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Sex Differences in Neurochemical Effects of Dopaminergic Drugs in Rat Striatum

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Previous data indicate that dopamine neurotransmission is differently regulated in male and female rats. The purpose of the present study was to investigate the dopamine transporter and autoreceptor as potential loci responsible for this sex difference. Fast cyclic voltammetry at carbon-fiber microelectrodes was used to monitor changes in electrically evoked levels of extracellular dopamine in the striata of anesthetized male and female rats before and after administration of an uptake inhibitor, a dopamine D_2 antagonist, or a D_3/D_2 agonist. Administration of 40 mg/kg cocaine ip increased electrically-evoked extracellular dopamine concentrations in both sexes, but to a significantly greater extent in female striatum at the higher stimulation frequencies. The typical antipsychotic, haloperidol, increased dopamine efflux in both sexes but the effect was twice as large in the female striatum. The D_3/D_2 agonist quinpirole induced an unexpected, transient increase in dopamine efflux following high-frequency stimulation only in females, and evoked dopamine was higher in females across this entire time course. More detailed analysis of cocaine effects revealed no fundamental sex differences in the interaction of cocaine with DAT *in vivo* or in synaptosomes. These results indicate that nigrostriatal dopamine neurotransmission in the female rat is more tightly regulated by autoreceptor and transporter mechanisms, perhaps related by greater autoreceptor control of DAT activity. Thus, baseline sex differences in striatal dopamine regulation induce different pharmacologic responses. These results contribute to understanding sex differences in stimulant-induced locomotor activity in rats and may have broader implications for neurologic disorders and their pharmacotherapies in humans.
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INTRODUCTION

Central dopaminergic systems mediate the reinforcing effects of drugs of abuse and are perturbed in many neurological disorders including schizophrenia, Parkinson's disease, and attention deficit disorder (Berridge and Robinson, 1998; Seeman and Kapur, 2000; Berke and Hyman, 2000; Solanto, 2002). Sex differences in several of these disorders have been reported. For instance, certain aspects of cocaine taking and addiction are more severe in women including prevalence of cocaine dependence in adolescence (Kandel *et al*, 1997), age and severity of use at intake (Robbins *et al*, 1999), and cocaine-induced craving (Kosten *et al*, 1993; Robbins *et al*, 1999). In contrast to stimulant addiction, women are somewhat protected from Parkinson's disease relative to men (Bower *et al*, 1999; Baldereschi *et al*, 2000), and the age of onset of schizophrenia is about 6 years later in women than men (Lindamer *et al*, 1997; Hafner *et al*, 1998; Leung and Chue, 2000). This set of clinical findings suggests that underlying sex differences in dopaminergic function exist in humans. Three studies using three different radioligands all showed that women have more dopamine transporters than men in the striatum (Lavalaye *et al*, 2000; Staley *et al*, 2001; Mozley *et al*, 2001).

Animal studies including those from this laboratory also demonstrate the existence of sex differences in dopaminergic function that contribute to significant sex differences in behavior. Our laboratory and others have shown that indirect dopamine agonists like cocaine induce greater behavioral (Bowman and Kuhn, 1996; Walker *et al*, 2001a) and neuroendocrine (Walker *et al*, 2001b) effects in female than male rats. Female rats trained to self-administer cocaine on a progressive ratio schedule have higher break points than males (Roberts *et al*, 1987; Carroll *et al*, 2002), and acquisition, maintenance and reinstatement of cocaine self-administration are greater in female rats (Lynch and Carroll, 1999, 2000; Lynch *et al*, 2000; Hu *et al*, 2004). However, Caine *et al* (2004) using a relatively high-training dose of cocaine found that male rats acquired

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self-administration earlier than females. This study found no sex or ovarian hormone effects on maintenance of cocaine self-administration using a fixed ratio five schedule of reinforcement.

Female and male rats also respond differently to direct-acting dopamine D_2 ligands. Male rats were more sensitive than females to acute and sub-chronic haloperidol impairment of escape-avoidance behavior (Parra *et al*, 1999; Arenas *et al*, 1999). Campbell *et al* (1988) showed that female Long-Evans rats were more sensitive than males to haloperidol-induced catalepsy. Weekly administration of haloperidol produced a greater hypersensitivity to haloperidol-induced catalepsy in female than male rats, although daily administration induced a tolerance in females but not males (Fujii and Ikeda, 1982). The D_2 agonists apomorphine and quinpirole have also been reported to induce sex differences in spontaneous behavior in rats (Savageau and Beatty, 1981; van Hartesveldt, 1997; Schindler and Cannona, 2002).

The basis of the substantially greater dopamine release and uptake rates in the striatum of female rats (Walker *et al*, 2000) remains unknown. The dopamine transporter (DAT) and/or the release-and uptake-regulating autoreceptor (Rouge-Pont *et al*, 2002) could be important molecules for regulating sex differences in extracellular dopamine. These baseline differences in dopaminergic neurotransmission could mediate sex differences in behavioral responses to dopaminergic drugs and potentially contribute to the sex differences in addiction and neurologic disease risk. The following studies probed the contributions of DAT and the autoreceptor to sex differences in dopamine by determining changes in dopamine overflow induced by protypical ligands for these sites. We have more extensively probed sex differences in the interactions of cocaine and DAT to better understand sex differences in the disregulation of dopaminergic neurotransmission.

MATERIALS AND METHODS

Subjects

Adult male and female CD rats were purchased from Charles River Laboratories (Raleigh, NC, USA). They were segregated by sex and were housed in plastic cages under a 12 : 12 light : dark cycle with lights on at 0600. Food and water were provided *ad libitum*. Animals were moved to the testing facility and weighed the day before observations. All animals were tested between 60 and 90 days of age, 2–3 weeks after shipment on average. Females were used without regard to estrous state because we have shown that dopamine uptake and release do not vary across the female estrous cycle (Walker *et al*, 2000). Animal care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 865-23, Bethesda, MD, USA) and approved by the Institutional Animal Care and Use Committee.

