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Effects of dopamine agonists and antagonists on locomotor activity in male and female rats

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Abstract

Male and female Sprague–Dawley rats were treated with cocaine, the specific dopamine uptake inhibitor GBR 12909, the dopamine D1 agonist SKF 82958 or the dopamine D2 agonist quinpirole. After treatment, the rats were placed in an activity chamber for 30 min and locomotor activity was monitored. Cocaine, GBR 12909 and SKF 82958 all increased locomotor activity in both males and females, but greater increases were observed in females. In contrast, quinpirole produced decreases in activity, with males showing greater decreases than females. Separate groups of animals were given SCH 23390 or eticlopride prior to cocaine. The D1 antagonist SCH 23390 reduced the locomotor activating effects of cocaine in both males and females, with females showing greater sensitivity to SCH 23390. The D2 antagonist eticlopride also reduced the locomotor activating effects of cocaine, with no clear differences between males and females. These results suggest that the differences between males and females in their locomotor response to cocaine can be at least partially attributed to differences in the function of dopamine D1 and D2 receptors.

Keywords: Cocaine; Dopamine; Activity; Sex; Rat

1. Introduction

Cocaine has been shown to produce differential effects on behavior depending on the sex of the subject. In particular, in rodents, females are more sensitive to the locomotor activating effects of cocaine. This is true both following acute administration (Quinones-Jenab et al., 1999; Sircar and Kim, 1999) and chronic administration. Following chronic cocaine administration, female rats are more likely to show sensitization of activity (Cailhol and Mormede, 1999; Sircar and Kim, 1999). Similarly, female rats are also more sensitive to the reinforcing effects of cocaine as reflected by both the acquisition of self-administration (Lynch and Carroll, 1999) and the reinstatement of extinguished self-administration (Lynch and Carroll, 2000). However, females are not more sensitive to all of the behavioral effects of cocaine. Male and female rats appear to be equally sensitive to the discriminative stimulus effects of cocaine (Craft and Stratmann, 1996; Anderson and van Haaren, 1999).

The mechanism for the observed gender differences does not appear to be related to difference in the metabolism of cocaine. The half-life of cocaine in plasma and brain is similar in males and females (Bowman et al., 1999), although there are differences in the patterns of metabolism. Female rats produce higher levels of ecgonine methyl ester following cocaine, while male rats produce higher levels of benzoylecgonine (Bowman et al., 1999). It is not clear whether active metabolites, like norcocaine, are produced differently in male and female rats.

While metabolism might not account for the differing effects of cocaine in male and female rats, dopamine receptor function could explain these differences. In females, estrogen acts to inhibit GABA neurons in the striatum and accumbens, which in turn increases dopamine function (Becker, 1999). In addition, estrogen acts to enhance dopamine release by down-regulating dopamine D2 receptor function (Becker, 1999). Males also have a higher density of D1 receptors in the nucleus accumbens than female rats (Andersen et al., 1997). These results suggest that differences in dopamine receptor function could contribute to differences in the behavioral effects of cocaine in male and female rats.

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The purpose of the present study was to further investigate the role of dopamine in the differences in cocaine's effects on locomotor activity in male and female rats. Male and female rats were treated with cocaine, the specific dopamine uptake inhibitor GBR 12909, the D1 agonist SKF 82958 and the dopamine D2 agonist quinpirole. If the effect of cocaine on locomotor activity were related solely to uptake, then a difference between the sexes would be expected for GBR 12909 but not for the two agonists. If the effect were mediated directly at a receptor, then we would expect to see differences between the sexes with the direct agonists. To further investigate the role of the D1 and D2 receptors, other groups of rats were treated with the D1 antagonist SCH 23390 or the D2 antagonist eticlopride prior to cocaine administration.

