

Sex differences in effects of dopamine D1 receptors on social withdrawal



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ABSTRACT

Dopamine signaling in the nucleus accumbens (NAc) plays a critical role in the regulation of motivational states. Recent studies in male rodents show that social defeat stress increases the activity of ventral tegmental dopamine neurons projecting to the NAc, and that this increased activity is necessary for stress-induced social withdrawal. Domestic female mice are not similarly aggressive, which has hindered complementary studies in females. Using the monogamous California mouse (*Peromyscus californicus*), we found that social defeat increased total dopamine, DOPAC, and HVA content in the NAc in both males and females. These results are generally consistent with previous studies in *Mus*, and suggest defeat stress also increases NAc dopamine signaling in females. However, these results do not explain our previous observations that defeat stress induces social withdrawal in female but not male California mice. Pharmacological manipulations provided more insights. When 500 ng of the D1 agonist SKF38393 was infused in the NAc shell of females that were naïve to defeat, social interaction behavior was reduced. This same dose of SKF38393 had no effect in males, suggesting that D1 receptor activation is sufficient to induce social withdrawal in females but not males. Intra-accumbens infusion of the D1 antagonist SCH23390 increased social approach behavior in females exposed to defeat but not in females naïve to defeat. This result suggests that D1 receptors are necessary for defeat-induced social withdrawal. Overall, our results suggest that sex differences in molecular pathways that are regulated by D1 receptors contribute to sex differences in social withdrawal behavior.

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1. Introduction

There is compelling evidence that the mesolimbic dopamine system has important effects on behavior in aversive contexts. Social defeat stress induces an immediate increase in dopamine turnover in ventral striatum (Mos and Van Valkenburg, 1979; Puglisi-Allegra and Cabib, 1990) and dopamine release in the nucleus accumbens (NAc) (Tidey and Miczek, 1996). In addition to these short term responses, defeat stress induces long lasting increases in burst firing of ventral tegmental area (VTA) dopamine neurons (Anstrom et al., 2009; Cao et al., 2010; Krishnan et al., 2007; Razzoli et al., 2011). Withdrawal from social contexts is linked to hyperactivity of VTA dopamine neurons (Krishnan et al.,

2007). Inhibition of burst firing by VTA dopamine neurons through overexpressing potassium channels (Krishnan et al., 2007) or direct optogenetic control (Chaudhury et al., 2013) increases social interaction behavior in male *Mus musculus* exposed to defeat. Increases in activity of VTA neurons projecting to the NAc, but not medial prefrontal cortex (mPFC) were especially critical for inducing social avoidance. This suggests that sustained increases in dopaminergic activity in the NAc are important for inducing social withdrawal behavior.

Social withdrawal is an important component of stress-induced mental disorders including anxiety and depression. These disorders are more commonly diagnosed in women than men, and there are important sex differences in neurobiological and endocrine responses to stress (Trainor, 2011). A handful of studies have examined the effects of social defeat in female rodents (Holly et al., 2012; Huhman et al., 2003; Solomon et al., 2007), but no study has tested whether the mesolimbic dopamine system is affected by defeat in females. The most widely studied rodent model species,

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M. musculus, is not optimal for studying sex differences because female aggression levels are low (Jacobson-Pick et al., 2013). We addressed this problem through studying the monogamous California mouse (*Peromyscus californicus*), a species in which both males and females (Silva et al., 2010) exhibit territorial aggression.

Female California mice exposed to three episodes of social defeat stress exhibit social withdrawal behavior whereas this effect is reduced or absent in males (Trainor et al., 2011, 2013). In females, defeat stress increased the number of phosphorylated CREB (pCREB) positive cells in the NAc shell, and social interaction behavior is negatively correlated with the number of pCREB cells in the NAc shell (Trainor et al., 2011). Activation of dopamine D1 receptors increases cyclic AMP production (Kebabian et al., 1972), which in turn facilitates phosphorylation of CREB (Yamamoto et al., 1988). We hypothesized that increased activation of D1 receptors in the NAc shell would inhibit social interaction behavior and that this effect would be enhanced in females compared to males. We also examined, for the first time, the effects of social defeat on dopamine content and receptor mRNA in the NAc of both males and females. Our results show that activation of D1 receptors is indeed necessary and sufficient to induce social withdrawal in female California mice, but that the mechanism for sex differences in behavior may be downstream of D1-signaling.

2. Materials and methods

2.1. Animals and housing conditions

Male and female California mice were obtained from our breeding colony at UC Davis. They were group housed (2–3 same-sex animals per cage) unless otherwise stated for each experiment. Animals were maintained in a temperature-controlled room on a 16L-8D cycle with *ad libitum* water and food (Harlan Teklad 2016, Madison, WI). Cages were polycarbonate plastic with corn-cob bedding, nestlets, and enviro-dri. All procedures were approved by the Internal Animal Care and Use Committee (IACUC) and conformed to NIH guidelines. All efforts were made to minimize animal suffering, to reduce the number of animals used.

2.2. Social defeat

Mice were randomly assigned to social defeat or control handling for three consecutive days (Trainor et al., 2011, 2013). Mice assigned to social defeat were introduced to the home cage of an aggressive, same-sex sexually-experienced mouse during the dark phase. Episodes of defeat were terminated following either 7 min or 10 bites from the resident, whichever occurred first. Control mice were introduced to an empty cage for 7 min. This approach more closely resembles methods used in rats (*Rattus norvegicus*) (Carnevali et al., 2012; Nikulina et al., 2012) and Syrian hamsters (*Mesocricetus auratus*) (Morrison et al., 2012; Taylor et al., 2011).

2.3. Open field and social interaction test

Social interaction tests consisted of 3 phases, 3 min each (Trainor et al., 2013). In the open field phase (OF), animals were introduced into a large open field (89 × 63 × 60 cm). Durations within a center zone located 14 cm from the sides were recorded using the Any-Maze video tracking system (Stoelting, Wood Dale, IL). During the acclimation phase a small wire cage was introduced against one side of the arena, the amount of time the mouse spent within 8 cm of the empty cage was recorded. During the social interaction phase an unfamiliar, same-sex virgin stimulus mouse was placed into the wire cage. We recorded the amount of time the focal mouse spent interacting with the wire cage and the duration spent in the two corners opposite the wire cage. We also calculated ratios for the interaction zone and corner zones defined as time during social interaction phase/time during acclimation phase × 100, as previously described (Krishnan et al., 2007; Vialou et al., 2010). Total distance traveled during the open field was used as an estimate of total activity.

