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Dopamine and Stress System Modulation of Sex Differences in Decision Making

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Maladaptive decision making is associated with several neuropsychiatric disorders, including problem gambling and suicidal behavior. The prevalence of these disorders is higher in men *vs* women, suggesting gender-dependent regulation of their pathophysiology underpinnings. We assessed sex differences in decision making using the rat version of the Iowa gambling task. Female rats identified the most optimal choice from session 1, whereas male rats from session 5. Male, but not female rats, progressively improved their advantageous option responding and surpassed females. Estrus cycle phase did not affect decision making. To test whether pharmacological manipulations targeting the dopaminergic and stress systems affect decision making in a sex-dependent manner, male and female rats received injections of a dopamine D_2 receptor (D_2R) antagonist (eticlopride), D_2R agonist (quinpirole), corticotropin-releasing factor 1 (CRF_1) antagonist (antalarmin), and α_2-adrenergic receptor antagonist (yohimbine; used as a pharmacological stressor). Alterations in mRNA levels of D_2R and CRF_1 were also assessed. Eticlopride decreased advantageous responding in male, but not female rats, whereas quinpirole decreased advantageous responding specifically in females. Yohimbine dose-dependently decreased advantageous responding in female rats, whereas decreased advantageous responding was only observed at higher doses in males. Antalarmin increased optimal choice responding only in female rats. Higher *Drd2* and *Crhr1* expression in the amygdala were observed in female *vs* male rats. Higher amygdalar *Crhr1* expression was negatively correlated with advantageous responding specifically in females. This study demonstrates the relevance of dopaminergic- and stress-dependent sex differences to maladaptive decision making.
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INTRODUCTION

Decision making is an essential part of everyday life. It is characterized by evaluating the advantages/disadvantages of potential actions and is regulated by the brain's impulsive/emotional systems, which react to immediate rewards and losses, as well as cognitive systems, which are sensitive to the long-term outcomes (Bechara, 2005). Impaired decision making has been linked with both the initiation and development of several neuropsychiatric disorders, including problem gambling (see Brevers *et al*, 2013), bipolar disorder (Christodoulou *et al*, 2006; Ono *et al*, 2015), and psychiatric disorders-associated suicidal behaviors (Gould *et al*, 2017; Jollant *et al*, 2007).

In humans, the Iowa Gambling task (IGT) measures decision making by mimicking real-life situations. During this task, individuals are presented with four decks of cards, each associated with different amounts of rewards and punishments (Bechara *et al*, 1994). Healthy individuals learn to differentiate between advantageous and disadvantageous cards, whereas individuals with maladaptive decision-making favor the disadvantegeous options (Bechara *et al*, 1994). Gender differences have been identified in the IGT, where men show higher preference for the long-term advantegeous cards compared with women (see van den Bos *et al*, 2013). Importantly, the prevalence of problem gambling (Wong *et al*, 2013) and rates of suicide (Hawton, 2000) are higher among men compared to women, suggesting gender-related differences in conditions characterized by impaired decision making.

Despite some evidence suggesting that decision making is differentially modulated by sex (Orsini *et al*, 2016; van den Bos *et al*, 2012), there are limited data for the underlying mechanisms, which are critical to identify sex-specific targets for the treatment of maladaptive decision making and its associated psychiatric conditions. Findings in male rats support a role of the dopaminergic and brain stress systems. For instance, administration of the non-selective dopamine receptor agonist *d*-amphetamine impaired decision making, whereas antagonist of the dopamine D_2 receptor (D_2R) improved decision making in the rat gambling task (rGT)

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(Zeeb *et al*, 2009). In addition, the α_2-adrenergic receptor antagonist, yohimbine (used as a pharmacological stressor) increased impulsivity in male rats (Sun *et al*, 2010b), a component of impaired decision making, whereas the cortocotropin-releasing factor (CRF) antagonist, alpha-helical CRF, attenuated stress-induced maladaptive decision making in male rats (Bryce and Floresco, 2016). Sex differences in stress responses have been observed (see Verma *et al*, 2011) and stress directly affects decision making in humans (see Starcke and Brand, 2012). Moreover, stress is associated with several psychiatric disorders characterized by maladaptive decision making including suicide (see Currier and Mann, 2008), problem gambling (Coman *et al*, 1997) and bipolar disorder (Dienes *et al*, 2006). Therefore, it is possible that baseline sex differences in stress reactivity might affect and/or be responsible for maladaptive decision making associated with psychiatric disorders. Although, the involvement of the dopaminergic and brain stress systems in decision making have been studied in males, their role in sex-dependent modulation of decision making needs to be investigated for the identification of sex-specific targets for the treatment of disorders associated with maladaptive decision making.

Here, we investigated sex differences in decision making using the rat version of the Iowa gambling task; the rGT. We utilized the reward or time-out operant design of the rGT, which is based on the ability of rats to discriminate the most advantegeous option from four reinforcement/punishment schedules presented. Using this task, we also investigated the effects of blockade (eticlopride) and activation (quinpirole) of the D_2R, as well as the effects of yohimbine and the CRF receptor 1 (CRF_1) antagonist antalarmin in the performance of male and female rats. To determine to what extent differences in the expression of D_2R and CRF_1 account for the sex differences in decision making, we measured the expression levels of these receptors in brain regions associated with decision making: orbitofrontal cortex (OFC), prelimbic cortex (PrL), nucleus accumbens (NAc), and amygdala (van den Bos *et al*, 2014).

MATERIALS AND METHODS

Animals

Male and female Long-Evans rats (7 weeks old at arrival; 10 weeks old at the start of experiment, Charles River Laboratories) were housed 3/cage (same sex) under a reverse 12 h light—dark cycle (lights off: 10:00am). Rats were food-restricted to 85% of their free-feeding weight and maintained at 85% throughout the experiment. To account for growth of rats, body weights were adjusted upwards by 5 g every week throughout the duration of the experiment. Water was available *ad libitum*. Behavioral testing occurred during the dark phase of the light-–dark cycle. All experimental procedures were approved by the University of Maryland, Baltimore Animal Care and Use Committee and conducted in full accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Behavioral Testing

Behavioral testing took place in sound-attenuated operant-conditioning boxes (Coulbourn Instruments, PA, USA). Detailed description of the behavioral apparatus used is provided in the Supplementary Information.

