Sex Differences in the Motivational Contrast between Sucrose and Cocaine in Rats

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Abstract
There are sex differences in the vulnerability to cocaine abuse and addiction. Understanding the differences is critical for developing the sex-tailored prevention and treatment strategies. Cocaine addiction is characterized by the pathological motivation for cocaine accompanied by the diminished motivation for natural rewards. Thus, the motivational impact of cocaine relative to natural rewards likely determines the attractiveness of cocaine and likely plays a role in the vulnerability to cocaine abuse and addiction. This study aimed to determine whether the relative magnitudes or contrast of the motivational impact between cocaine and sucrose is different between sexes. To this end, cocaine-naïve out bred Wistar rats were trained to self-administer sucrose pellets and the motivation for different amounts of sucrose was then determined as the breakpoints under the progressive-ratio schedule of reinforcement. Following the sucrose tests, the same rats were trained to self-administer cocaine and the motivation for different doses of cocaine was similarly measured. For the female rats, the motivation was also measured during the diestrus and proestrus/estrus, respectively, to determine the impact of the estrous cycle on the motivational effects of cocaine and sucrose. The differences between the breakpoints of cocaine and sucrose were significantly larger in the males. The enhanced motivational contrast may contribute to the increased vulnerability to recreational cocaine abuse and addiction in the males.

ABBREVIATIONS
SA: Self-Administration; PR: Progressive-Ratio; BP: Breakpoint

INTRODUCTION
There are sex differences in the vulnerability to cocaine abuse and addiction [1-4]. For example, the rates of cocaine use and addiction are higher in men than women [5,6] although women appear to have a faster transition from recreational use to addiction [1,3,4,7,8]. Understanding the mechanisms underlying these differences will provide important insights into sex differences in the vulnerability to cocaine addiction and pave the way for developing the sex-tailored prevention and treatment strategies.

The euphoric effects of cocaine play an important role in initiation and maintenance of recreational use [9]. However, transition to addiction is not driven by intensification of these effects. In fact, addicts typically report less intense euphoric effects [10-13] but a much higher level of motivation for cocaine compared with recreational users [14]. Notably, the pathologically enhanced motivation for cocaine accompanied by simultaneously impaired motivation for natural rewards is a key feature of cocaine addiction [15,16]. Thus, the relative magnitudes of the motivational effects between cocaine and natural rewards in recreational users likely play an important role in the development of cocaine abuse and addiction. Sex differences in such a motivational contrast may contribute to sex differences in the vulnerability. Preclinical studies have demonstrated sex differences in the motivational effects of cocaine [17-21]. However, sex differences in the motivational contrast between cocaine and natural rewards have not been examined.

MATERIALS AND METHODS
Subjects and drugs
Male (n=41, 300-390 g) and female (n=32, 240-320 g) outbred Wistar rats (Charles River) were housed individually in plastic home cages in a temperature- and humidity-controlled colony room on a 12-h reverse light-dark cycle (lights off at 08:00). Experiments were conducted during the dark phase (between 09:00 and 18:00). All procedures followed the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.
and were approved by University of Tennessee Health Science Center Animal Care and Use Committee.

Cocaine hydrochloride (the National Institute on Drug Abuse, Bethesda, MD) was dissolved in physiological saline to prepare the solutions with concentrations of 2.5, and 5 mg/ml (salt), respectively.

**Sucrose self-administration (SA) training**

Before operant training, rats were placed on a restricted diet to reach ~85% of free feeding body weights and thereafter, fed daily with ~20 and ~15 g regular rat chow for the males and females, respectively. The rats were first trained to press a lever for a sucrose pellet (45 mg, Research Diet, New Brunswick, NJ) in a standard operant chamber (Med Associates Inc., St Albans, VT). They were trained to press the left and right lever on alternate days as described previously [22]. Once the rats learned to obtain the maximum reinforcements (100 and 60 for the males and females, respectively), the training with the chained schedule of reinforcement began. The reason for the different maximum reinforcements for the two sexes was based on the observation that the females often left some pellets untouched in the food tray when the maximum was set at 100. Under such a schedule, reward-seeking and reward-taking behaviors can be investigated separately [22-24]. The goal of this study was to determine the motivation related to cocain-seeking behavior. With this schedule, sequential responses on the two levers, designated as the seeking and taking levers, respectively, are required to earn a reward. Only one lever is available at one time. Pressing the seeking lever leads to lever retraction and access to the taking lever after a delay. Pressing the taking lever results in reward delivery and lever retraction followed by next trial after a timeout period. In this study, 2-second delay and 5-second timeout were used in daily 30-minute sessions [22]. The session ended either when 30 minutes had passed or when the maximum reinforcements were earned, whichever occurred first. Training continued until the rats reached the criteria: the rates of reinforcement varied < 10% for three consecutive sessions and a minimum of 10 training sessions.