Electrochemistry

Voltammetry procedures were similar to previously published methods (Walker *et al*, 2000). Fast-scan cyclic voltammetry (Millar *et al*, 1985) was conducted with an EI-400 potentiostat (Ensman Instrumentation, Bloomington, IN, USA). Locally written computer software triggered the potentiostat to apply a triangle wave (−0.4 to 1 V and back, at 300 V/s) to a carbon fiber electrode. Cyclic voltammograms were recorded every 100 ms and digitized using a DMA Labmaster board (Scientific Solutions, Solon, OH). Carbon-fiber microcylinder electrodes prepared from 7 μm diameter T-300 fibers (Amoco, Greenville, SC, USA.) were used in the *in vivo* experiments (Cahill *et al*, 1996) along with a Ag/AgCl reference (BAS Inc., West Lafayette, IN).

Changes in extracellular dopamine were determined by monitoring the current over a 200 mV window at the peak oxidation potential for dopamine. Background subtracted cyclic voltammograms were used to identify the electroactive substance as dopamine. These voltammograms were obtained by subtracting voltammograms collected during stimulation from those collected during baseline recording. To convert oxidation current to dopamine concentration, electrodes were calibrated with dopamine standard solutions in a flow injection system following experimental use.

In Vivo Procedures

Rats were anesthetized with urethane (1.5 g/kg i.p.) and positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Body temperature was maintained at 37°C with a Deltaphase Isothermal Pad (Braintree Scientific, Braintree, MA). A bipolar stimulating electrode (Plastics One Inc., Roanoke, VA) was positioned in the medial forebrain bundle (MFB) and electrical stimulation parameters were 300 μA, biphasic, 2 ms each phase. The stereotaxic coordinates (in mm) anteroposterior (AP) and mediolateral (ML) from the bregma and dorsoventral (DV) from the dura that follow are based on a brain atlas (Paxinos and Watson, 1986). The stimulating electrode was placed at: −4.6 AP, +1.4 ML, −7.5 to −9.0 DV. For the cocaine and quinpirole experiments the carbon-fiber microelectrode was directed at the center of the caudate (+1.2 AP, 2.0 ML, −4.5 to −6.2 DV). Haloperidol effects were determined at medial and lateral locations within the striatum because the density of dopamine D_2 receptors has been reported to vary across its medial-lateral axis (Falardeau and Di Paolo, 1987; Joyce *et al*, 1985). The medial and lateral coordinates for haloperidol experiments were: +1.2 AP, 1.4 or 2.7 ML, −4.5 to −6.2 DV, respectively. The electrode placed in the lateral aspect of the caudate was angled toward the midline at 12°.

The locations of the stimulating and working electrodes were optimized to give maximal dopamine responses. A 60 Hz 120-pulse stimulation was applied to evoke extracellular concentrations of dopamine 10-fold greater than the K_m to measure V_{max} directly from the slope of the decay curve (Wightman *et al*, 1988). Dopamine efflux induced by frequencies from 10, 20, 30, 40, 50, and 60 Hz was recorded. Haloperidol and quinpirole experiments used 120 pulse train duration at each frequency and post-drug recordings occurred at 5–10 min intervals beginning 10 min after drug administration. A shorter stimulus train duration (30 pulses) was used in the cocaine experiments to enable more frequent data collection so that all frequency responses could be recorded over the time of cocaine's

peak behavioral effects (Walker *et al*, 2001a). Since sex differences in extracellular striatal dopamine concentrations develop with increasing stimulus train duration, the shorter stimulus duration also served to minimize baseline sex differences (Walker *et al*, 2000). Thirty stimulus pulses were applied at each frequency except for 40 Hz, which used 28 pulses. Immediately after the final baseline data collection, rats were administered 40 mg/kg cocaine i.p, to saturate dopamine transporters. At 2.5 min post cocaine injection the 20 Hz stimulations commenced and were made at 2.5 min intervals until 20 min postinjection. Postcocaine responses to stimulations at the other frequencies were recorded between 20 and 50 min following cocaine administration.

Synaptosomal Uptake

Male and female CD rats (Charles River), aged 70–90 days, were killed by decapitation and the brains were rapidly removed. Dorsal striata were dissected on ice, weighed, and homogenized with a glass-teflon homogenizer using five to seven strokes by hand in 10 volumes of 0.32 M sucrose, 25.0 mM tris and 10.0 μM iproniazid phosphate. Homogenates were centrifuged at 1000*g* for 10 min. The resulting supernatants were centrifuged for 20 min at 17500*g*. The P2 pellet was resuspended with a glass-teflon homogenizer using two to three strokes by hand in 100 volumes of modified Kreb's-bicarbonate buffer. This buffer consisted of 118 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM $NaH_2PO_4 \cdot H_2O$, 1.2 mM $MgCl_2$, 11.1 mM dextrose, 2.0 μM ascorbic acid, and 1.0 μM iproniazid phosphate to inhibit monoamine oxidase activity. The buffer was bubbled with 95% O_2/5% CO_2 and the pH was adjusted to 7.4.

The synaptosomes (100 μl in 1 ml total incubation volume) were preincubated with buffer and cocaine for 5 min in a shaking 37°C water bath. The various cocaine solutions were serially diluted from a 10 mM stock solution. Uptake was initiated with the addition of 50 nM [^{3}H] dopamine (New England Nuclear, Natick, MA). Synaptosomes from one male and one female rat were run in each assay ($N=4$). Nonspecific binding was determined in duplicate tubes kept on ice. Tubes were incubated for 4 min in a 37°C shaking water bath. Uptake was terminated by the addition of 4 ml of ice-cold Kreb's-bicarbonate buffer followed by rapid vacuum filtration over GF/B glass fiber filter paper. Samples were then washed two more times with 4 ml of ice-cold buffer. Radioactivity was counted using liquid scintillation spectrometry. Protein was determined by the method of Bradford.

