, although this remains to be tested experimentally.

CONCLUSIONS

In summary, these experiments suggest that acute stress interferes somewhat selectively with cost/benefit evaluations concerning rewards of different magnitudes and the relative effort costs associated with obtaining them. These effects do not appear to be attributable to increased impulsivity, or reductions in motivation to work for food rewards or for larger rewards in general, nor do they seem to be caused solely by increased CORT activity. Acute stress also induces greater hesitation to make a choice, which appears to be mediated by increased DA activity. Collectively, these studies complement a growing literature investigating how acute stress may alter different types of cognitive processes, showing here that stress may change the manner in which animals value larger rewards associated with greater effort costs. One byproduct of this effect would be to cause an organism to conserve the energy that would otherwise be

spent on the higher effort requirement associated with the larger reward. This in turn emphasizes that the stress response would reinforce the selection of less physically demanding options, possibly in an attempt to increase energy conservation. Given that the stress response is designed to be adaptive, biasing response selection towards low-effort options may help to promote energy conservation for the time immediately following a stressor.

Negative affect and depressed mood are the most recognized phenotypes of depression. However, individuals with clinical diagnosis of depression also suffer from a number of energy-related deficiencies, such as psychomotor retardation, anergia, and fatigue (Tylee *et al*, 1999; Stahl, 2002). Given the intertwined relationship between stress and depression (Heit *et al*, 1997), this model of effort-based decision-making and its sensitivity to the acute stress may help clarify some of the underlying behavioral symptoms associated with depression. As such, this approach may not only further our understanding of the neural circuitry/neurotransmitter systems associated with the stress-induced alterations in cost/benefit decision-making, but may also serve as a model for the anergia and motivational deficits associated with depression. This in turn may prove particularly useful in the development of novel treatments for this aspect of the disorder.

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