The Rat Gambling Task. The rGT was performed as previously described (Zeeb *et al*, 2009), with minor modifications. Details for the acquisition of operant responding are included in the Supplementary Information. In brief, rats were

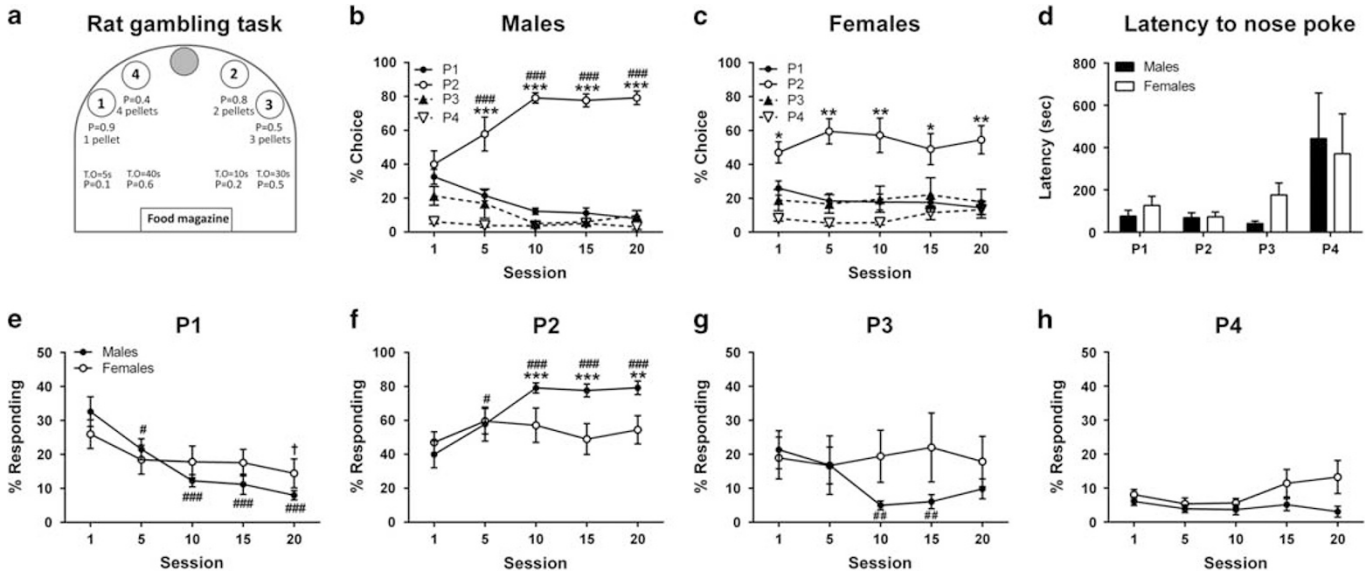

Figure 1 Sex-dependent modulation of decision making in the rat gambling task (rGT). (a) Schematic representation of the rGT. Percent choice of the different schedules of (b) male and (c) female rats in the gambling task. (d) Latency to nose poke in the hole associated with the difference schedules of reinforcement/punishment in both male and female rats during the first free-choice session. Percent responding of male and female rats in the (e) P1, (f) P2, (g) P3, and (h) P4 choices for the 20 sessions of the free-choice task. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.01$ *vs* P1, P3, P4, or *vs* females; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.01$ *vs* session 1; $\dagger p<0.05$ *vs* session 1; $n=8$–9.

placed in operant boxes and all four response holes were illuminated. A response in any illuminated hole turned off all stimulus lights, and resulted in either the delivery of reward (tray-light on) or the start of a time-out 'punishment' period. If the trial was punished, no reward was delivered and the stimulus light within the chosen hole was flashing at 0.5 Hz throughout the time-out period. A 5-s inter-trial interval (ITI) was used, when all lights were turned off. Premature responses made at any hole during the ITI were punished by a 5-s time-out period, in which the house light was illuminated. Perseverative responses made at the response holes, both after reward and during punishing time-outs, were recorded but not punished. The rGT comprised of four different schedules of reinforcement/punishment associated with different amounts of rewards (either 1, 2, 3, or 4 pellets). The probability to get rewarded/punished for the P1 choice was $p = 0.9/0.1$ with 5-s punishment time-out period and 1-pellet reward, for the P2 choice was $p = 0.8/0.2$ with 10-s punishment time-out period and 2-pellets reward, for P3 was $p = 0.5/0.5$ with 30-s punishment time-out period and 3-pellets reward, and for the P4 was $p = 0.4/0.6$ with 40-s punishment time-out period and 4-pellets reward (Figure 1a). Therefore, as Zeeb *et al* calculated, if each choice was exclusively chosen during a 30-min test session, rats would have earned the following rewards: P1-295; P2-411; P3-135, and P4-99 rewards. Thus, the most optimal choice was the P2 choice, followed by the P1. The two disadvantageous options are the P3 and P4 choices (lower probability of reward/higher probability of punishment). To avoid side bias, location of the different pellet choices was counterbalanced such that half of the rats were tested on version 1 and the other half on version 2. The order of the pellet choices for version 1 was P2, P3, P1, and P4 and for version 2 was P3, P2, P4, and P1. The trial was scored as an omission when the animal failed to respond within 10-s, where all the stimulus lights were turned off and re-illuminated allowing the animals to start a new trial.

Determination of the Estrus Cycle Phase

To determine whether female hormones influence the differential decision making responses, vaginal smears were collected by lavage using distilled water following the end of the experimental sessions between days 7–20 of the free-choice task. To ensure that any stress associated with the vaginal smear procedure was controlled, male rats were handled in the same manner as females and water was applied to the genital area. Moreover, the procedures were conducted following the end of the behavioral testing on the days specified. The estrous cycle was monitored for three cycles. The average of each stage from the three cycles was calculated for each animal, which was used for the group means result.

Drug Treatments

The different doses of each drug were administered according to a balanced Latin Square design as previously described (Zeeb *et al*, 2009). Injections were given on a 3-day cycle (Day 1—baseline, Day 2—drug/vehicle treatment, Day 3—no test). The pharmacological manipulations occurred following stable performance being achieved. The criteria set for a stable performance were $<15\%$ variation on 3 consecutive days on each response hole and completing at least 75% of the total trials. Eticlopride, yohimbine, and quinpirole were administered 10 min prior to the beginning of the task (Sun *et al*, 2010b; Zeeb *et al*, 2009), whereas antalarmin 60 mins before testing (Zorrilla *et al*, 2002). The timing for drug administrations were based on the pharmacokinetic profiles of these compounds (Hubbard *et al*, 1988; Norman *et al*, 2011; Whitaker and Lindstrom, 1987; Zorrilla *et al*, 2002). Eticlopride was administered at the doses of 0.01, 0.025, and 0.075 mg/kg, quinpirole at 0.0125, 0.0375, 0.125 mg/kg, yohimbine at 0.5, 1.5, and 4.5 mg/kg, and antalarmin at 5 and 15 mg/kg. The doses used for each drug were chosen based on previously published reports; eticlopride and quinpirole (Zeeb *et al*, 2009), yohimbine (Sun *et al*, 2010a; Sun *et al*, 2010b), and antalarmin (Zorrilla *et al*, 2002).