Data Analysis

For the cocaine experiment the dynamic changes of extracellular dopamine evoked by electrical stimulation were modeled as a balance between dopamine release and uptake (Wightman *et al*, 1988; May *et al*, 1988; Wightman and Zimmerman, 1990). The equation employed to extract kinetic parameters is $d[DA]/dt = f[DA]_p - V_{max}/((K_m/[DA]) + 1)$ where f is the frequency of stimulation, $[DA]_p$ is the concentration of dopamine released per stimulus pulse, and V_{max} and K_m are Michaelis–Menten parameters for the maximal uptake rate and the affinity of the transporter, respectively. Non-linear curve fitting using a simplex algorithm calculated V_{max}, K_m, and $[DA]_p$ from *in vivo* data at each frequency from 20 to 60 Hz (Wu *et al*, 2001b). All three parameters were typically allowed to float in this analysis, although occasionally V_{max} was fixed to the value determined from the slope of the clearance phase of 60 Hz 2 s overflow curves where $[DA] \gg K_m$ (Wu *et al*, 2001b).

Where applicable, data are expressed as the mean ± SEM and N is the number of rats. The effects of cocaine on dopamine overflow were analyzed using three-way ANOVA (sex by frequency × treatment). Effects of cocaine and sex on changes of *in vivo* kinetic parameters were also analyzed by two-way ANOVA. Sex differences in cocaine effects on evoked dopamine overflow were determined by ANOVA with repeated measures. Differences in haloperidol responses in the medial and lateral striatum were determined by paired *t*-test and then medial and lateral electrode responses were averaged per rat. Effects of sex and frequency on percent haloperidol changes in dopamine efflux were analyzed by ANOVA with repeated measures. The effect of sex on maximal extracellular dopamine elicited by 60 Hz stimulations following quinpirole administration was analyzed by ANOVA with repeated measures on time after injection. *Post hoc* analysis used the Newman–Keuls Multiple Comparison Test. Differences were considered to be significant when $p < 0.05$.

Cocaine inhibition of synaptosomal dopamine uptake data was fitted using a one-site competition model (Prism 3.3, Graphpad Software, San Diego, CA). Sex differences in IC_{50}'s and maximal dopamine uptake (determined in tubes without cocaine) were analyzed by paired *t*-tests.

Drugs and Reagents

(−) Cocaine HCl was obtained from NIDA through the Research Triangle Institute. Cocaine solutions were prepared fresh in 0.9% saline and injected i.p. (±) Quinpirole (R.B.I., Natick, MA) was injected subcutaneously at 0.5 mg/kg in a saline vehicle. Haloperidol (Sigma, St Louis, MO, USA) was prepared in 0.1% tartaric acid and injected subcutaneously at 0.5 mg/kg. The dose volume for each drug was 1 ml/kg.

RESULTS

Sex-Dependent Changes in Dopamine Efflux Induced by Dopamine Transporter and Autoreceptor Ligands

Cocaine. Figure 1 shows voltammetric recordings of electrically stimulated dopamine overflow in the dorsal striatum of anesthetized male and female rats at baseline and following 40 mg/kg cocaine i.p. Short stimulus trains of 30 pulses were employed so that overflow at all frequencies including 60 Hz would be approximately equal in males and females at baseline (Walker *et al*, 2000). The maximal effect of cocaine occurred within 15 min after injection (data not shown). Increasing stimulation frequency induced greater dopamine overflow and systemic cocaine administration increased overflow at each frequency. Cocaine induced proportionally larger increases in the overflow in female striatum at higher frequencies.