2.4. Experiment 1: effects of defeat stress on dopamine content

Males and females were randomly assigned to social defeat or control conditions. Two weeks after defeat or control handling, all mice were tested in the social interaction test. The morning following social interaction testing (during lights on), cages were moved to the necropsy area 30–45 min before euthanasia. After a brief increase in activity after transfer, the mice returned to nests and were inactive. Each mouse was then lightly anesthetized and decapitated. It should be noted that the isoflurane anesthesia can inhibit the dopamine transporter and increase dopamine levels after >15 min of anesthesia (Baba et al., 2013; Byas-Smith et al., 2004; Votaw et al., 2003, 2004). However, because our mice experienced 90 s of isoflurane anesthesia we interpret these levels as baseline differences in dopaminergic tone.

Brains were rapidly removed and 2 mm slices were dissected using a brain matrix (Trainor et al., 2003). The NAc and medial prefrontal cortex were dissected using a 1 mm punch tool and samples were frozen on dry ice and stored at –40 °C. Punch samples were homogenized in .3 M perchloric acid and passed through .22 μm filters (Ultrafree Millipore, Billerica, MA). Total protein content in each sample was assessed using the Pierce Protein Assay (660 nm). Samples were then frozen at –40 °C and then shipped to the Wisconsin National Primate Research Center for high pressure liquid chromatography (HPLC) analysis.

For measurement of norepinephrine, epinephrine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin and homovanillic acid (HVA), 150 μl of perfusate was thawed and aliquotted into polypropylene inserts for HPLC vials. To this, 50 μl of internal standard, 3,4-dihydroxybenzylamine (DHBA), was added at a concentration of .1 μg/mL. Samples were loaded onto an autosampler (ESA #542) cooled to 5 °C. The injection volume was 50 μl using 100 μl partial loop onto a 4.6 × 250 mm C18 100A column (#00G-4252-E, Luna, Phenomenex, Torrance, CA). The detection system consisted of ESA (Chelmsford, MA, USA) isocratic pumps with a Coluochem III electrochemical detector. The mobile phase consisted of 10% acetonitrile in phosphate buffer (pH 2.75) containing 1.73 mM 1-octanesulfonic acid. The flow rate was 1.0 mL/min and the pressure at that flow rate was approximately 120 bar. The voltage was as follows: guard at –250 mV, E1 at –250 mV and E2 at +250 mV. The gain for E1 and E2 was set at 500 nA. The analytes were purchased in high purity powder form from Sigma (Sigma–Aldrich, St Louis, MO), and fresh stocks were prepared on a weekly basis (10 μg/mL in .2 N perchloric acid). The standard curve was 10-points, ranging from 250 to .488 ng/mL also in .2 N perchloric acid with DHBA as the internal standard. Linearity for each analyte was at least .999. The CV was 3.5% for norepinephrine, 4.0% for epinephrine, 4.7% for dopamine, 5.2% for DOPAC, 4.6% for serotonin and 9.1% for HVA.

2.5. Experiment 2: effects of defeat stress on dopamine receptor expression

Males and females were randomly assigned to social defeat or control conditions. Two weeks after defeat or control handling, all mice were tested in the social interaction test. Immediately after testing each mouse was lightly anesthetized with isoflurane and decapitated. Punch samples of the NAc were collected as in experiment 1.

RNA was extracted from punch samples using RNAqueous kits (Life Technologies) and reverse transcribed using iScript kits (BioRad). Transcripts were quantified using SYBR Green chemistry on an ABI 7500 Sequencing Detection System. To detect specific dopamine receptor subtypes, primer pairs were based on previously published sequences. Each primer pair was tested with California mouse cDNA and sequenced via Sanger Sequencing to confirm specificity (Table 1). For each sample, dopamine receptor gene expression was normalized to an average of GAPDH and β-actin expression. There were no significant differences in cycle thresholds between groups for GAPDH or β-actin.

2.6. Experiment 3: effects of D1 agonist infusion in males and females naïve to defeat

Males and females were anesthetized with isoflurane (3–5% in 1% O₂) and implanted with bilateral stainless steel guide cannula (Plastics One, C2351/SPC) aimed at the NAc shell (Fig. 1, AP = .51 mm, LM = 1.1 mm, DV = 6.85). The guide cannula (26 ga, o.d. = .46 mm; i.d. = .24 mm; length = 5.85 mm), was lowered into burr holes (#105 dremel bit, 1/16" tip) and attached to the skull using acrylic dental cement and skull screws (plastics one, 00-96 × 1/16). Guide cannulae were maintained patent using bilateral dummy caps (Plastics One, C235DC). Animals were given 3–7 days for recovery, during which the mice were observed and handled daily.

Infusions were made using bilateral internals (Plastics One, C2351/SPC, 33ga, o.d. = .21 mm; i.d. = .11 mm) that projected 1 mm past the cannula guide (6.85 mm total length). The D1 agonist SKF38393 (Sigma, St. Louis, MO) was dissolved in artificial cerebrospinal fluid (aCSF) and prepared fresh on the day of injection. Males and females were randomly assigned to receive a 200 μl infusion containing either aCSF, 5 ng, 50 ng, or 500 ng of SKF38393. Hamilton syringes were attached to an automatic micropump delivery apparatus (PHD 2000, Harvard Apparatus, Cambridge, MA) set to deliver 100 nl/min. Internal guides were kept in place for 1 min after injection to ensure delivery after which dummy guides were placed back into cannula guide. Each mouse was returned to its home cage and after 30 min was tested in social interaction tests as described above. Immediately following testing, each animal received a 200 nl infusion of blue food coloring to visualize both the injection site and fluid diffusion. Each mouse was then anesthetized with isoflurane

Table 1
Primer pair sequences.