Dopamine D_2R and Corticotrophin Releasing Factor Receptor 1 Expression

The animals used in the behavioral studies were euthanized by a brief exposure to CO_2 followed by decapitation 15 days after the last treatment injection. Brains were collected and dissected using a rat brain matrix on ice. Details on brain dissection, RNA extraction and analysis of the results for the *Drd2* and *Crhr1* expression are provided in the Supplementary Information.

Statistical Analysis

All values are expressed as the mean $\pm$ S.E.M. Statistical analyses were performed using STATISTICA *v7.0* (StatSoft, Oklahoma, USA). Details for the statistical tests are provided in the Supplementary Information. All statistical analyses and animal numbers are summarized in Supplementary Table S1.

RESULTS

Sex-Dependent Modulation of Decision Making

Male rats did not initially discriminate P2 as the most advantageous option, but did so from session 5 onward ($p<0.001$) and following session 10 maintained a stable choice behavior until the end of testing ($F_{(12,240)}=5.667$, $p<0.001$; Figure 1b). Unlike male rats, female rats discriminated P2 as the most advantageous option from the first session ($p<0.05$) and maintained stable choice behavior until the end of the task ($F_{(12,240)}=5.667$, $p<0.001$; Figure 1c). Female rats had a clear preference for the most advantageous option (P2) compared with all the other options from session 1, whereas male rats did not show significant preference to respond to the P2 choice during the same session (Figures 1b and c). Nevertheless, there are no sex differences in P2 responding *per se* ($F_{(1,60)}=0.00$, $p=1.00$; Figure 1f). No significant differences were observed in the latency to nose poke during the first session ($F_{(3,45)}=0.350$, $p=0.789$; Figure 1d) or in the choice patterns over time during the first session ($F_{(9,180)}=1.802$, $p=0.071$; Supplementary Figure S1). As the task progressed, both male and female rats decreased their preference for the P1 schedule ($p<0.05$; Figure 1e). Although male rats increased their preference for the P2 choice as the task progressed ($p<0.05$), female rats

maintained a stable preference (Figure 1f). Following 10 training sessions, and until the end of the task, male rats showed greater preference for the most optimal, P2 choice compared with female rats ($p<0.001$; Figure 1f). No significant difference was observed between male and female rats in their choice for the disadvantageous options of P3 (Figure 1g) and P4 (Figure 1h). Male rats decreased their responding to the P3 schedule following 10 training sessions and sustained this decrease until the end of the 20 sessions ($p<0.05$; Figure 1g). No differences were observed between the different sessions and between male and female rats in premature ($F_{(4,60)}=0.177$, $p=0.950$) and perseverative responses ($F_{(4,60)}=1.733$, $p=0.155$; Supplementary Table S2). Female rats had significantly higher omissions compared with male rats throughout the duration of the task ($F_{(1,60)}=12.00$, $p<0.01$; Supplementary Table S2). This difference in omissions does not result from differences in the total nose pokes between the two sexes (Sex: $F_{(1,60)}=0.945$, $p=0.346$; Interaction: $F_{(4,60)}=1.349$, $p=0.263$; Supplementary Table S2).

Effects of Estrous Cycle in the Performance of Female Rats in the Rat Gambling Task

Representative images of vaginal cells are provided from each cycle stage (Figure 2a). No effect of estrous cycle stage was observed in the choice patterns ($F_{(9,72)}=1.522$, $p=0.157$; Figure 2b), omissions ($F_{(3,24)}=2.250$, $p=0.108$; Figure 2d), perseverative responses ($F_{(3,24)}=1.546$, $p=0.249$; Supplementary Fig S2), and total responding ($F_{(3,24)}=1.472$, $p=0.247$; Figure 2e). A significant effect of reinforcement schedule irrelevant of estrous phase was observed in the choice patterns ($F_{(3,24)}=8.099$, $p<0.001$; Figure 2b). Females showed higher premature responses during the proestrus compared with the other cycle phases ($p<0.05$), indicating higher levels of impulsivity ($F_{(3,24)}=3.027$, $p<0.05$; Figure 2c).

Sex Differences in Decision Making Following Pharmacological Treatments Targeting Dopamine D_2R

Eticlopride. The $D_{2/3}R$ antagonist eticlopride had no effect on choice patterns of females at any dose administered ($F_{(9,96)}=1.182$; $p=0.315$; Figures 3a-d). In contrast, eticlopride administration affected choice patterns of males ($F_{(9,84)}=2.391$; $p<0.05$). Although low and medium doses of eticlopride (0.01 and 0.025 mg/kg, respectively) did not affect the choice behavior of male rats (Figure 3b), the high dose (0.075 mg/kg) decreased their percent responding to P2 choice ($p<0.001$; Figure 3b), suggesting that blockade of D_2R differentially affects decision making in male and female rats. Although it did not reach statistical significance, there is a statistical trend ($p=0.06$) toward an increase in the percent responding to the P4 choice induced by the high dose of eticlopride only in male rats. Eticlopride had no effect in P1 (Figure 3a) and P3 (Figure 3c) in male rats. No effect of eticlopride was observed in omissions ($F_{(3,45)}=0.576$; $p=0.364$), premature responses ($F_{(3,45)}=1.547$; $p=0.215$), perseverative responses ($F_{(3,45)}=0.133$; $p=0.940$), and total responding ($F_{(3,45)}=0.468$; $p=0.706$; Supplementary Table S2). A sex-specific effect, irrelevant of treatment, was observed in the omissions, where females showed higher omissions compared with males ($F_{(1,45)}=10.58$; $p<0.01$; Supplementary Table S2).