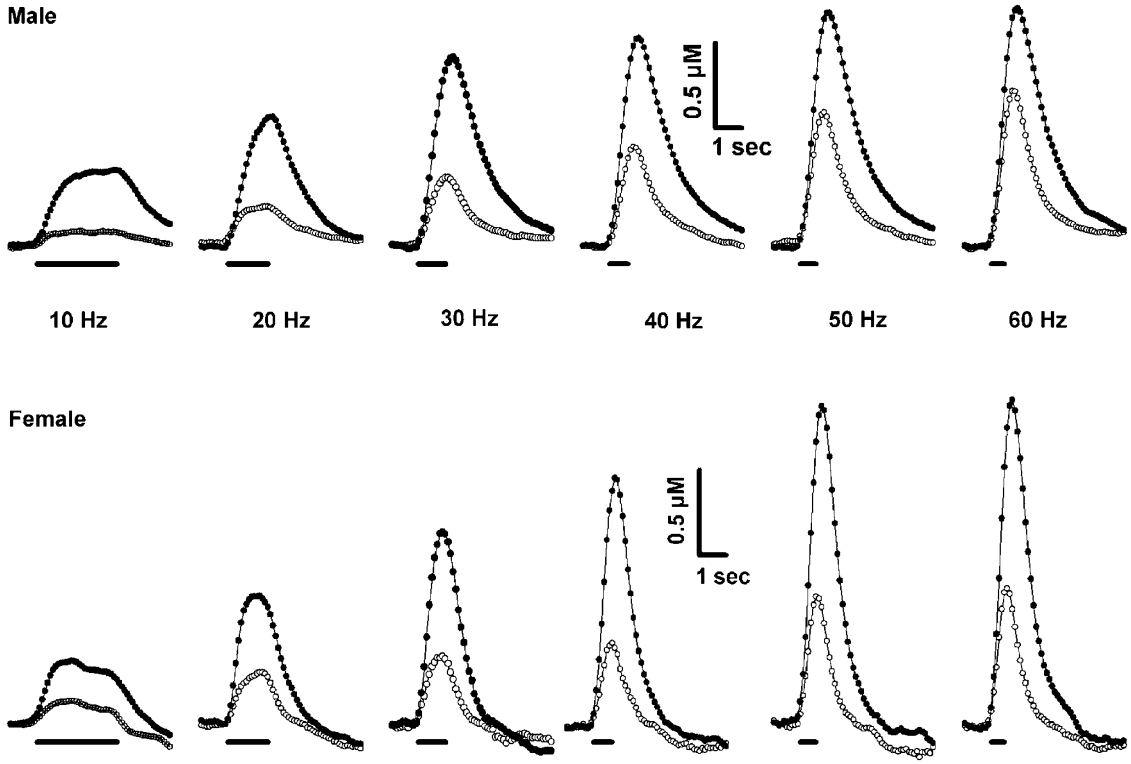

Figure 1 Voltammetric recordings in anesthetized rat striata before and after 40 mg/kg cocaine. Dopamine release was evoked by 30 pulse electrical stimulations (300 µA) of the medial forebrain bundle at the frequencies indicated. Cyclic voltammograms obtained from each recording matched those of a standard dopamine solution obtained from the same electrodes *in vitro* following the experiment. The frequency of stimulation is indicated between the male and female recordings. The identical scale bars for each rat apply to all frequencies. The open circles represent baseline dopamine concentrations determined at 100 msec intervals and the filled circles show the effects of cocaine on stimulated dopamine efflux in each rat. The lines under the recordings indicate application of the electrical stimulation. Cocaine effects were determined between 15 and 50 min after i.p. injection.

Figure 2 shows group averages of data obtained as in Figure 1. In all, 30 pulse stimulus trains elicited the same extracellular dopamine concentrations in male and female striata prior to cocaine administration. Therefore, the main effect of sex did not reach statistical significance ($p = 0.08$). Cocaine increased dopamine overflow three to five-fold in males and females and ANOVA confirmed a significant overall effect of treatment ($F(1,12) = 106$, $p < 0.001$). Stimulation frequency exerted a significant overall effect on dopamine efflux ($p < 0.001$) and significantly interacted with sex ($F(5,57) = 2.59$, $p = 0.035$). A significant interaction of sex, frequency, and treatment ($F(5,55) = 2.39$, $p = 0.049$) was also found. Subsequent ANOVA excluding baseline data showed that sex differences in cocaine effects on dopamine efflux were frequency dependent (sex × frequency, $F(5,56) = 3.07$, $p = 0.016$). *Post hoc* analysis indicated that maximal dopamine concentrations elicited by 50 and 60 Hz stimulations following cocaine administration were significantly greater in females than in males ($p < 0.05$).

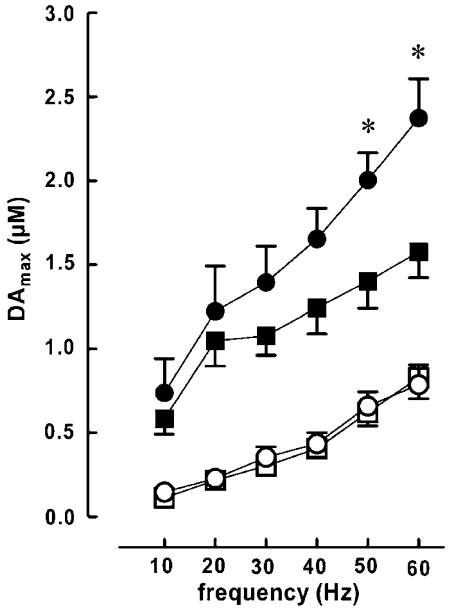

Figure 2 Sex differences in the effects of 40 mg/kg cocaine in rat striata *in vivo*. Maximal dopamine overflow (DA_{max}) was determined from recordings obtained as described in Figure 1 and averaged across groups of male ($N = 8$) and female ($N = 8$) rats. Square symbols represent data from males while circles represent female data. Open symbols represent baseline data and filled symbols represent after cocaine measurements. *significantly greater than males at the same frequency ($p < 0.05$).

Haloperidol. Figure 3 shows voltammetric recordings of electrically stimulated dopamine overflow in the dorsal striatum of anesthetized male and female rats before and after 0.5 mg/kg haloperidol i.p. Haloperidol was maximally effective within 15 min after injection (data not shown). All the data in Figure 3 were determined at the medial site