	Forward	Reverse
D1	GGCTCCATCTCCAAGGACTGTA	AGCTTCTCCAGTGGCITAGCTATTC
D2	CGCTCGGAAGCGGGTCAACA	TCGGCGGGCAGCATCCATTC
D3	TGCGGCTGCATCCCATTCGG	GCTTGGGTGCCATGGTGGGG
D5	GGGCCTTTCGATCACATGTCT	AAGGAAACCTCTTCTCACAGTCA

Dopamine Agonist SKF 38393

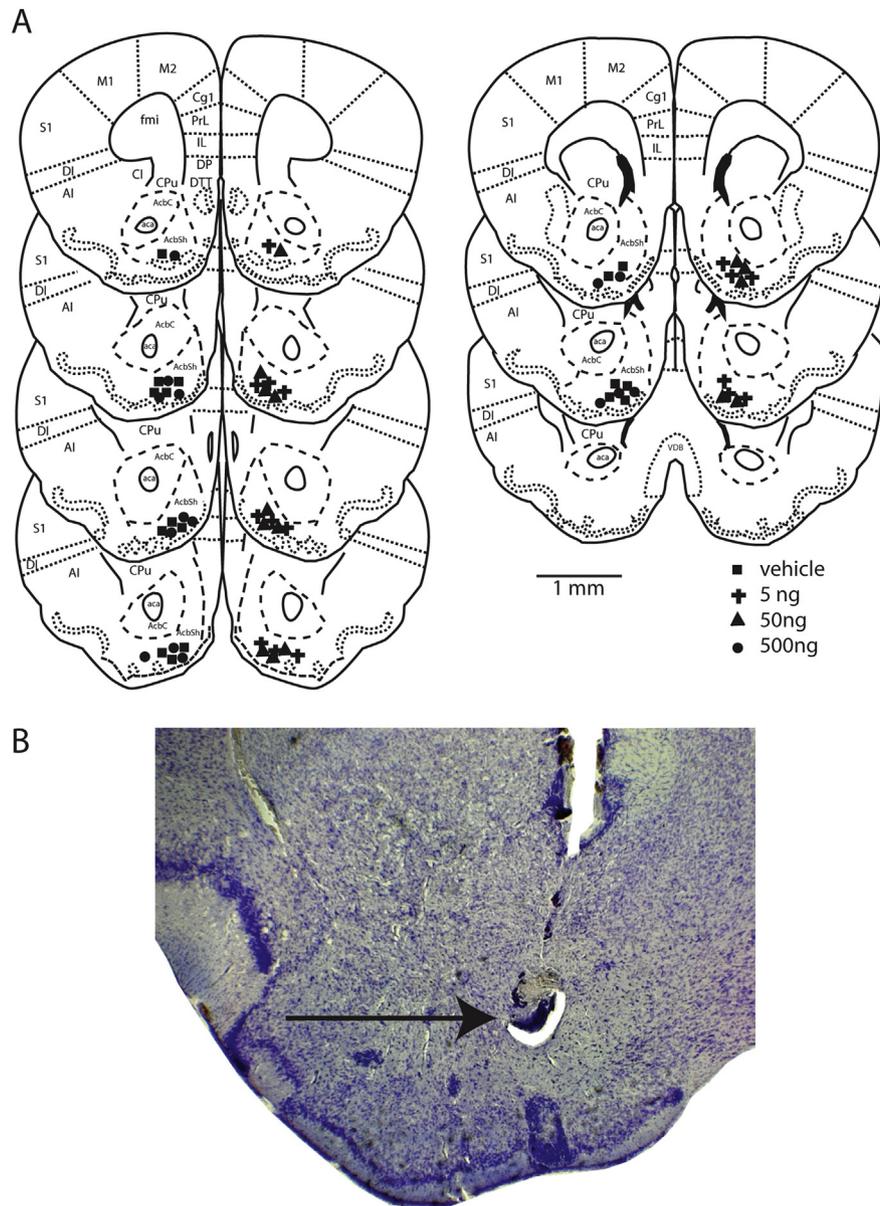


Fig. 1. (A) Reconstructions of a coronally cut series of sections through the nucleus accumbens showing the histological verification of injection of D1 agonist SKF38393 placement into the nucleus accumbens shell. AcbC nucleus accumbens core; AcbSh nucleus accumbens shell; aca anterior commissure; AI agranular insula; DI dysgranular insula; S1 primary somatosensory cortex; Cg1 cingulate cortex; PrL prelimbic cortex; M1 primary motor cortex; M2 secondary motor cortex; IL infralimbic cortex; DP dorsal peduncular cortex; DTT dorsal tenia tecta; fmi forceps minor or the corpus callosum; CPu caudate putamen; cl claustrum; DEn dorsal endopiriform nucleus; VDB vertical limb of diagonal band nucleus. All images are original work of the authors drawn from California mouse sections. (B) Photomicrograph of a Nissl stained section showing correct cannula placement and microinjection into the nucleus accumbens shell. Injection sites are represented by filled squares for vehicle, plus symbols for 5 ng SKF38393, filled triangles for 50 ng SKF38393 and filled circles for 500 ng SKF38393. Scale bar is 1 mm. Black arrow indicates microinjection site.

and decapitated. Brains were removed and fixed in 5% acrolein and processed to confirm needle placement. The brains were immersed in 20% sucrose overnight, frozen and sectioned coronally at 40 μ m on a cryostat. In order to confirm needle placement, tracks were assessed in sections stained using cresyl violet (in the web version) (Fig. 1B). In addition, diffusion of fluid was examined by visualization of blue dye in pictures of each brain during cryostat sectioning. Data from mice with needle tracks outside of the NAc shell were included in statistical analysis as anatomical controls (Tables 4 and 5).

2.7. Experiment 4: effects of D1 antagonist infusion in females exposed to defeat or control conditions

Female California mice were randomly assigned to social defeat or control conditions as described above. Two weeks later each female was implanted with guide cannula aimed at the NAc shell as described in Experiment 3 (Fig. 2). After

recovery, each female was randomly assigned to receive an infusion of aCSF or 2.5 μ g of the D1 receptor antagonist SCH23390 (Sigma, St. Louis, MO). The infusion procedure and behavioral testing was conducted exactly as described in Experiment 3. After testing, dye infusion and histology were performed as in Experiment 3.