Quinpirole. Quinpirole had no effect on choice patterns of males at any dose administered ($F_{(9,84)}=0.491$; $p=0.877$; Figures 3e-h). In contrast, quinpirole administration affected choice patterns of females ($F_{(9,96)}=2.881$; $p<0.01$). No effect of quinpirole was observed on percent responding to P1 (Figure 3e) and P4 (Figure 3h) in females. Low and medium doses (0.0125 and 0.0375 mg/kg, respectively) of quinpirole did not significantly affect responding of female rats to the P2 and P3 choice (Figures 3f and g). However, the high dose of quinpirole (0.325 mg/kg) shifted the preference of female rats from P2 to the P3 choice ($p<0.05$; Figures 3f and g). No effect of quinpirole was observed in either of the two sexes in premature responses ($F_{(3,45)}=1.010$; $p=0.397$), perseverative responses ($F_{(3,45)}=0.343$; $p=0.794$) and total responding ($F_{(3,45)}=1.315$; $p=0.281$; Supplementary Table S2). A sex-specific effect, irrelevant of treatment, was observed in the omissions ($F_{(1,45)}=7.160$; $p<0.05$) and premature responses ($F_{(1,45)}=11.46$; $p<0.01$) where females showed higher omissions and premature responses compared with males (Supplementary Table S2).

Sex Differences in Decision Making Following Pharmacological Treatments Targeting Brain Stress Systems

Yohimbine. Yohimbine increases norepinephrine release by blocking the inhibitory autoreceptors on noradrenergic neurons in rats (Szemeredi *et al*, 1991) and cortisol levels in humans (Stine *et al*, 2002), which are indications of an increased stress response. A significant interaction effect was observed with yohimbine in both males ($F_{(9,84)}=3.043$; $p<0.01$) and females ($F_{(9,96)}=2.568$; $p<0.05$). Yohimbine, did not affect the percent responding in either male or female rats on P1 (Figure 4a) and P4 (Figure 4d). In male rats, low doses of yohimbine (0.5 mg/kg) had no significant effect on the percent responding to the P2 choice; however, decreased responding to the P2 choice was observed following administration of the medium and high doses (1.5 and 4.5 mg/kg; Figure 4b; $p<0.01$). In female rats, administration of yohimbine at all three doses, significantly decreased the percent responding to the advantageous P2 choice compared with vehicle treatment ($p<0.05$; Figure 4b). The medium dose of yohimbine increased responding of male rats on the P3 choice ($p<0.05$; Figure 4c). In female rats, a statistical trend was observed with the high dose of yohimbine shifting responding towards the P3 choice; however, this did not reach statistical significance ($p=0.07$; Figure 4c). In male rats, there was a significant effect of yohimbine administration on omissions ($F_{(3,45)}=7.418$; $p<0.001$) and premature responses ($F_{(3,45)}=13.731$; $p<0.001$; Supplementary Table S2) with the high dose of yohimbine increasing omitted trials ($p<0.01$) and the medium dose increasing premature responses ($p<0.05$). The low dose of yohimbine increased total responding in male rats ($p<0.001$) and the medium dose of yohimbine increased total responding in both male ($p<0.001$) and female rats ($p<0.05$; Supplementary Table S2). Total responding was also higher in males treated with low and medium doses of yohimbine compared with females ($p<0.05$; Supplementary Table S2).

Antalarmin. CRF1 antagonists inhibit stress-induced HPA-axis activation (De Souza, 1995; Koob and Heinrichs,

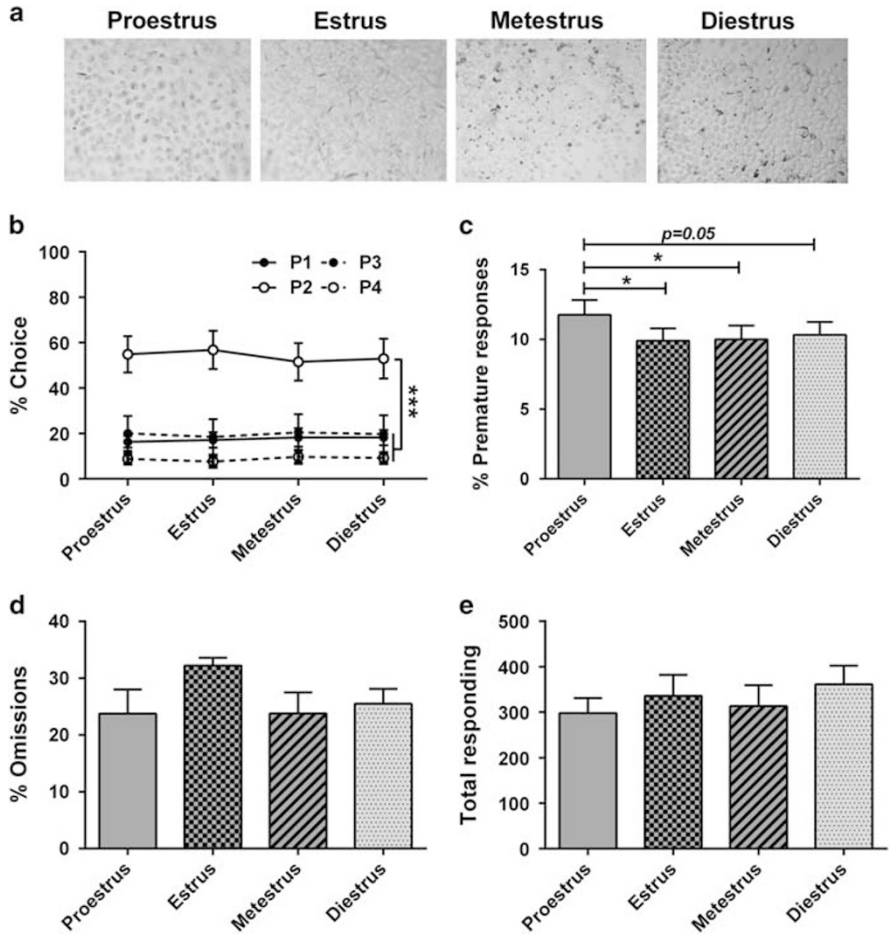

Figure 2 Effect of estrous cycle of female rats in the rat gambling task (rGT). Representative images of the (a) proestrous, estrous, metestrous, and diestrous stages of the rat's estrous cycle. (b) Percent choice, (c) percent premature responses, (d) percent omissions, and (e) total responding of female rats during the four stages of the estrous cycle in the rGT. *$p<0.05$; $n=9$.