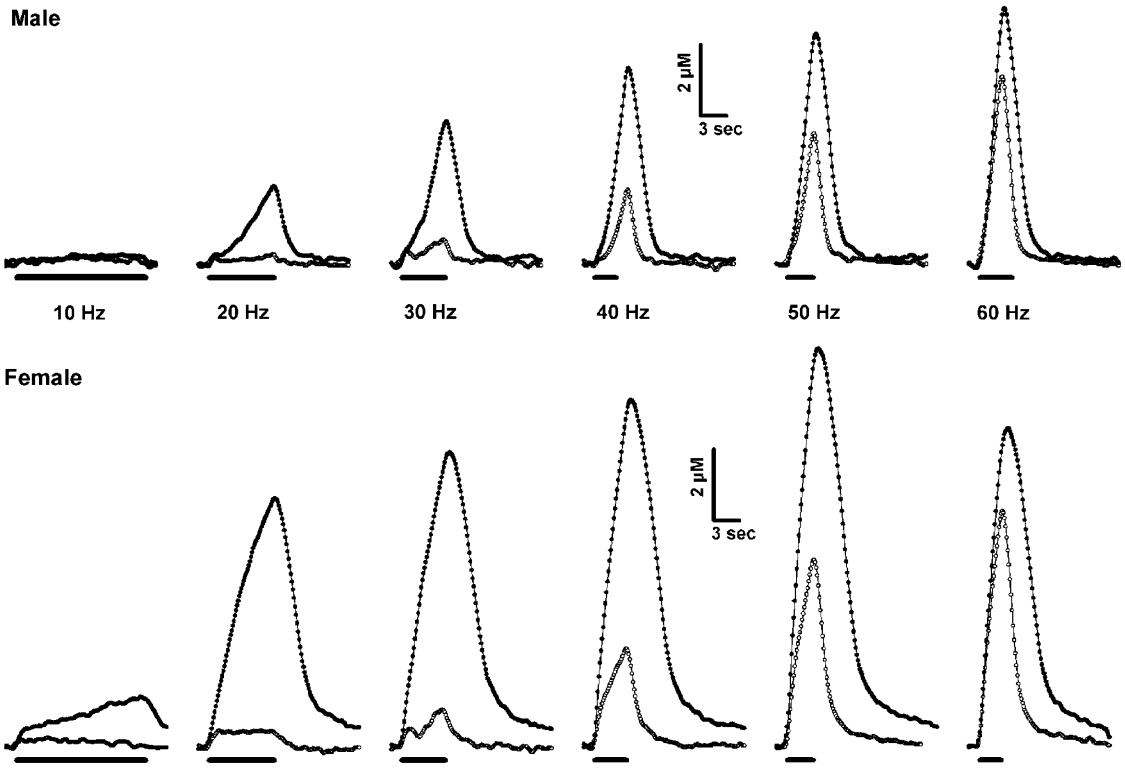

Figure 3 Voltammetric recordings in anesthetized rat striata before and after 0.5 mg/kg haloperidol. Data were collected as in Figure 1 except that 120 pulse electrical stimulations of the medial forebrain bundle were used. The lines under the recordings indicate application of the electrical stimulation. Data are from a single male and a single female rat at the medial electrode location. Haloperidol effects were determined between 15 and 70 min after i.p. injection.

and show that haloperidol-induced increases in dopamine overflow were greater in the female.

Haloperidol had identical effects on dopamine overflow at both the medial and lateral sites in the striatum and therefore, the average haloperidol effect per rat is shown for clarity. Figure 4 shows group averages of data obtained at both sites in the striatum as in Figure 3. Data are expressed as percent effect of haloperidol relative to the baseline to emphasize the most robust relative changes in extracellular dopamine. The greatest change in dopamine overflow induced by haloperidol was at 20 Hz in both sexes. Two-way ANOVA indicated that frequency exerted a significant effect on haloperidol-induced changes ($p<0.001$) but the main effect of sex did not reach statistical significance ($p=0.06$). The effect of haloperidol in each sex differed by frequency as indicated by a significant interaction of sex and frequency ($F(5,51)=3.8$, $p=0.008$).

Quinpirole. Figure 5 shows effects of administration of 0.5 mg/kg quinpirole s.c. in the striata of anesthetized male and female rats. The recordings on the left (top and bottom) show dopamine efflux at baseline following typical 60 Hz 2 s electrical stimulations. Quinpirole increased dopamine efflux 10 min after injection in both rats but was somewhat more effective in this female. Evoked dopamine was slightly higher or equal to baseline at 30 and 60 min, respectively, in the female. In contrast, evoked dopamine decreased slightly below baseline levels in the male at 30 and 60 min. A rigorous examination of quinpirole effects at other frequencies was not conducted in all rats (as in the previous experiments) in order to describe completely the time course of this unexpected increase at 60 Hz. Stimulated efflux was determined at a single, representative low frequency of 20 Hz in all rats. The two panels on the right

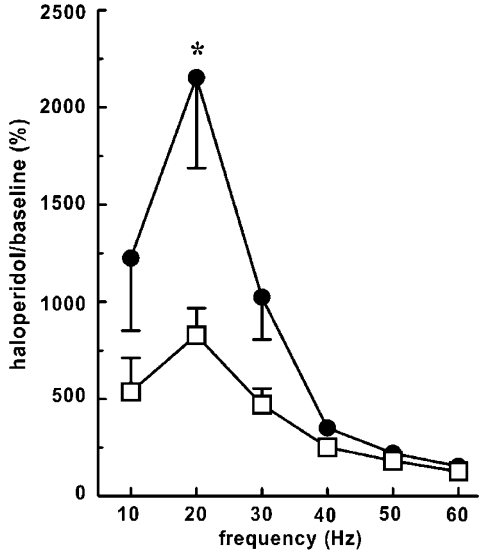

Figure 4 Sex differences in the effects of 0.5 mg/kg haloperidol in rat striata *in vivo*. Maximal dopamine overflow (DA_{max}) was determined from recordings obtained as described in Figure 3 and averaged across groups of male ($N=5$) and female ($N=4$) rats. The $[DA]_{max}$ after haloperidol was divided by that obtained at baseline and shown as the percentage. The average response per rat is shown because the drug effect was the same at the medial and lateral electrode locations. ANOVA indicated significant effects of frequency and the interaction of sex and frequency. *significantly greater than males at the same frequency ($p<0.05$).