2.8. Statistical analyses

In experiments 1 and 2, HPLC and gene expression data were log transformed and analyzed with two way ANOVA (sex and stress). Analyses of Q–Q plots revealed that these data were not normally distributed and so log transformations were used. For experiments 3 and 4 variation in behavioral data was heterogeneous across treatment groups, so nonparametric analyses were used. Specifically, in experiment 3 Kruskal–Wallis tests were used to test for an effect of SKF38393, followed by pairwise Mann–Whitney *U* tests. In experiment 4 Mann–Whitney analyses were used to compare the effect of SCH23990 in control and stressed females. In experiment 3

Dopamine Antagonist SCH 23390

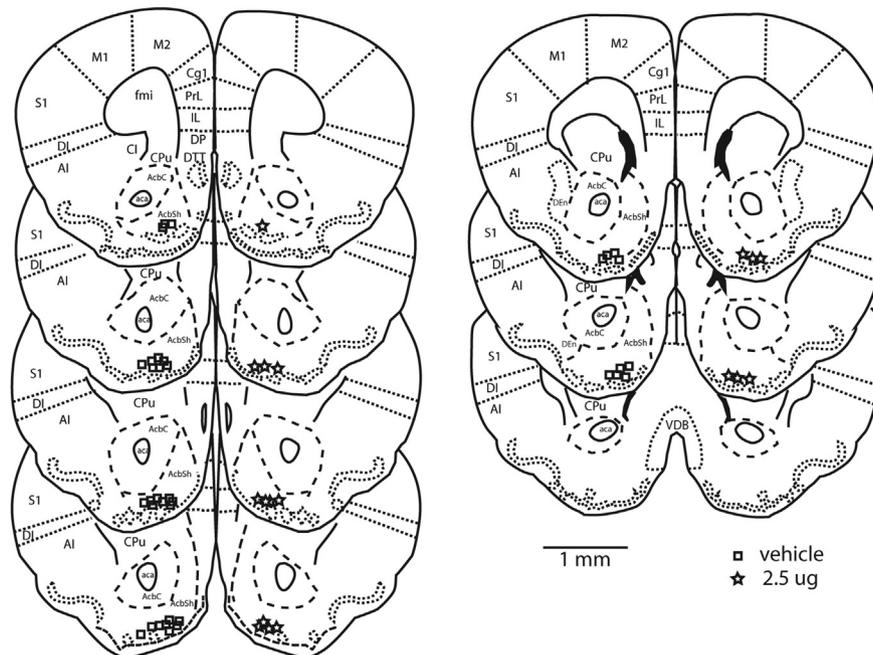


Fig. 2. Reconstructions of a coronally cut series of sections through the nucleus accumbens showing the histological verification of injection placement of D1 antagonist SCH23390 into the nucleus accumbens shell. AcbC nucleus accumbens core; AcbSh nucleus accumbens shell; aca anterior commissure; AI agranular insula; Dl dysgranular insula; S1 primary somatosensory cortex; Cg1 cingulate cortex; PrL prelimbic cortex; M1 primary motor cortex; M2 secondary motor cortex; IL infralimbic cortex; DP dorsal peduncular cortex; DTT dorsal tenia tecta; fmi forceps minor or the corpus callosum; CPU caudate putamen; cl claustrum; DEN dorsal endopiriform nucleus; VDB vertical limb of diagonal band nucleus. All images are original work of the authors drawn from California mouse sections. Injection sites are represented by squares for vehicle and stars for 2.5 µg SCH23390. Scale bar is 1 mm.

male and female experiments were run at separate times, so male and female data were analyzed separately. In experiments 3 and 4, we also calculated an interaction ratio, defined as the time spent in a zone during the social interaction period divided by the time spent in the zone during the acclimation period (Krishnan et al., 2007; Vialou et al., 2010). Interaction ratios were calculated for both the cage zone and corners opposite the cage.

3. Results

3.1. Experiment 1: effects of defeat stress on dopamine content

In the NAC, males had higher dopamine (Fig. 3A, $F_{1,31} = 16.5$, $p < 0.001$), DOPAC (Fig. 3B, $F_{1,31} = 10.8$, $p < 0.01$), and HVA (Fig. 3C, $F_{1,31} = 12.5$, $p < 0.001$) content compared to females. In addition, defeat stress induced a significant increase in dopamine ($F_{1,31} = 8.6$, $p < 0.01$), and modest increases in DOPAC ($F_{1,31} = 3.7$, $p = 0.06$) and HVA ($F_{1,31} = 3.2$, $p < 0.08$) content. There were no sex \times stress interactions (all p 's > 0.4). Norepinephrine levels were significantly higher in males (Table 2, $F_{1,31} = 17.3$, $p < 0.001$) but there were no effects of stress or interaction. There were no significant differences in 5-HT content (all p 's > 0.15). In the mPFC, females had higher 5-HT content than males ($F_{1,31} = 9.8$, $p < 0.01$) but there was no effect of stress or interaction. There were no significant differences in dopamine, NE, DOPAC, or HVA.

3.2. Experiment 2: effects of defeat stress on dopamine receptor expression

In the NAC, males had higher levels of D3 mRNA expression in NAC compared to females (Table 3, $F_{1,50} = 10.34$, $p < 0.01$). However, there was no effect of stress or interaction on D3 expression. There were no significant differences in D1, D2, or D5 gene expression.

3.3. Experiment 3: effects of D1 agonist infusion in males and female naïve to defeat

In females, SKF38393 infusions reduced time spent in the cage zone during the social interaction phase in a dose dependent fashion (Fig. 4B, Kruskal–Wallis $H_3 = 9.44$, $p = 0.02$). Females treated with 500 ng of SKF38393 spent significantly less time in the interaction zone in the presence of a novel mouse compared to females treated with aCSF (Mann–Whitney $U = 2.60$, $p < 0.01$), the low dose (Mann–Whitney $U = 1.99$, $p < 0.05$), or the medium dose (Mann–Whitney $U = 2.65$, $p < 0.01$). Similar differences were observed in the social interaction ratio (Table 4, Kruskal–Wallis $H_3 = 8.76$, $p < 0.03$). The effects of SKF38393 were specific to social contexts because there were no differences in time spent in the cage zone during the acclimation phase (Fig. 4A). There were also no effects on locomotor behavior or time spent in the center of the arena during the open field phase (all p 's > 0.62). In males, there were no significant effects on time spent in the interaction zone during the social interaction phase or during the acclimation phase (p 's > 0.50), and there were no differences in the social interaction ratio. Furthermore, there were no significant differences in locomotor behavior or time spent in the center during the open field phase (Table 4, all p 's > 0.43). No significant difference on any behavioral measure was observed in the anatomical misses (Table 4, all p 's > 0.50).