1999; McElroy *et al*, 2002). A significant Treatment × Schedule × Sex interaction was observed with administration of antalarmin ($F_{(6,120)}=2.655$; $p<0.05$). Antalarmin had no effect on the choice patterns of males at any dose administered (Figures 4e-h). No effect of antalarmin was observed in the percent responding on P1, P3, and P4 (Figures 4e, g and h) in females. However, 5 and 15 mg/kg of antalarmin increased percent responding on the P2 choice in female rats ($p\leq0.05$; Figure 4f). A treatment-independent effect of sex was observed in omissions ($F_{(1,30)}=7.569$; $p<0.05$) and premature responses ($F_{(1,30)}=11.04$; $p<0.01$), where females showed higher omissions ($p<0.01$) and premature responses ($p<0.05$) compared with males (Supplementary Table S2).

Relative mRNA Expression of *Drd2* and *Crhr1* in Male and Female Rats

We quantified mRNA expression of *Drd2* and *Crhr1* in the OFC, PrL, NAc, and amygdala. Correlation analysis was performed to determine whether the relative expression of *Drd2* and *Crhr1* were associated with the performance of rats in the rGT.

Drd2. Higher relative expression of *Drd2* was observed in female rats compared with male rats in the PrL ($t_{(11)}=2.908$, $p<0.05$; Figure 5b) and amygdala ($t_{(12)}=2.19$, $p<0.05$; Figure 5j). We did not observe statistically significant difference in the *Drd2* mRNA expression in the OFC ($t_{(15)}=0.147$, $p=0.885$; Figure 5a) and NAc ($t_{(10)}=0.604$, $p=0.559$; Figure 5g).

Pearson's correlation coefficient analysis revealed no significant correlations between relative expression of *Drd2* and % advantageous responses in both male and female rats in OFC (Figure 5b), PrL (Figure 5e), NAc (Figure 5h) and amygdala (Figure 5k). A significant correlation was observed between relative expression of *Drd2* and % premature responses in male rats in the NAc ($r=-0.84$; Bonferroni correction adjusted $p<0.036$; Supplementary Fig S3). No other correlation was observed between *Drd2* expression and premature responses (Supplementary Fig S3), omission (Supplementary Fig S4), perseverative responses (Supplementary Fig S5), and total responses (Supplementary Fig S6).

Crhr1. Higher relative expression of *Crhr1* was observed in female rats compared with male rats in the amygdala ($t_{(14)}=2.140$, $p<0.05$; Figure 5j). A statistical trend for decrease in *Crhr1* expression in the NAc was observed in female rats compared with male rats ($t_{(15)}=1.193$, $p=0.06$; Figure 5g). No significant difference in the relative

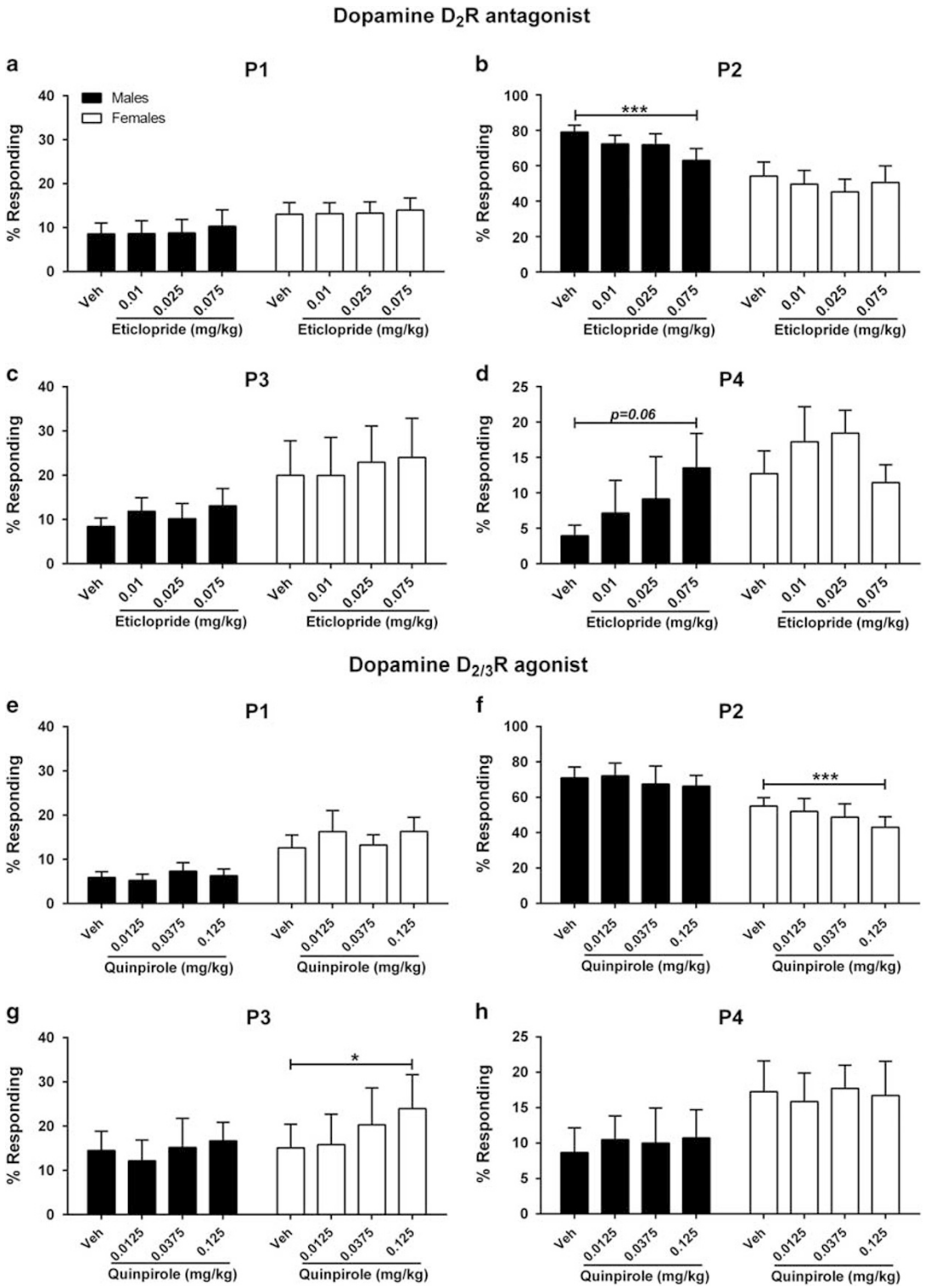

Figure 3 Effect of D_2R antagonist and agonist in the performance of male and female rats in the rat gambling task. Percent responding of male and female rats on (a) P1, (b) P2, (c) P2, and (d) P4 schedules following administration of eticlopride. Percent responding of male and female rats on (e) P1, (f) P2, (g) P2, and (h) P4 schedules following administration of quinpirole. *$p<0.05$, ***$p<0.01$; $n=8$–9.

expression of *Crhr1* mRNA was observed in the OFC ($t_{(14)}=0.036$, $p=0.971$; Figure 5a) and PrL ($t_{(13)}=0.594$, $p=0.563$; Figure 5b).