2. Materials and methods

2.1. Subjects

Male and female Sprague–Dawley rats weighing approximately 275 g at the start of the experiment were housed in groups of three with fresh drinking water and food available ad lib. They were maintained on a 12:12-h light/dark cycle, with lights on at 7:00 am. All animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). All procedures were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the NIDA/IRP and the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.2. Apparatus and procedure

Six identical locomotor activity monitors (Columbus Instruments, Columbus, OH) were enclosed in three sound attenuation chambers (BRS/LVE, Laurel, MD). A smaller 40×40-cm Plexiglas chamber was situated inside each locomotor activity monitor. Each monitor consisted of a 15×15 infrared photocell array. The monitors were interfaced to a computer that tabulated distanced traveled (in centimeters) over 30-min intervals. All rats were placed in the activity chamber for 30-min/day, 5 days/week. Over the first 7–10 days, the rats were treated with saline just prior to being placed in the chamber. In general, responses to saline stabilized within the first 2–5 days of habituation. From that point on, activity following saline administration was rarely outside of 1000 counts from the saline mean. Following this habituation phase, the rats were given various drug treatments each separated by at least 2 days of saline treatment. For one group of male and one group of female rats, these treatments consisted of various doses of cocaine, GBR 12909, SKF 82958 and quinpirole ($n = 10–12$/sex). For another group of male and female rates, these treatments consisted of cocaine, SCH 23390 or the combination of SCH 23390 and cocaine ($n = 10–12$/sex). An additional group of male and female rats were treated with cocaine, eticlopride or the combination of eticlopride and cocaine ($n = 10–12$/sex). For these three drug treatment regimens, drugs and doses were given in a semirandom order, although all animals in any group received the drugs and doses in the same order and the comparable male and female groups received the drugs and doses in the same order. Therefore, if there were order effects, these should have been similar across male and female rats. Finally, one group of male and one group of female rats were given repeated treatments with cocaine to determine if the repeated treatment procedure itself produced shifts in the dose–effect functions for cocaine ($n = 10$/sex). Here, a complete dose–effect function was completed before the next was started, although within a dose–effect determination, the order of doses was semirandom. All drugs were administered intraperitoneally.

2.3. Drugs

Cocaine hydrochloride (NIDA, Baltimore) was dissolved in sterile saline. GGR 12909 dihydrochloride, (±)-quinpirole dihydrochloride, SCH 23390 hydrochloride and (−)-eticlopride hydrochloride (RBI, Natick, MA) were dissolved in sterile water. All drugs were administered in a volume of 1 ml/kg and doses are given as the salt. All drugs were administered just prior to placement in the activity chamber, except SCH 23390 and eticlopride that were given as a 5-min pretreatment to cocaine or saline.

2.4. Data analysis

Distance traveled was measured in centimeters and summed over the entire 30-min testing period. The session before drug administration was used as the saline control, and for any one drug, these sessions were averaged across doses. All data were subjected to multifactor ANOVA with follow-up tests to determine individual effects using the method of Fisher (Wilkinson, 1992).

3. Results

Fig. 1a shows the effects of cocaine in male and females rats on distance traveled. Each drug was tested in 10–12 rats. As expected, females were clearly more sensitive to the locomotor activating effects of cocaine [Gender × Dose ANOVA showed significant effects of gender $F(1,88) = 49.6$, $P < .001$, dose $F(3,88) = 78.2$, $P < .001$ and Gender × Dose $F(3,88) = 17.2$, $P < .001$]. At the two higher doses, distance traveled was significantly higher than for animals treated with saline for both sexes. However, activity was significantly higher for females at both of these doses. When treated with saline, however, there were no differences between males and females.