3.4. Experiment 4: effects of D1 antagonist infusion in females exposed to defeat or control conditions

Although there were no significant differences in the absolute amount of time spent in the interaction zone in this study (Table 5), significant differences were observed for ratio scores (social interaction phase/acclimation phase) for the interaction and corner

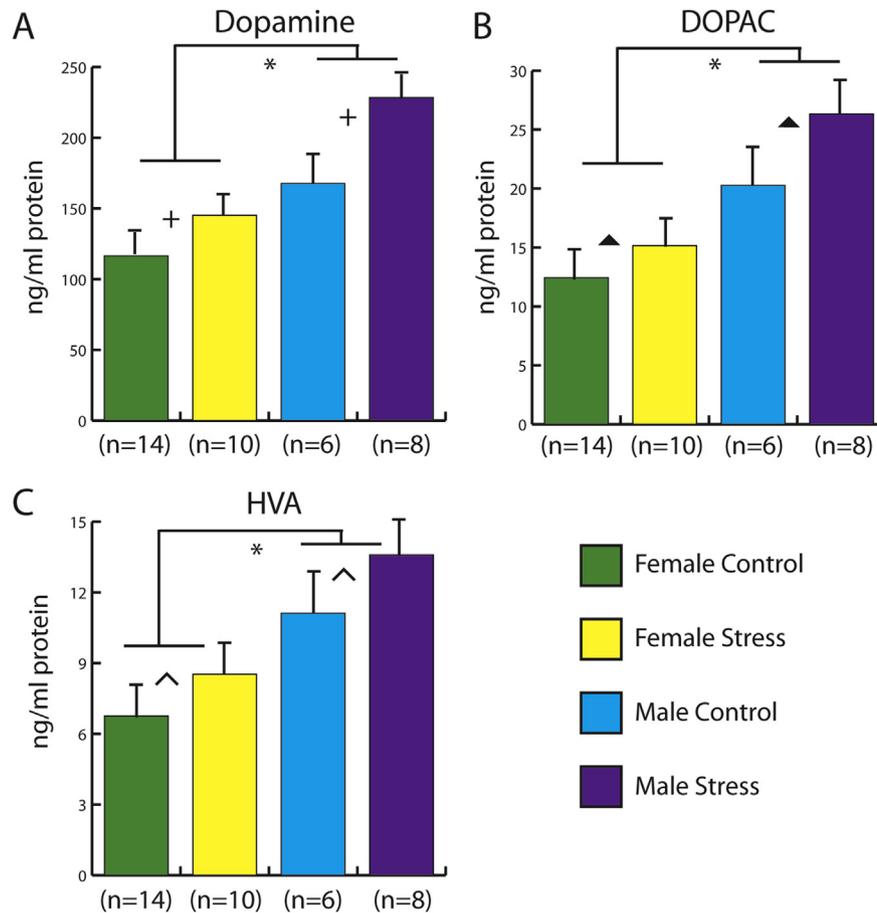


Fig. 3. Effect of stress and sex on dopamine (A), DOPAC (B), and HVA (C) levels in the nucleus accumbens of control and stressed mice in both females and males using HPLC. Green bars represent control females. Yellow bars represent stressed females. Blue bars represent control males. Purple bars represent stressed males. Error bars are SEM. * indicates main effect of sex, $p < 0.05$. + indicates main effect of stress, $p < 0.05$. Caret indicates a trend for effect of stress, DOPAC $p = 0.06$; HVA $p = 0.08$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

zones. In stressed females, infusions of the D1 antagonist SCH23390 significantly increased the ratio of time spent in the interaction zone in the presence of a novel mouse (Fig. 5A, Mann–Whitney $U = 152$, $p < 0.01$) and significantly decreased the ratio of time spent in the corner zones (Fig. 5B, Mann–Whitney $U = 42$, $p = 0.01$) compared to aCSF treatment. In control females there was no effect of SCH23390 infusions on the ratio of time spent in the interaction zone in the presence of a novel mouse or the corner zones (p 's > 0.5). In contrast to our study with SKF38393, infusions of SCH23390 did have an inhibitory effect on locomotor behavior. Infusion of SCH23390 reduced locomotor activity in the open field for stressed females (Mann–Whitney $U = 41$, $p = 0.03$). A similar trend was observed in control females but was not statistically significant. No significant difference on any behavioral measure was observed in the anatomical misses (Table 5, all p 's > 0.50).

4. Discussion

Our results indicate that although defeat stress increases dopaminergic signaling in both male and female California mice, dopamine D1-like receptor signaling induces social withdrawal in females but not males. These data suggest that previous observations that defeat stress increases the activity of VTA dopamine neurons (Anstrom et al., 2009; Krishnan et al., 2007; Razzoli et al., 2011) generalize not only to different species of rodents, but also to females. The sex difference in sensitivity to D1-like receptors does not appear to occur at the level of receptor gene expression in the NAC, because there were no sex differences in dopamine receptor gene expression. We hypothesize that sex differences in the behavioral effects of D1-like receptors are mediated downstream of D1 neurons.

Table 2

Neurotransmitter protein (ng/ml) measured by HPLC (mean \pm SEM). Main effect of sex * $p < 0.05$, ** $p < 0.01$.

		Nucleus accumbens					Frontal cortex					
		NE	Epi	5-HT	DOPAC/DA	HVA/DA	DA	DOPAC	HVA	NE	Epi	5-HT
Female	Control	7.41 \pm 2.26	.23 \pm .08	4.93 \pm .61	.11 \pm .02	.06 \pm .01	1.02 \pm .12	.56 \pm .08	.25 \pm .03	2.54 \pm .35	.29 \pm .15	.85 \pm .16*
	Stress	7.05 \pm 2.16	.31 \pm .07	5.39 \pm .90	.11 \pm .005	.06 \pm .005	1.01 \pm .24	.38 \pm .12	.21 \pm .03	2.91 \pm .50	.19 \pm .06	1.06 \pm .15*
Male	Control	15.03 \pm 2.92**	.39 \pm .10	3.27 \pm .93	.12 \pm .01	.06 \pm .005	1.37 \pm .34	.45 \pm .05	.24 \pm .03	2.28 \pm .40	.21 \pm .04	.50 \pm .20
	Stress	14.98 \pm 2.53**	.48 \pm .08	6.10 \pm 1.43	.11 \pm .01	.06 \pm .005	.97 \pm .28	.36 \pm .06	.25 \pm .08	2.04 \pm .16	.36 \pm .19	.54 \pm .18

Table 3Dopamine receptor mRNA relative expression. **Main effect of sex $p < 0.01$.