Pearson's correlation coefficient analysis revealed a significant negative correlation between *Crhr1* expression and % advantageous responses in the amygdala in female ($r=$ -0.76, Bonferroni correction adjusted $p<0.03$; Figure 5l) but not male rats (Figure 5l). Although Pearson's correlation coefficient analysis revealed a positive correlation in male OFC ($r=0.72$; $p=0.043$), this does not reach statistical significance following the Bonferroni correction for multiple comparisons (adjusted p-value for reaching significance is $p<0.028$; Figure 5c). No

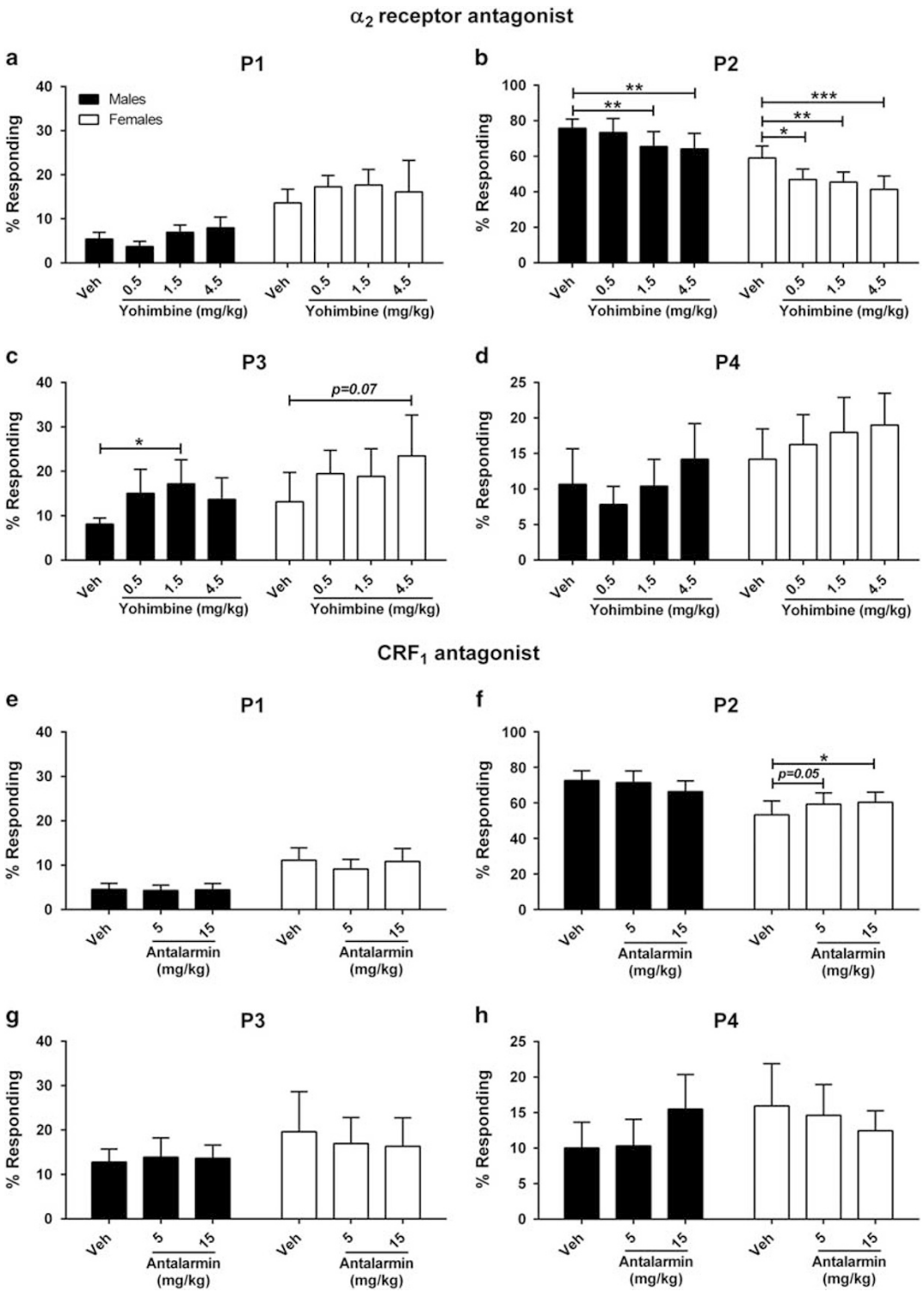

Figure 4 Effect of α2 receptor and CRF1 receptor antagonists in the performance of male and female rats in the rat gambling task. Percent responding of male and female rats on (a) P1, (b) P2, (c) P2, and (d) P4 schedules following administration of yohimbine. Percent responding of male and female rats on (e) P1, (f) P2, (g) P2, and (h) P4 schedules following administration of antalarmin. *$p<0.05$, **$p<0.01$, ***$p<0.001$; $n=8$–9.

significant correlation was observed in PrL (Figure 5f) and the NAc (Figure 5i) in both sexes. No other correlation was observed between *Crhr1* expression and premature responses (Supplementary Fig S3), omission (Supplementary Fig S4), perseverative responses (Supplementary Fig S5) and total responses (Supplementary Fig S6).