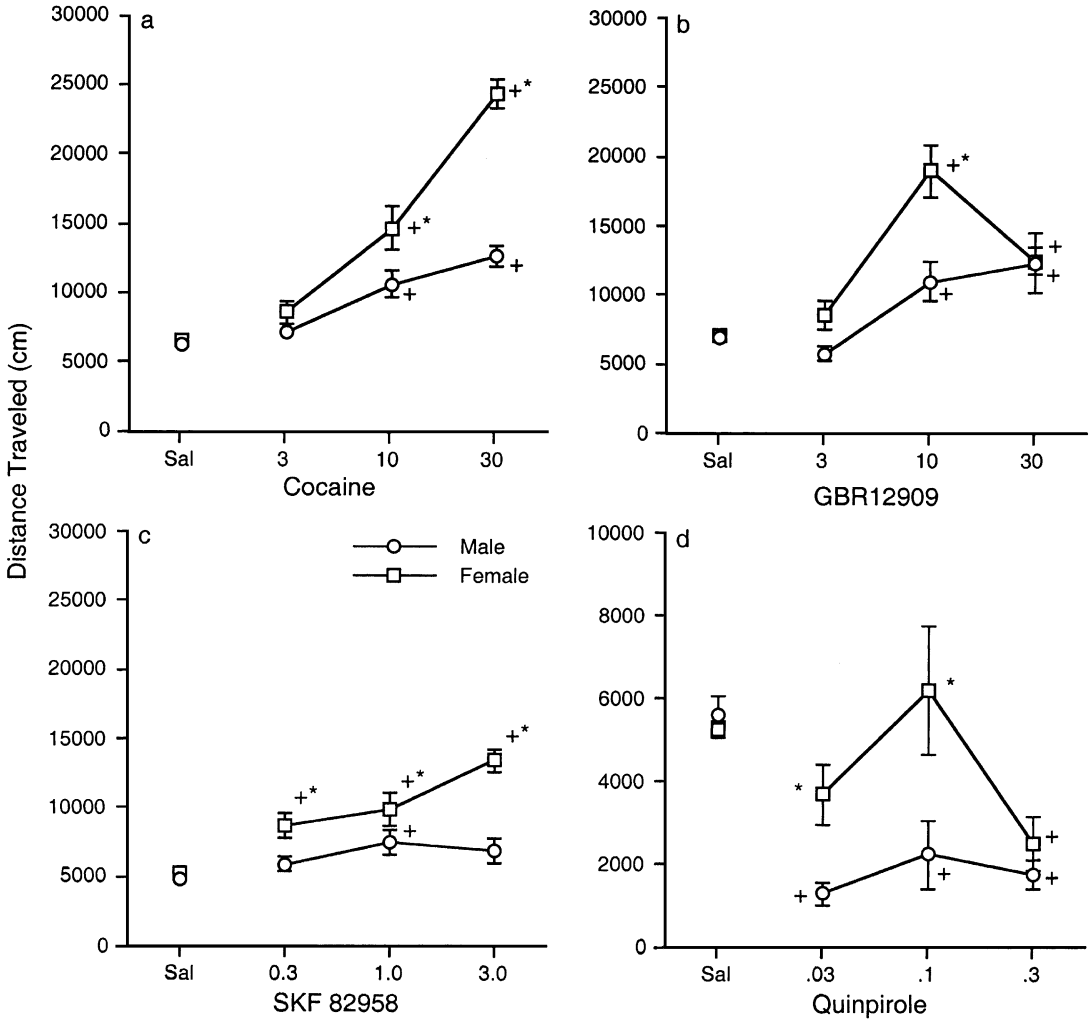

Fig. 1. Effects of cocaine, GBR 12909, SKF 82958 and quinpirole on locomotor activity as measured by distance traveled (cm) over 30-min activity sessions. All drugs were given just prior to the sessions and doses are in mg/kg. Drug treatments were at least 3 days apart and at least one session in the activity monitor following a saline injection. For each drug, male and female rats were compared. The "+" symbol indicates a significant ($P<.05$) difference from saline for that gender, while "*" represents a significant difference ($P<.05$) from males at the comparable dose. Each point is the mean ± S.E.M. of 10–12 rats. If no error bars are shown, they fall within the range covered by the point.

Also shown in Fig. 1b are the effects of the specific dopamine uptake inhibitor GBR 12909. Again, there were no differences between males and females during saline sessions. Like cocaine, GBR 12909 also increased distance traveled and females were significantly more activated at the intermediate dose than were males [Gender × Dose ANOVA showed significant effect of gender $F(1,86)=9.2$, $P<.01$, dose $F(3,86)=19.6$, $P<.001$ and Gender × Dose $F(3,86)=4.3$, $P<.01$]. At the highest dose tested, however, the effects of GBR 12909 in females were similar to that of the males.