		D1R	D2R	D3R	D5R	Prodynorphin
Female	Control	2.24 ± .36	1.44 ± .20	3.22 ± .87	1.80 ± .21	1.72 ± .25
	Stress	1.57 ± .36	1.12 ± .20	2.61 ± .87	1.20 ± .21	1.14 ± .25
Male	Control	2.17 ± .41	1.38 ± .23	6.37 ± 1.00**	1.18 ± .24	1.51 ± .28
	Stress	1.47 ± .45	1.03 ± .25	5.99 ± 1.10**	1.30 ± .27	1.32 ± .31

4.1. Effects of defeat stress on dopamine content and receptor expression

In male rats and *Mus*, social defeat induces a long term increase in dopamine activity. Both male and female California mice exposed to social defeat stress had elevated levels of dopamine and DOPAC in the NAc, two weeks after the last episode of defeat stress. Punch samples were collected during the inactive phase, which suggests that these increases are due to an increase in baseline dopaminergic tone. Three weeks after defeat, baseline rates of *in vivo* VTA neuronal burst firing are increased in male *Mus* (Razzoli et al., 2011). In *Mus*, there is strong evidence that this increase in neuronal activity inhibits social approach behavior. *In vivo* recordings showed that the baseline activity of VTA neurons is negatively correlated with social interaction behavior, and that the activity of VTA neurons can be normalized by chronic

antidepressant treatment (Cao et al., 2010). Reducing burst firing of VTA dopamine neurons by overexpressing potassium channels (Krishnan et al., 2007) or direct optogenetic control (Chaudhury et al., 2013) also increases social interaction behavior in male mice exposed to defeat. Interestingly, studies in *Mus* follow a standardized protocol of 10 episodes of defeat combined with prolonged sensory contact (Golden et al., 2011). In our studies, only three relatively brief episodes of defeat were sufficient to increase dopamine and DOPAC levels in both males and females. However, this raises the question of why social withdrawal was not observed in male California mice.

Increased reuptake could induce resistance to increased dopamine signaling. Studies using the visible burrow system showed that subordinate rats had reduced dopamine transporter (DAT) binding in NAc shell (Lucas et al., 2004). If DAT activity was elevated in stressed males, we would expect defeat stress to have little or no

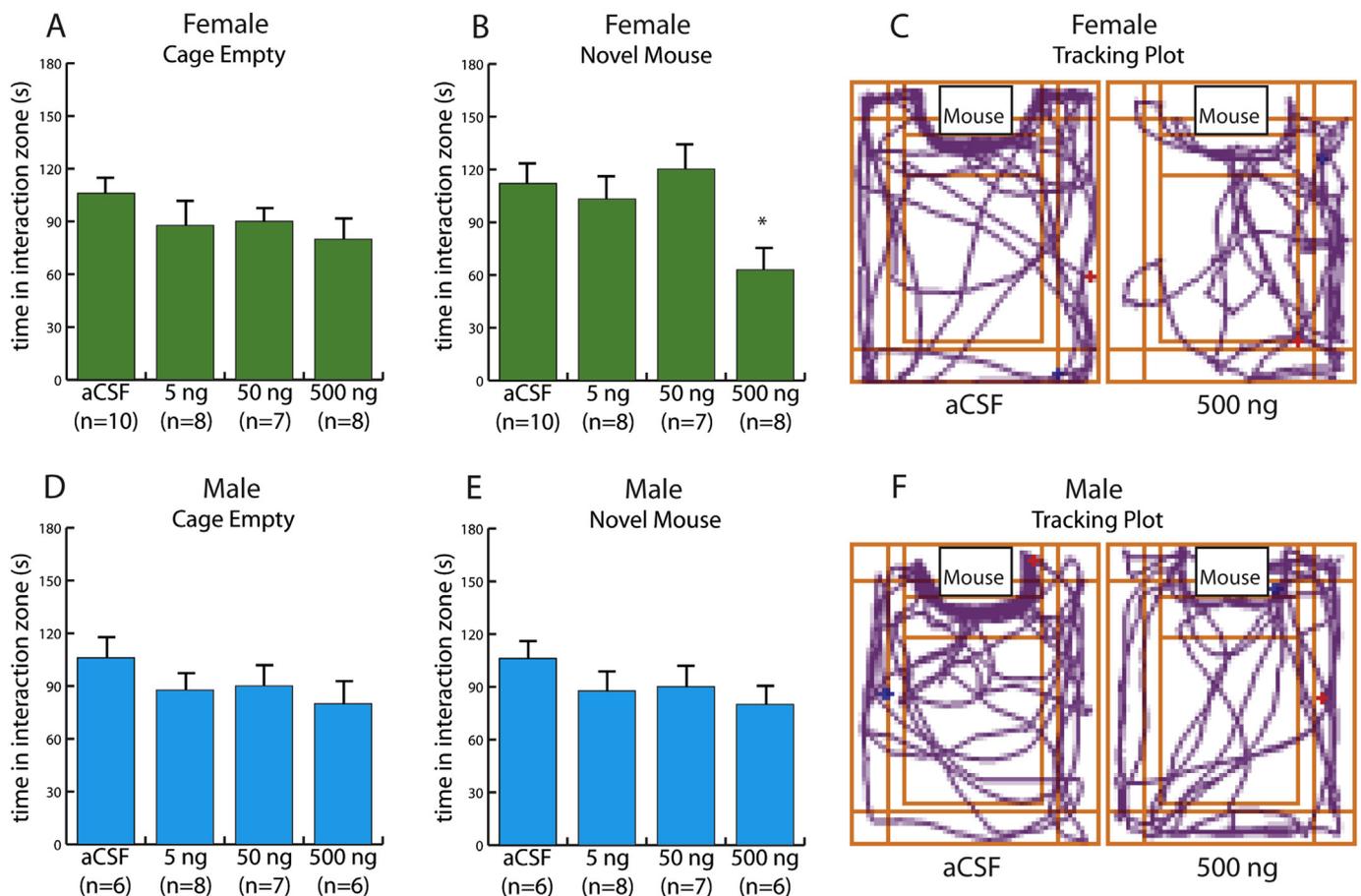


Fig. 4. Effect of the D1 agonist SKF38393 in the nucleus accumbens shell on time spent in the cage zone in females (A–C) and males (D–F) naïve to defeat. (A) Time spent in the cage zone during the acclimation phase by females. (B) Time spent in the cage zone during the social interaction phase by females. (C) Tracking plot of a representative female mouse path when a same-sex novel mouse was present after aCSF (left) or 500 ng SKF38393 (right) microinjection. (D) Time spent in the cage zone during the acclimation phase by males. (E) Time spent in the cage zone during the social interaction phase by male mice. (F) Tracking plot of a representative male mouse path during the social interaction phase after aCSF (left) or 500 ng SKF38393 (right) microinjection. * indicates $p < 0.05$ vs aCSF.

Table 4
Social interaction data after D1 agonist infusion. **p* < 0.05 vs aCSF.