DISCUSSION

In the present study, we demonstrate that male rats require five sessions to discriminate the advantageous choice, which is in agreement with previous reports (Zeeb *et al*, 2009); however, this is the first study to report that female rats are

able to discriminate the most advantageous option from the initial session of the rGT. Previous research has demonstrated that males are more risk-prone than females in both rodent tests (Jolles *et al*, 2015) and in humans (Pawlowski *et al*, 2008). As a consequence, females show greater risk aversion than males, manifested by their preference for a

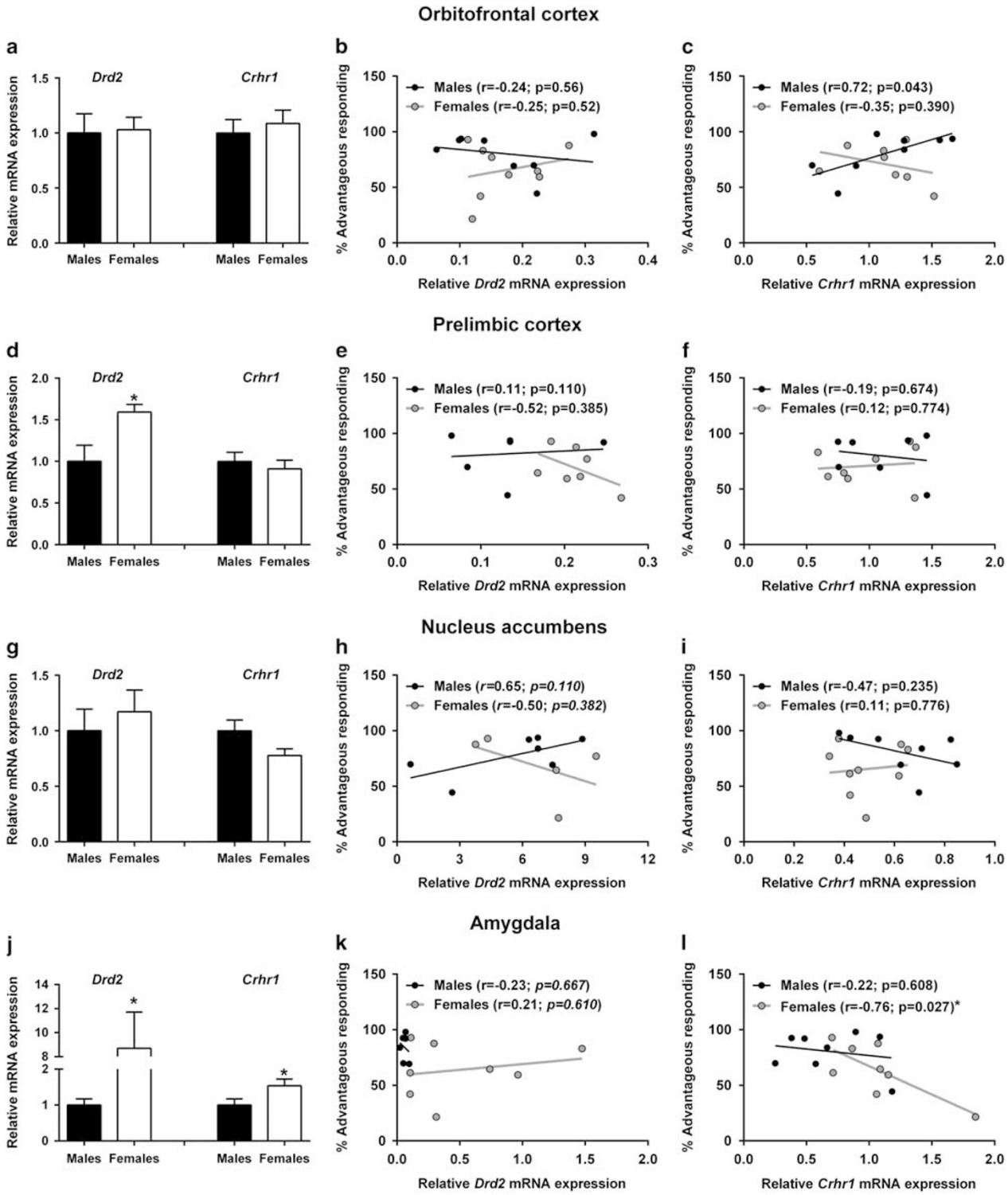

Figure 5 Effect of sex in the expression of D_2R and CRF1 and its association with the rat gambling task performance. Relative mRNA expression of D_2R and CRF1 in male and female rats in the (a) orbitofrontal cortex (D_2R: $n=8$–9; CRF1: $n=8$), (d), prelimbic cortex (D_2R: $n=6$–7; CRF1: $n=7$–8), (g) nucleus accumbens (D_2R: $n=7$–5; CRF1: $n=8$–9), and (j) amygdala (D_2R: $n=6$–8; CRF1: $n=8$). Pearson correlation coefficient analysis of D_2R expression and percent advantageous responding in male and female rats in the (b) orbitofrontal cortex, (e) prelimbic cortex, (h) nucleus accumbens, and (k) amygdala. Pearson correlation coefficient analysis of CRF1 expression and percent advantageous responding in male and female rats in the (c) orbitofrontal cortex, (f) prelimbic cortex, (i) nucleus accumbens, and (l) amygdala. *$p<0.03$ (adjusted p-value for reaching significance following Bonferroni correction).

choice associated with lower punishment probability, whereas males respond to all four options ignoring the risks associated with the disadvantageous choices. Our results also demonstrate that as the task progresses, male, but not female rats, increase their preference to the advantageous option, thus improving their overall performance and net gain. This sex difference in the task progression is in line with data from human studies (de Visser *et al*, 2010; Overman *et al*, 2011; Reavis and Overman, 2001; van den Bos *et al*, 2009), highlighting the translational validity of the sex-dependent differences in the decision making responses demonstrated in the present study. The sex differences in the task progression might be attributed to differences in information processing. For instance, women performing the IGT consider both the frequency of wins and losses of each choice as well as the long-term payoff, whereas males consider solely the long-term payoff of each deck (van den Bos *et al*, 2013). This difference in information processing might, at least partly, explain why females do not show the same task progression as males in both the human, as well as the rat version of the Iowa gambling task. Another interpretation for the observed sex difference in the task progression is the evolutionary differences between males and females. As Orsini and Setlow (2017) suggested, males tend to be more risk-prone due to their hypersensitivity to reward and hyposensitivity to punishments, whereas females are more risk averse because they are more hyposensitive to reward and hypersensitive to punishments. Therefore, the hypersensitivity of females to punishments might be related to the lack of improvement in the rGT.

Fluctuating levels of hormones in female rats, inferred by estrous cycle phase, did not affect decision making, which is in agreement with previously published findings (Orsini *et al*, 2016; Pellman and Setlow, (2017). However, this is the first study to report that female rats manifest higher premature responses during the proestrous phase, which is line with clinical findings (Diekhof, 2015).