The effects of the direct dopamine agonists are also shown in Fig. 1. The D1 agonist SKF 82958 (Fig. 1c) increased distanced traveled over saline levels for both male and female rats, although this effect was clearly less than that of either cocaine or GBR 12909. In fact, the effect in males was only significant at the 1.0 mg/kg dose. The activating effect in females was significant at every dose and was significantly above the males for every dose [Gender × Dose ANOVA showed significant effects of gender $F(1,88)=28.2$, $P<.001$, dose $F(3,88)=14.8$, $P<.001$ and Gender × Dose $F(3,88)=5.1$, $P<.01$]. Finally, Fig. 1d shows the effect of the D2 agonist quinpirole. Unlike the other drugs, quinpirole tended to decrease distance traveled and this effect was more prominent in the male rats [Gender × Dose ANOVA showed significant effects of gender $F(1,80)=10.2$, $P<.01$, dose $F(3, 80)=8.4$, $P<.001$ and Gender × Dose $F(3,80)=3.2$, $P<.05$]. For males, every dose was significantly below the saline level, while for females, the effect of quinpirole was significant only at the highest dose. Distance traveled for males was lower than that of females at the two lower doses.

Fig. 2 shows the effects of pretreatment drugs. Each drug was tested in 10–12 rats. Fig. 2a and b show the effect of