		Cage area time (s)		Corners time (s)		Open field		Interaction ratio	
		Empty cage	Novel mouse	Empty cage	Novel mouse	Center time (s)	Distance (m)	Cage area	Corners
Anatomical hits									
Females	aCSF (<i>n</i> = 10)	106.01 ± 8.87	111.98 ± 8.74	4.44 ± 1.61	7.12 ± 3.06	24.78 ± 5.15	22.91 ± 2.73	109.09 ± 8.03	194.44 ± 61.26
	5 ng (<i>n</i> = 8)	87.69 ± 13.85	103.20 ± 18.20	7.84 ± 1.71	6.06 ± 2.57	39.76 ± 10.15	20.15 ± 2.44	153.53 ± 43.10	328.68 ± 252.95
	50 ng (<i>n</i> = 7)	90.09 ± 7.37	120.23 ± 10.75	11.87 ± 3.59	6.10 ± 3.84	23.23 ± 4.78	20.10 ± 4.19	135.66 ± 9.92	66.33 ± 19.95
	500 ng (<i>n</i> = 9)	79.96 ± 11.43	62.99 ± 11.01*	8.30 ± 2.47	10.49 ± 3.12	32.59 ± 7.33	24.15 ± 4.02	84.04 ± 10.22*	164.24 ± 54.17
Males	aCSF (<i>n</i> = 7)	76.39 ± 11.11	88.47 ± 20.18	11.07 ± 1.71	34.90 ± 22.14	31.83 ± 7.99	16.20 ± 1.59	105.75 ± 17.30	263.92 ± 175.41
	5 ng (<i>n</i> = 8)	67.84 ± 9.05	93.08 ± 17.21	15.36 ± 3.64	8.89 ± 3.01	22.38 ± 4.61	20.23 ± 3.01	149.52 ± 28.66	82.41 ± 27.66
	50 ng (<i>n</i> = 8)	75.15 ± 11.32	80.34 ± 19.11	8.83 ± 2.82	5.56 ± 1.58	31.08 ± 3.89	15.37 ± 2.32	102.97 ± 17.81	169.21 ± 94.90
	500 ng (<i>n</i> = 6)	66.60 ± 13.00	77.33 ± 16.51	19.08 ± 9.54	33.33 ± 28.41	33.52 ± 5.18	14.44 ± 2.32	124.36 ± 39.38	227.65 ± 116.02
Anatomical misses									
Females	aCSF (<i>n</i> = 8)	91.43 ± 8.77	115.63 ± 8.94	4.26 ± 1.53	1.71 ± .86	25.03 ± 1.96	19.01 ± 1.96	129.08 ± 6.94	105.71 ± 80.94
	5 ng (<i>n</i> = 5)	80.90 ± 14.96	120.94 ± 19.51	11.26 ± 4.99	3.22 ± 1.66	33.24 ± 5.26	19.94 ± 3.72	163.85 ± 35.47	105.55 ± 86.63
	50 ng (<i>n</i> = 5)	58.46 ± 14.30	83.40 ± 15.75	9.46 ± 2.10	6.12 ± 1.63	28.68 ± 6.17	25.59 ± 6.39	157.64 ± 32.96	63.07 ± 17.03
	500 ng (<i>n</i> = 3)	79.53 ± 6.30	89.37 ± 42.51	10.27 ± 3.50	5.03 ± 3.98	23.10 ± 3.98	16.75 ± 4.25	111.84 ± 54.09	111.86 ± 101.57

effect on dopamine metabolites (Huotari et al., 2002). However, DOPAC and HVA were elevated in stressed males. Alternatively, resistance to increased dopamine activity could be achieved via reduced expression of dopamine receptors. While male rats have been reported to have more intense D1 receptor binding in NAc compared to females (Andersen and Teicher, 2000) this effect has not been observed in every study (Ferris et al., 2007). We observed no sex differences in D1-like receptor (D1 or D5) expression, nor did we observe any effects of stress. Thus there is little support for the hypothesis that sex differences in behavioral responses to defeat stress are mediated by differences in dopamine receptor expression. Sex differences in behavior might instead be mediated by mechanisms downstream of receptor expression. Indeed, most evidence suggests that the behavioral effects of psychostimulants (which increase dopamine transmission) are stronger in females than males (Carroll and Anker, 2010). For example, amphetamine injections have stronger effects on rotational behavior in female rats compared to males (Robinson et al., 1980). Similarly, female rats form cocaine-based conditioned place preferences (CPP) at lower doses and with fewer conditioning sessions than males (Russo et al., 2003). These data suggest that behavioral effects of dopaminergic signaling may be stronger in females compared to males.

4.2. Sex differences in effects of D1 receptors on social withdrawal

Two observations suggested that D1 receptors would have important effects on stress-induced social withdrawal. First, D1 receptors are more likely than D2 receptors to be expressed in a low affinity state (Richfield et al., 1989), and several studies suggest that

D1 activation occurs primarily in the presence of high dopamine levels (Cheer et al., 2007; Di Chiara and Bassareo, 2007). Second, defeat stress increases the number of phospho-CREB cells in the NAc shell of females but not males (Trainor et al., 2011). Thus, we used D1 receptor agonists and antagonists to test whether D1 receptors mediated stress-induced social withdrawal in California mice. Increased dopamine D1 signaling induced social aversion in females naïve to defeat, suggesting that D1 receptor activation is sufficient to induce social withdrawal behavior in females. Inhibition of D1 receptors in stressed females increased social approach behavior, suggesting that D1 signaling plays an important role in mediating the social withdrawal phenotypes. However, this effect appeared to be more subtle than what was observed in experiment 3 (D1 agonist), because only social interaction ratios were significantly changed. If social defeat induces a sustained increase in dopamine signaling in the NAc, this might induce neuroadaptations that would reduce the sensitivity of the NAc to dopaminergic signaling (Self, 2004).

Although D1 receptors in the NAc have been found to modulate locomotor behavior (Dreher and Jackson, 1989; Essman et al., 1993; Smith-Roe and Kelley, 2000), our results can not be explained by simple changes in locomotor behavior. The dose of SKF that reduced social interaction behavior in females was lower than those doses found to increase locomotor behavior, and we observed no changes in locomotor behavior of California mice in the open field test. While SCH infusions did reduce locomotor behavior in control and stressed females, only stressed females showed an increase in social interaction behavior. Furthermore, in stressed females SCH increased the relative time spent in the interaction zone and decreased the relative time spent in the corners opposite the

Table 5
Social interaction data after D1 antagonist infusion in females. **p* < 0.05 vs aCSF. ***p* < 0.01 vs aCSF.