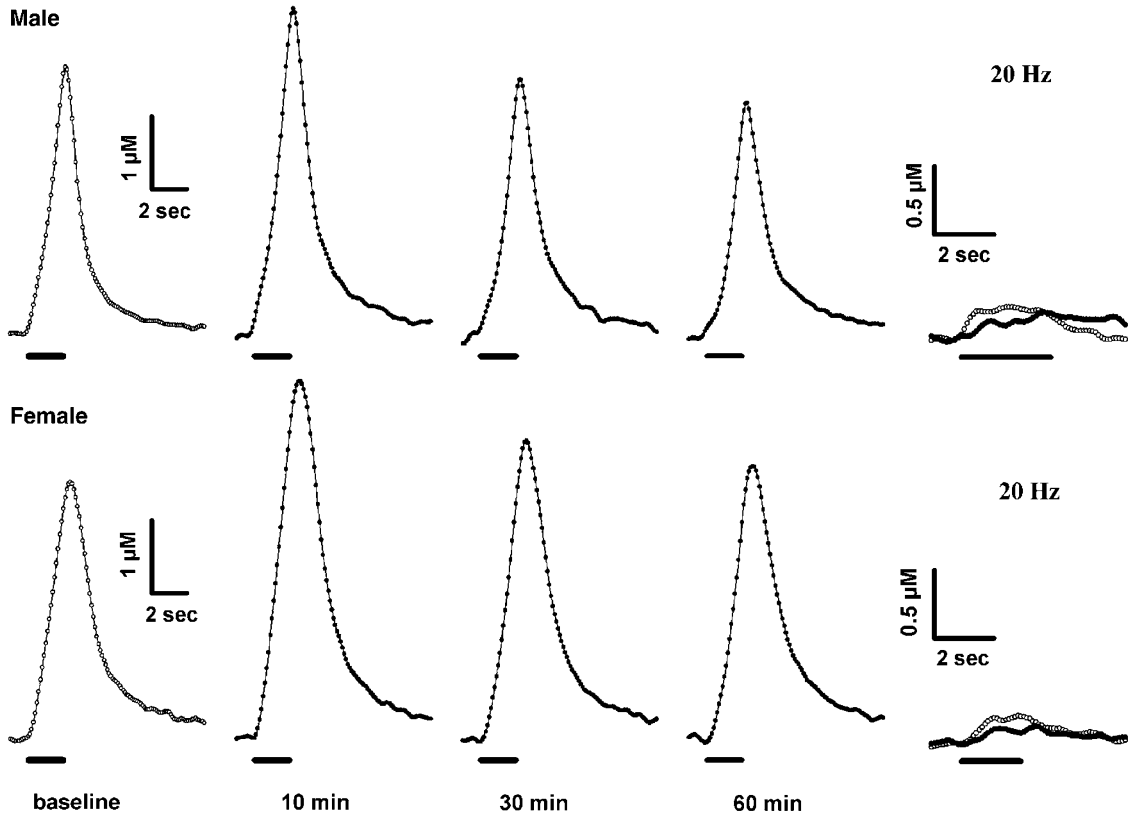

Figure 5 Time course of quinpirole effects on dopamine overflow following 60 Hz stimulations. Voltammetric recordings in striatum of anesthetized male and female rats following 60 Hz 120 pulse electrical stimulations of the medial forebrain bundle. A baseline recording, prior to drug administration, and recordings 10, 30 and 60 min after 0.5 mg/kg quinpirole s.c. are shown. The two recordings on the right side of the figure illustrate that quinpirole never increased dopamine overflow induced by 20 Hz stimulations at any point in the time course, in contrast to the effects at 60 Hz. For clarity the scale was increased for the 20 Hz recordings and the scale bars for each sex are identical at each frequency.

of Figure 5 show dopamine efflux following 20 Hz stimulations in the same animals before and after quinpirole. Quinpirole attenuated dopamine efflux at 20 Hz in both these rats and never increased electrically stimulated efflux at 20 Hz in any rat at any time after administration. Quinpirole decreased dopamine levels evoked by 20 Hz stimulations to an average of $63 \pm 6\%$ of baseline in males and $53 \pm 5\%$ in females by 50 min after injection (not significant, $p = 0.75$).

The average effects of quinpirole on dopamine efflux evoked by 60 Hz stimulations on male ($N = 6$) and female ($N = 5$) rats are shown in Figure 6. Maximal evoked dopamine concentrations over the entire time course were significantly greater in the female striatum ($F(1,49) = 5.1$, $p = 0.049$). The effects of quinpirole in all rats varied over the one hour time course ($F(4,49) = 13.7$, $p < 0.001$) and the interaction of sex and time was significant ($F(4,49) = 2.7$, $p = 0.049$). *Post hoc* analysis showed that quinpirole increased evoked dopamine at 10 and 20 min in the females ($p < 0.05$) and males and females were significantly different at 10, 20, and 30 min ($p < 0.05$).

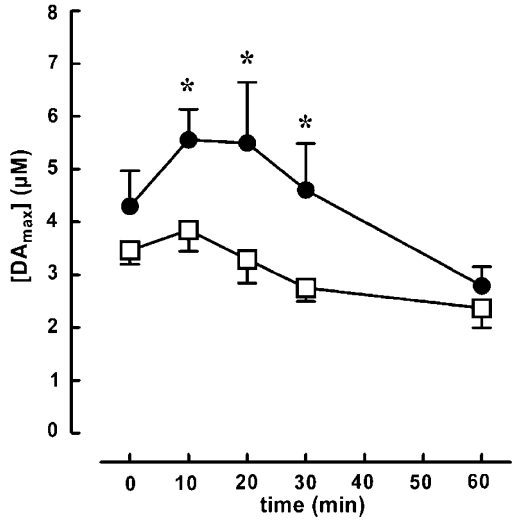

Figure 6 Sex differences in the time course of quinpirole effects on dopamine overflow in anesthetized rat striatum. Maximal dopamine overflow (DA_{max}) was determined from data as described in Figure 5. The average $[DA]_{max}$ before administration of quinpirole was 3.4 ± 0.2 (males, $N = 6$) and 4.3 ± 0.7 μM (females, $N = 5$) ($p = 0.3$). Significant effects of sex and time and their interaction were found. Quinpirole increased evoked dopamine 10 and 20 min after injection in females ($p < 0.05$). *significantly greater than males at the same time ($p < 0.05$).