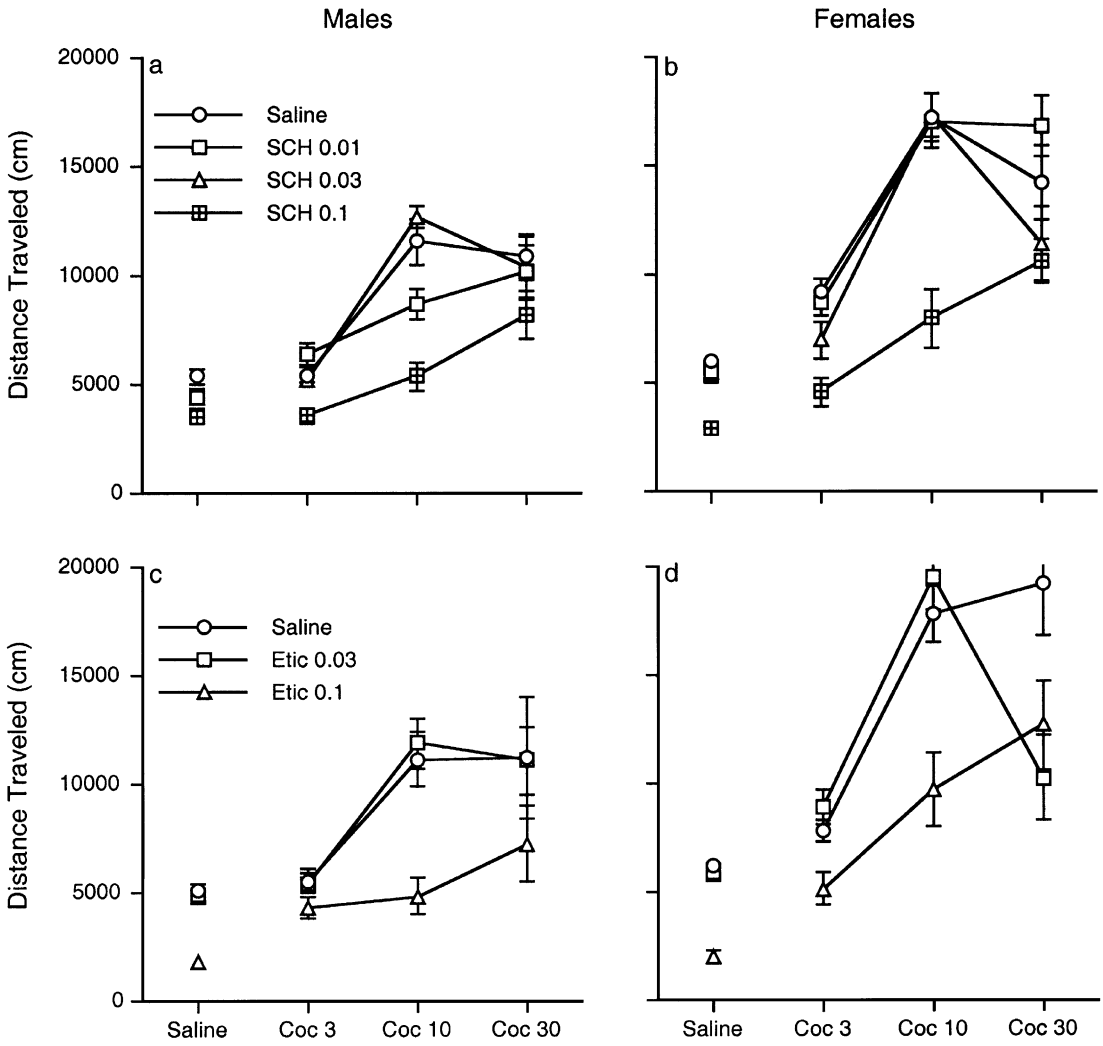

Fig. 2. Effect of pretreatment with various doses of SCH 23390 (top panels) or eticlopride (bottom panels) on locomotor activity following cocaine in male and female rats. The pretreatments were given 5 min prior to cocaine, which was given just prior to being placed in the activity monitor for a period of 30 min. Drug treatments were at least 3 days apart with at least one session in the activity monitor following a saline injection before each drug treatment. Each point is the mean ± S.E.M. of 10–12 rats. If no error bars are shown, they fall within the range covered by the point. For SCH 23390, follow-up tests on the significant Gender × Pretreatment interaction revealed that 0.1 mg/kg SCH 23390 significantly antagonized the effects of cocaine for both males and females, while 0.03 mg/kg SCH 23390 antagonized the effects of cocaine in females only. No effects involving gender were significant for eticlopride.

pretreatment with the dopamine D1 antagonist SCH 23390 and the effect of cocaine in both male and female rats, respectively. Cocaine again increased distance traveled in the female rats more than the males (compare Fig. 2a and b saline pretreatment curves [Gender × Cocaine dose × Pre-Pretreatment dose ANOVA revealed a significant effect gender $F(1,330) = 79.0$, $P < .001$]. For both males and females, the effect of cocaine was at least partially reversed by the 0.1 mg/kg dose of SCH 23390. However, the females appeared to be more sensitive to SCH 23390, as the 0.03 mg/kg dose was also significant [Gender × Pretreat-Pretreatment dose $F(3,330) = 4.8$, $P < .01$]. This effect most likely resulted from the slight reduction in distance traveled at both the 3 and 30 mg/kg cocaine doses for the 0.03 SCH 23390 pretreatment condition. While the 0.1 mg/kg dose of SCH 23390 antagonized the effect of cocaine, this dose also significantly reduced distance traveled when given prior to saline administration [Pretreatment dose × Cocaine dose $F(9,330) = 5.2$ $P < .001$].

The dopamine D2 antagonist eticlopride (Fig. 2c and d) also antagonized the effect of cocaine at the highest dose tested [Gender × Cocaine dose × Pretreatment dose ANOVA showed significant effect of pretreatment dose $F(2,256) = 28.2$, $P < .01$]. However, in contrast to SCH 23390, none of the effects involving pretreatment or gender was significant. Like with SCH 23390, the 0.1 mg/kg dose of eticlopride also reduced distance traveled when given prior to saline [Pretreatment dose × Cocaine dose $F(6,256) = 4.5$, $P < .001$].

Fig. 3a shows the effect of repeated determinations of a cocaine dose–effect function in male rats. Ten rats received