		Cage area time (s)		Corners time (s)		Open field		Interaction ratio	
		Empty cage	Novel mouse	Empty cage	Novel mouse	Center time (s)	Distance (m)	Cage area	Corners
Anatomical hits									
Control	aCSF (<i>n</i> = 16)	91.27 ± 6.65	91.97 ± 10.62	7.22 ± 1.92	16.11 ± 9.31	31.58 ± 4.03	22.48 ± 3.50	95.62 ± 10.22	206.26 ± 52.79
	2.5 μg (<i>n</i> = 9)	81.80 ± 16.20	88.74 ± 16.27	9.69 ± 3.55	6.57 ± 2.36	30.19 ± 6.90	15.92 ± 2.24	113.09 ± 26.19	258.52 ± 121.06
Stress	aCSF (<i>n</i> = 19)	103.17 ± 7.60	77.40 ± 10.17	5.36 ± 1.33	17.98 ± 5.92	32.12 ± 2.88	22.53 ± 2.26	76.22 ± 10.51	451.18 ± 111.12
	2.5 μg (<i>n</i> = 10)	79.64 ± 15.99	93.24 ± 18.37	7.00 ± 16.60	20.08 ± 17.78	28.65 ± 5.59	15.22 ± 3.53*	135.18 ± 19.92**	108.67 ± 30.39**
Anatomical misses									
Control	aCSF (<i>n</i> = 3)	136.10 ± 4.39	143.83 ± 8.44	1.77 ± 1.52	.2 ± .06	23.1 ± 1.43	18.39 ± 3.73	105.80 ± 6.06	92.36 ± 57.80
	2.5 μg (<i>n</i> = 4)	88.18 ± 28.58	89.88 ± 20.00	1.73 ± .92	7.5 ± 5.71	14.18 ± 4.92	20.17 ± 3.63	183.02 ± 102.65	245.37 ± 245.37
Stress	aCSF (<i>n</i> = 3)	112.25 ± 28.99	117.48 ± 16.60	7.88 ± 3.76	13.25 ± 13.25	18.43 ± 3.34	18.11 ± 5.07	90.40 ± 14.67	58.33 ± 44.49
	2.5 μg (<i>n</i> = 7)	75.24 ± 14.10	73.13 ± 30.44	8.77 ± 4.86	27.8 ± 6.68	27.64 ± 6.68	20.43 ± 4.3	95.73 ± 29.29	186.33 ± 76.81

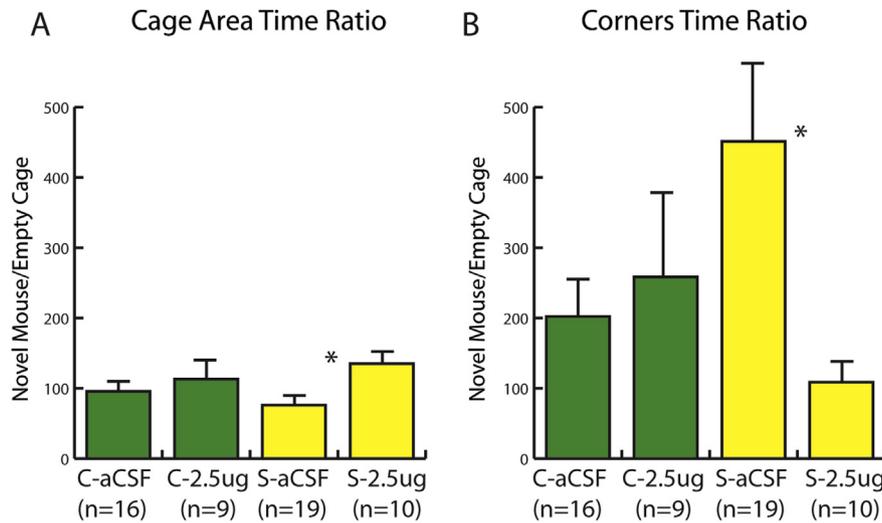


Fig. 5. Effect of the D1 antagonist SCH23390 in the nucleus accumbens shell on the interaction ratios (social interaction/acclimation \times 100) for the cage zone (A) and corner zones (B). Infusions of SCH23390 had no effect on control (C) mice but increased social interaction behavior in stressed (S) mice. ** indicates $p < 0.01$ vs aCSF.

interaction zone during the social interaction phase of the test. Together, these data indicate that D1 receptors in NAc shell have important effects on social withdrawal behavior.

Systemic injection of D1 agonists reduces social interaction behavior in males (Sams-Dodd, 1998), but only a few studies have considered whether D1 receptors regulate neural circuits controlling social behavior. Dopamine D1 receptors in the NAc shell play an intriguing role in the formation and maintenance of pair bonds between male and female prairie voles (Aragona and Wang, 2009). In males, activation of D1 receptors in the NAc shell prevents the formation of new pair bonds (Aragona et al., 2006). However, once a pair bond is formed, D1 receptor expression in the NAc is enhanced and facilitates the maintenance of the bond by increasing aggressive behavior towards unfamiliar females (Aragona et al., 2006). This effect appears to be mediated by endogenous opioid signaling. Medium spiny neurons in the NAc that express D1 receptor also express dynorphin (Hara et al., 2006), the primary endogenous ligand for the kappa opioid receptor (KOR). Infusion of the KOR antagonist norbinaltorphimine (nor-BNI) into the NAc shell of pair-bonded prairie voles reduces aggressive behavior (Resendez et al., 2012). These results suggest the possibility that effects of D1 receptors on social withdrawal are mediated in part by KOR signaling. However, we did not examine D1 receptor affinity or efficacy which could differ between the sexes and be differentially altered by social stress between the sexes, thus the effects could still be at the level of the D1 receptor.

Stress-induced anxiety and depression disorders are more common in women versus men (Kessler et al., 1993). Rodent studies have demonstrated that females have exaggerated glucocorticoid responses to stress (Weiser and Handa, 2009). Chronic stressors also lead to stronger inductions of depression-like behaviors, such as anhedonia, in females compared to males (Dalla et al., 2005, 2008; Konkle et al., 2003). Less is known about potential sex differences in neural circuits that may mediate these behavioral responses. Our findings indicate that sex differences in the strength of D1 receptor signaling in the NAc may contribute to increased vulnerability to psychosocial stress in females. These results suggest that further study of molecular pathways downstream of D1 receptor expressing neurons, particularly the dynorphin-KOR pathway, will provide new insights to understanding sex differences in the behavioral effects of psychosocial stress.

Conflict of interest

The authors declare no competing financial interests.

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