Probing Sex Differences in Cocaine Interactions with the Dopamine Transporter

Kinetic modeling of baseline and postcocaine data indicated that cocaine induced a large increase in the K_m in both male

and female striata and minor, insignificant increases in the release term ($[DA]_p$) (see Table 1). Cocaine significantly increased the K_m in both male and female rats ($F(1,30) = 36.5$, $p < 0.001$) and no sex difference existed for K_m after cocaine ($p = 0.76$). Cocaine did not change $[DA]_p$ significantly ($p = 0.13$). $[DA]_p$ values were significantly larger in females than in males ($F(1,30) = 13.7$, $p = 0.001$). V_{max} values were also greater in the female striatum overall ($F(1,30) = 17.2$, $p < 0.001$) and cocaine did not alter the maximal uptake rate ($p = 0.44$). There were no interactions between sex and drug treatment on any of the three kinetic parameters indicating a similar mechanism of action for cocaine in both sexes.

Possible sex differences in interactions of cocaine with dopamine transporters were investigated *in vitro*. Figure 7 shows that cocaine was equally potent for inhibition of dopamine uptake in striatal synaptosomes from both males and females ($N = 4$). The average IC_{50}s determined from one-site, sigmoidal regression analysis in individual experiments in male and female synaptosomes were virtually identical, 359 ± 37 and 358 ± 32 nM, respectively. These data are expressed as percent of maximal uptake in each sex because uptake tended to be higher in synaptosomes from female than male rats (19.8 ± 7.2 and 10.9 ± 3.5 pmol/mg protein/min, respectively, $p = 0.18$). Thus, the sex differences observed *in vivo* did not result from sex differences in molecular interactions of cocaine with DAT.

Table 1 *In Vivo* Dopamine Release and Uptake Kinetics

	Baseline		Cocaine (40 mg/kg)	
Parameter	**Male**	**Female**	**Male**	**Female**
$[DA]_p$ (nM)*	60 ± 8	99 ± 13	72 ± 7	125 ± 19
V_{max} (μM/s)*	2.07 ± 0.27	3.99 ± 0.49	1.76 ± 0.19	3.60 ± 0.74
K_m (μM)#	0.2 ± 0.02	0.2 ± 0.02	1.03 ± 0.13	1.12 ± 0.29

Values represent the mean ± SEM for eight rats per group.
*Significant overall effect of sex ($p < 0.005$).
#Significant overall cocaine effect ($p < 0.001$).

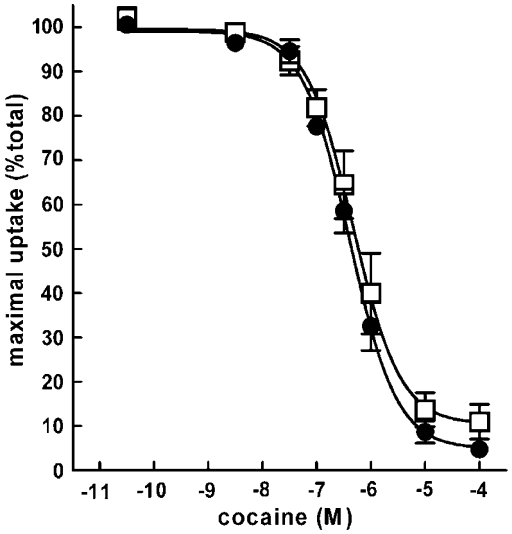

Figure 7 Cocaine inhibition of dopamine uptake in rat striatal homogenates. P2 synaptosomal fractions were prepared from freshly dissected male and female striata ($N = 4$) and exposed to 50 nM ^{3}H-dopamine for four min in the presence of different concentrations of cocaine. The maximal uptake rates for female and male rats in the absence of any inhibitor were determined (19.8 ± 7.2 and 11.0 ± 3.6 pmol dopamine/mg protein/min, respectively) and cocaine inhibition was then expressed as a percentage of the maximal uptake. The IC_{50}'s were 358 ± 32 and 359 ± 37 nM in female and male striatum, respectively.

DISCUSSION

The present results suggest that pharmacologic disruption of either DAT or the dopamine autoreceptor causes exaggerated changes in regulation of extracellular dopamine in the female striatum compared to that in males. While cocaine increased dopamine overflow three-to–five fold in the striatum of anesthetized male and female rats, it induced significantly more dopamine efflux in females than in males, despite equivalent levels following 30 pulse baseline stimulations. Antagonism of the D_2 autoreceptor by haloperidol induced more dopamine overflow in female than male rats. Evoked dopamine was also increased only in the female striatum at early time points following administration of the D_2 agonist quinpirole. These differences in dopaminergic function may partly explain sex differences in cocaine-stimulated locomotion and other behaviors.

Sex differences in cocaine-induced dopamine overflow likely reflect the same processes that mediate previously observed sex differences in basal dopamine overflow following 60 Hz but not 20 Hz stimulations (Walker *et al*, 2000). Rates of uptake and release are significantly greater in the female striatum, but the net of these competing processes in both sexes produces equivalent extracellular dopamine concentrations at 20 Hz stimulations. As less time is available for uptake to occur in between stimulus pulses at high-stimulation frequency, extracellular dopamine saturates uptake and rise rapidly (Wightman and Zimmerman, 1990). The greater female release rate overwhelms its own rapid uptake rate faster than the analogous competition in the male with slower rates for both processes. We hypothesize that the sex difference in extracellular dopamine following cocaine occurred mainly at the upper range of frequencies because uptake inhibition from cocaine accentuated the normal propensity of the female striatum to produce higher concentrations of extracellular dopamine than the male striatum when stimulated at high frequency. The synaptosomal uptake data confirmed the *in vivo* modeling results showing that cocaine did not have any preferential effect at the dopamine transporter in female tissue.