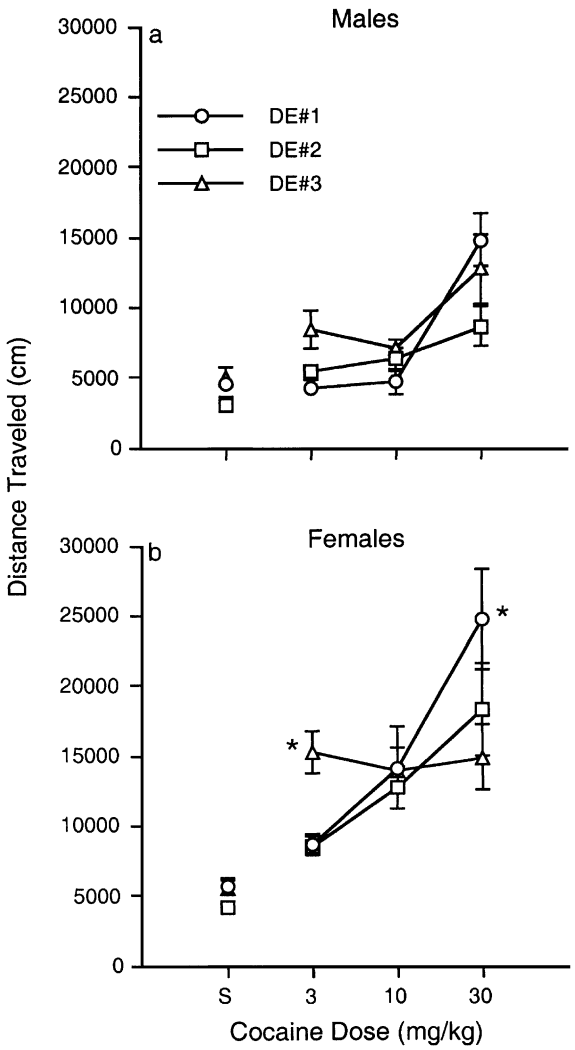

Fig. 3. Repeated determinations of the cocaine dose–effect function in male and female rats. Cocaine was given just prior to placement in the activity monitor for 30 min. Each cocaine dose was separated by at least 3 days and at least one session in the activity monitor following a saline injection. Each point is the mean ± S.E.M. of 10 rats. If no error bars are shown, they fall within the range covered by the point. For the males, there were slight differences between determinations as reflected by a significant main effect of determination number, with Determination 2 being different from Determination 3. Determinations 1 and 3 did not differ. For females, the main effect of determination number was not significant, but there was a significant Determination number × Cocaine dose interaction (* $P < .01$ from the other determinations).

all three dose–effect determinations. Cocaine clearly increased distance traveled [Cocaine dose × Determination number ANOVA showed a significant effect of cocaine dose $F(3,101) = 25.2$, $P < .001$]. There was a slight difference between determinations as reflected in a significant effect of dose determination number [$F(2,101) = 4.3$, $P < .05$], with Determination 2 differing significantly from Determination 3. Determinations 1 and 3 did not differ. For females (Fig. 3b), cocaine again dose-dependently increased distance traveled [Cocaine dose × Determination number ANOVA showed a significant effect of cocaine dose $F(3,108) = 26.4$, $P < .001$]. Unlike males, the dose determination number effect was not significant, although the Dose determination number × Cocaine dose interaction was significant [$F(6,108) = 3.0$, $P < .01$]. At the 3 mg/kg cocaine dose, Determination 3 was different from Determinations 1 and 2. At the 10 mg/kg dose, none of the determinations differed. At the 30 mg/kg dose, Determination 1 was different from Determinations 2 and 3, while Determinations 2 and 3 were not different. Following saline, the amount of activity did not change over the course of the study for either sex (points above "S").

4. Discussion

The results showed clearly that female rats were more sensitive to the locomotor activating effects of cocaine than were male rats, particularly at the higher doses of cocaine. This result is in complete agreement with previous research (Quinones-Jenab et al., 1999; Sircar and Kim, 1999). The procedure used in the current experiment was different from that used in most previous activity experiments (but see Craft and Stratmann, 1996), in that cocaine was repeatedly tested in the same rats. However, the procedure of repeatedly testing cocaine in the same rats would be comparable to that used in studies of operant self-administration (Lynch and Carroll, 1999) or drug discrimination (Craft and Stratmann, 1996). Certain aspects of the procedure should minimize potential order effects. Animals were well adapted to the activity cages and the continued presentation of non-drug sessions was designed to minimize changes in baseline behavior. Baseline behavior following saline administration was comparable between male and female rates and remained stable throughout the experiment. Drug administration was also separated by at least 3 days to minimize the effects of tolerance or sensitization.

To determine if this particular procedure may have influenced the effects of drugs, in separate groups of rats, the cocaine dose–effect function was determined three consecutive times. For males, the only slight difference was seen between the second and third determinations, while the first and third determinations did not differ. Thus, there would not appear to be any consistent change in the effect of cocaine for males over the course of the experiment. For females, there were no significant differences between determinations, although there were some differences at individual doses. In particular, the dose–effect function appeared flatter for the third determination. However, not every dose showed a significant change for the final determination, and even for doses where effects were observed, they were not consistently different from the other two determinations. None of these differences would have produced any greater differences between males and females as may have been expected if sensitization were more likely in female rats (Cailhol and Mormede, 1999;

Sircar and Kim, 1999). Therefore, the procedure itself would not appear to be a factor in observing the male–female differences in the response to cocaine or any of the other drugs.