It was previously demonstrated that manipulations targeting the dopaminergic system affect decision making (Baarendse *et al*, 2013; Stopper and Floresco, 2015; Zeeb *et al*, 2009). However, the existing literature is mainly based on findings in males. As baseline sex-dependent differences in dopamine release and uptake exist (Walker *et al*, 2000), we assessed whether administration of compounds targeting the dopaminergic system will affect decision making differently in male *vs* female rats. We specifically demonstrated that blockade of $D_{2/3}R$ impaired decision making only in male rats, as shown by a shift of choice behavior to a riskier choice. This finding is in contrast with previous studies, where eticlopride administration improved decision making in male rats (Zeeb *et al*, 2009) and induced risk aversion in a risk-based decision making task (St Onge and Floresco, 2009). However, controversies exist between findings from studies assessing the effect of D_2R antagonists on decision making. For example, administration of haloperidol (Paine *et al*, 2013) and L741626 (Di Ciano *et al*, 2015) showed no effect on decision making in the rGT in males. In line with these findings, administration of eticlopride had no effect on a task assessing risky decision making, whereas administration of the D2 receptor agonist, bromocriptine, decreased risky decision making (Simon *et al*, 2011). These discrepancies might be due to the different dosing regimens (0.075 mg/kg in present study *vs* 0.05 mg/kg in the study by Simon *et al*), administration routes, time of administration, or different baseline responding (~80% in the present study *vs* ~ 60% in the initial study by Zeeb *et al*). We note that our findings are in line with previous reports, demonstrating that D_2R antagonists decrease the choice of the high reward choice in different effort-based decision making tasks (Bardgett *et al*, 2009; Mott *et al*, 2009; Robles and Johnson, 2017). Furthermore, Stopper *et al* (2014) demonstrated that electrical stimulation of the ventral tegmental area, resulting in terminal dopamine release, resulted in a shift towards riskier decision making following an initial risky choice in male rats. Therefore, considering that eticlopride, by blocking D_2R autoreceptors, induces an increase in dopaminergic neurotransmission (Ford, 2014), it is likely that the shift towards riskier choices observed in our study might be due to increased dopamine levels following responding to a disadvantageous option, as described by Stopper *et al* (2014). However, as we did not measure dopamine levels in the brain, nor specifically modulate individual circuits relying on dopaminergic neurotransmission, the role of dopamine on the effects of eticlopride and quinpirole in male *vs* female decision making and the circuits they are acting upon require further investigation. Nevertheless, our findings are in line with a previous report demonstrating that administration of a D_2R antagonist in pathological gamblers, who are characterized by maladaptive decision making, increases the rewarding effects of gambling and the desire to gamble (Zack and Poulos, 2007), which indicate further impairment in decision making.

Importantly, we found that blockade of the D_2R did not affect decision making of female rats, which indicates the presence of sex differences in the neurochemical underpinning of decision making. This is further supported by our finding that activation of the $D_{2/3}R$ via administration of quinpirole reduces optimal choice responding and increases disadvantageous option choice specifically in female, but not male rats. However, Orsini *et al* (2016) demonstrated that administration of the non-selective dopamine agonist amphetamine decreases risky decision making in female rats using a different model of decision making. Here, we used pharmacological treatments that are more selective for $D_{2/3}R$, which might explain the differential responses compared with the amphetamine findings. Indeed, eticlopride and amphetamine administration had opposing effects in decision making in male rats (Zeeb *et al*, 2009). The sex-dependent differences following pharmacological manipulation of the dopaminergic neurotransmission in the rGT demonstrated in the present study, might be due to the higher levels of D_2R we observed in the amygdala and PrL of female *vs* male rats. However, we did not find any correlation between *Drd2* expression and decision making. Our findings do not preclude a functional role of the D_2R or its downstream pathways in decision making and warrant further investigation. Interestingly, we observed a significant negative correlation between NAc *Drd2* expression and premature responses solely in male rats. This finding is in line with previous findings by Dalley *et al* (2007), where lower $D_{2/3}R$ availability in the NAc of male rats was associated with increased impulsivity.

Another factor that impacts decision making is stress (Preston *et al*, 2007; Starcke and Brand, 2012; van den Bos *et al*, 2009), however the direction of the effect depends on the task and the specific stress paradigm used, as well as individual responsiveness to each stressor (Preston *et al*, 2007; Starcke and Brand, 2012; van den Bos *et al*, 2009). Here, we assessed the effects of the pharmacological stressor yohimbine in the performance of both male and female rats in decision making. We showed females to be more sensitive to yohimbine administration, since it impaired their decision making at lower doses compared with male rats. As yohimbine increases stress responses by increasing norepinephrine release (Szemeredi *et al*, 1991) and via increasing cortisol levels (Stine *et al*, 2002), our findings suggest that females might perform worse in the rGT because of their higher responsiveness to stress compared with males. In line with this hypothesis, we show that antalarmin administration, which reverses CRF-induced activation of the hypothalamic-pituitary-adrenal axis (Webster *et al*, 1996) and exerts anxiolytic effects (eg, Zorrilla *et al*, 2002), improved decision making specifically in female rats.

Basolateral amygdala lesions prior to the acquisition of the rGT delayed the development of preference for the advantegeous option, whereas lesions of this region after task acquisition increased disadvantegeous option choice (Zeeb and Winstanley, 2011), indicating a key role of the amygdala in modulating decision making. In agreement, lesions of the basolateral amygdala increased risky choices, in a different model of decision making (Orsini *et al*, 2015). These findings, along with previous data revealing that increased *Crhr1* mRNA in the amygdala is correlated with higher anxiety traits in rodents (Sotnikov *et al*, 2014), led us to hypothesize that the sex-dependent effects of antalarmin might be due to sex differences in the amygdalar CRF_1 system. Indeed, we found higher expression of *Crhr1* in the amygdala of female compared with male rats. Importantly, we show for the first time that higher levels of *Crhr1* in the amygdala are directly correlated with suboptimal performance, specifically in female rats, suggesting a critical role of the amygdalar CRF_1 system to regulate the observed sex differences in decision making.

Overall, our data reveal that decision making is differentially modulated by sex and that drugs targeting the dopaminergic and stress systems affect decision making in a sex-specific manner. We specifically identified differential responses in decision making of females and males upon manipulations of the dopaminergic and brain stress systems. Importantly, we demonstrated that higher amygdalar CRF_1 expression levels in females are associated with increased propensity for risky decision making, which is of considerable importance for the understanding of the neurobiological underpinnings of neuropsychiatric disorders characterized by maladaptive decision making in females. Our data suggest that CRF_1 antagonists might be more effective in improving decision making of females compared with males. Collectively, our findings highlight the importance of considering 'sex' as a biological factor in the development of novel pharmacotherapies for the treatment of psychiatric disorders characterized by maladaptive decision making.

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