The greater increase in dopamine after cocaine in females is consistent with the theoretical simulations of Wu *et al* (2001a) which show that cocaine should induce greatest percent increases in dopamine efflux in regions where release rates are lower and uptake rates are higher. Analysis of present baseline data from the cocaine-treated rats indicated that V_{max} was 93% higher in females than in males, and $[DA]_p$ was 65% higher in females. Although the greater release rate in the female striatum could serve to attenuate the greater percent increase in the female striatum induced by cocaine, the disparity in V_{max} favors the greater

increase in the female striatum. These data suggest that V_{max} more tightly regulates extracellular dopamine in female striatum and inhibition of the more tightly regulated system leads to greater disruption, that is, more extracellular dopamine following cocaine administration. The sex differences are statistically significant only at high firing frequencies, which exhibit linear increases in extracellular dopamine caused by release overwhelming uptake (Wightman and Zimmerman, 1990). An important caveat is that these frequencies are substantially higher than those seen physiologically in neurons identified unequivocally as dopaminergic (Bunney *et al*, 1973; Grace and Bunney, 1983).

A number of reports suggest that certain aspects of cocaine taking and addiction are more severe in women (Griffin *et al*, 1989; Kosten *et al*, 1993; Mendelson *et al*, 1991). Human data show that cocaine cues induced craving more in women than in men (Robbins *et al*, 1999) and cocaine craving overall is generally greater in women (Elman *et al*, 2001). Dopamine neurons fire more rapidly (burst) when they are activated by stimuli with reward salience (Schultz, 2001). An interesting speculation is that the present data showing that cocaine induces sex differences in extracellular dopamine at high frequencies may be related to human sex differences in cocaine craving and addiction.

The present results showing robust increases in electrically stimulated dopamine levels after haloperidol that were greater in female than in male rats confirm previous studies of enhanced dopamine overflow after D2 antagonist treatment (Wiedemann *et al* (1992). The effect of haloperidol showed a significant sex difference at 20 Hz, in contrast to the effect of cocaine that was apparent at the high frequency range. Haloperidol exerted similar effects in medial and lateral striatum suggesting that the reported medial–lateral gradient of D_2 receptors in the rat striatum (Falardeau and Di Paolo, 1987; Joyce *et al*, 1985) is not due to presynaptic autoreceptors that represent only a small fraction of all striatal D_2 receptors.

Haloperidol increases electrically stimulated dopamine signals by blocking presynaptic autoreceptors that inhibit dopamine release and stimulate uptake (Meiergerd *et al*, 1993; Wolf and Roth, 1987, 1990; Starke *et al*, 1989; Cass and Gerhardt, 1994; Wu *et al*, 2002). These mechanisms play a primary role in the increased basal extracellular dopamine levels that result from D_2 antagonist administration (Imperato and Di Chiara, 1985). The present results are consistent with previous findings that female rats are more sensitive to haloperidol-induced catalepsy (Campbell *et al*, 1988) and have a higher striatal D_2 receptor density (Bosse and DiPaolo, 1996; Bazzett and Becker, 1994). Further, they suggest that females may also possess more presynaptic D_2 receptors.

The present data showing that haloperidol increases evoked dopamine release more in female rats may be relevant to observations in humans treated with antipsychotics. Women schizophrenics require lower doses of antipsychotics and respond more quickly and better than men (Seeman, 2002). Tardive dyskinesia is also more common and more severe in women than men treated with neuroleptics (Seeman, 1985; Yassa and Jeste, 1992). We postulate that enhanced dopamine release by equivalent doses could produce more depolarization blockade in women leading to both greater therapeutic benefit and a greater risk of side effects (Grace and Bunney, 1986; Boye and Rompre, 2000). Nondopaminergic mechanisms could also contribute to these differences. Sex and estrous cycle-related differences in tissue neurotensin concentrations in rats have been reported (Kinkead *et al*, 2000) and could also be involved in sex differences in severity of schizophrenic pathology (Garver *et al*, 1991).

The sex- and frequency-specific effects on evoked dopamine concentrations after quinpirole are also consistent with enhanced autoreceptor function in females. Quinpirole increased extracellular dopamine concentrations transiently in the female striatum following 60 Hz stimulations. A possible explanation for this increase in dopamine efflux may be a decrease in cell firing due to somatic autoreceptor stimulation resulting in enhanced accumulation of vesicular, releasable dopamine. The sex difference is consistent with the report that low doses of quinpirole (0.03 and 0.1 mg/kg) depress locomotor activity in males but not in females (Schindler and Cannona, 2002). This modest effect seen only in females could reflect tighter uptake regulation by autoreceptors. Chiodo and Caggiula (1983) have similarly shown that estradiol increases the sensitivity of nigral D_2 autoreceptors on Type A and B neurons that change the firing frequency of these neurons.

In summary, this work shows that pharmacologic antagonism of both DAT and the autoreceptor produces exaggerated responses in extracellular dopamine regulation in the striatum of female rats. These responses could be related through autoreceptor control of DAT activity. Stimulation of D_2 autoreceptors increases dopamine uptake (Meiergerd *et al*, 1993; Cass and Gerhardt, 1994; Wu *et al*, 2002). D_2 receptor knockout mice have only half the dopamine clearance rate of wild-type mice although DAT expression is identical (Dickinson *et al*, 1999). Greater autoreceptor stimulation of uptake in females could produce the greater V_{max} in the female striatum observed in our prior report (Walker *et al*, 2000) and the current study. Thus, sex differences in the regulation of dopamine neurotransmission by the dopamine autoreceptor and DAT may contribute to sex differences in addiction and neurologic diseases.

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