Another potential confounding factor is the stage of the estrus cycle during which drug was administered for the females. In general, drug was administered every 3–4 days. With a typical estrus cycle length for the rat being 4 days, the 3-day interval would insure that drug was administered in different phases of the estrus cycle for any individual rat. Thus, while we cannot rule out an effect specific to a particular phase of the estrus cycle, any effect of the estrus cycle should have been comparable across drug and doses, as test doses should have been presented in each phase of the estrus cycle for at least some of the rats in each group.

In addition to cocaine, locomotor activity in males and females differed for the other drugs tested as well. Females were more sensitive to the locomotor activating effect of GBR 12909 than were males. This is not surprising as both cocaine and GBR 12909 are potent dopamine reuptake blockers. Whether dopamine uptake alone was responsible for the differences between male and female rats was investigated by looking at the effects of dopamine D1 and D2 agonists. If differences between males and females in dopamine uptake alone were responsible for the observed effects, then the response to the D1 and D2 agonist may be similar. However, females were more sensitive to the locomotor activating effects of the D1 agonist SKF 82958, while males were more sensitive to the locomotor depressant effect of quinpirole. Both of these effects would lead to females showing more locomotor activation after cocaine than males. This result supports previous findings that male and females differ in a number of ways related to the dopamine system in brain (Andersen et al., 1997; Becker, 1999).

While the effects of GBR 12909, SKF 82958 and quinpirole have apparently not been compared across males and females before, the effects of these drugs on locomotor activity have been determined in male rodents. In general, the results of those studies agree with the results in the present study. For example, GBR 12909 has been shown to increase locomotor activity in rodents (Kelley and Lang, 1989; Hooks et al., 1994), as was shown here. Dopamine D1 agonists have been shown to increase activity, while dopamine D2 agonists decrease activity (Geter-Douglass et al., 1997). In the present study, the D1 agonist SKF 82958 increased activity in both males and females, and previous studies have also shown that this compound increases locomotor activity in rodents (Meyer and Shults, 1993; Geter-Douglass et al., 1997). The dopamine D2 agonist quinpirole decreased activity for both sexes in the current study. In previous studies, the effects of quinpirole in rodents have been shown to depend on dose and time after injection. In general, at lower doses, only decreases in activity are observed (Eilam and Szechtman, 1989; Eilam et al., 1992; Geter-Douglass et al., 1997). However, at higher doses, increases in activity can be seen 60–120 min after injection (Eilam et al., 1992; Geter-Douglass et al., 1997). The doses in the current study were in the low range and activity was measured for 30 min following injection. Therefore, the effects of quinpirole are also consistent with the effects observed previously.

The results of the antagonism experiments also support a role for dopamine receptors in the observed gender difference. Both the D1 antagonist SCH 23390 and the D2 antagonist eticlopride were able to antagonize the effects of cocaine in both male and female rats. That is, clear antagonism of the locomotor activating effect of cocaine was seen at the 0.1 mg/kg dose for SCH 23390 and eticlopride in both males and females. At the 0.03 mg/kg SCH 23390 dose, significant antagonism of the effect of cocaine was also seen, but only in female rats. Thus, females again appear to show increased sensitivity in D1 receptor function. The degree of antagonism may also have been different between the genders. In fact, this appears to be the case with female showing greater reductions in cocaine-induced activity with SCH 23390. However, females also have higher levels of activity following cocaine, making conclusions about degree of antagonism difficult. It is interesting to note that antagonism was primarily seen at antagonist doses that also significantly decreased activity on their own. Here, females did show greater decreases following 0.1 mg/kg SCH 23390 than males, even with similar baselines for comparison.

In conclusion, females were clearly more sensitive to the locomotor activating effects of cocaine than were males. Females were also more sensitive to the locomotor activating effects of the specific dopamine uptake inhibitor GBR 12909 and the D1 agonist SKF 92958. These results suggest that the difference in the locomotor activating effect of cocaine between male and female rats is at least partially medicated by dopamine D1 receptor function. The role of D2 receptor function in the effect is supported by the fact the males were more sensitive to the locomotor depressant effect of the D2 agonist quinpirole. This depressant effect in males may work to lower the overall activity of males following cocaine.

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