**Measuring the motivation for sucrose**

The progressive-ratio (PR) schedule [17] was used to measure the breakpoints (BPs), defined as the last ratio at which the rats failed to acquire the reward. As described previously [22], the PR schedule was effective for the seeking lever and the schedule for the taking lever remained unchanged. The session ended if the rats had failed to respond within a half hour or 4-hour had passed, whichever occurred first. To determine whether the motivation depends on the amount of sucrose, the BPs for one and three pellets were measured in the same rats, respectively, and the order of the two tests was counterbalanced. To determine the impact of the estrous cycle on the motivational effects of sucrose, the BPs were measured during the proestrus/estrus and diestrus in a subpopulation of the females using a mixed-subject design with estrous cycle and sucrose amount as the between- and within-subject factors, respectively. The choice for the phases of the estrous cycle was based on the previous studies demonstrating an increase in the BPs for cocaine during the estrus compared with other phases [17,25,26]. The vaginal smears were collected half hour before the test sessions and the phases were assessed by the vaginal cytology [27]. The rats were allowed to go through two normal estrous cycles (4-5 days per cycle) before testing.

**Surgery**

A subpopulation of the rats (male = 30; female = 25) were catheterized under the anesthesia with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), intraperitoneally. The catheter was made of a ~12-cm polyurethane tubing (MRE-037; Braintree Scientific, Inc. MA, USA) connected to the rat vascular access button (VAB950S; Inspetech, PA, USA). The button was placed subcutaneously in the mid-scapular region and the tubing was tunneled under the skin and inserted into the right external jugular vein with a length of ~3.5 cm. Catheter patency was evaluated by injecting 0.1 ml Brevital (1%) through the catheters as necessary and loss of muscle tone within five seconds indicated a patent catheter.

**Cocaine SA training**

After 5- or 6-day recovery from surgery, the rats began to self-administer 0.125 mg of cocaine in a daily 2-hour sessions under the same chained schedule as described above except that the timeout period was 20 seconds and a 10-second compound stimulus consisting of the flashing cue lights and tone was presented after onset of cocaine infusions. The session ended when two hours passed or 80 infusions were self-administered, whichever occurred first. The training was conducted 6-7 days per week until they reached the criterion: the number of cocaine infusions varied by < 20% for three consecutive sessions and a minimum of 10 training sessions. After measuring the BPs for 0.125 mg (see below), the rats began to self-administer 0.25 mg of cocaine and were trained for another 10 sessions. Cocaine (0.05 ml of 2.5 mg/ml or 5 mg/ml) was infused over a period of 0.55 second.

**Measuring the motivation for cocaine**

Measuring the BPs occurred after the rats reached the training criteria. The PR schedule was the same as described above except that the timeout was 20 seconds and a compound stimulus was presented as in cocaine SA. The BPs during the proestrus/estrus and diestrus were determined with a mixed-subject design as described above.

**Statistics**

Two-way mixed analysis of variance (ANOVAs) were used to analyze the effects of sex or estrous cycle and reward size (sucrose amount or cocaine dose) with sex or estrous cycle as the between-subject factor and reward size as the within-subject factor, respectively. To analyze sex differences in the motivational contrast between sucrose and cocaine, the interaction between sex and reward type (sucrose vs cocaine) was analyzed with a two-way mixed ANOVA with the former and the latter as the between- and within-subject factors, respectively.
Because the sucrose amount or cocaine dose may not be equivalent between two sexes due to the differences in their body weights, it is difficult directly to compare the BPs for sucrose or cocaine between sexes. The interaction between sex and reward size, however, allows to determine whether the same magnitude of changes in the reward size evokes different magnitudes of the motivational changes between sexes. Such a difference would indicate the sensitivity or responsiveness of the motivational system to the reward. To further address this issue, we scaled the reward size with the body weight and computed the motivational sensitivity as ratio between the percentage changes in the BP to the percentage changes in the reward size. The nonparametric Mann-Whitney test was used to compare sex differences in the sensitivity because the sensitivity values failed to pass the normality test (the D’Agostino & Pearson omnibus normality test). The significance level was set at 0.05. All the statistical analyses were conducted with the GraphPad Prism version 6.07 for Windows (GraphPad Software, La Jolla California).

RESULTS AND DISCUSSION

Sex differences in the intrinsic response to natural rewards could play a role in the vulnerability to cocaine abuse and addiction. Thus, we investigated how the motivational responses to a natural reward, sucrose pellets in cocaine-naïve rats may differ between sexes. Because males and females may perceive the value of the same amount of sucrose pellet differently, sex differences in the BPs could be a value-dependent phenomenon rather than the functional differences in their motivational systems. Thus, we aimed to determine the motivational responsiveness or sensitivity by analyzing the interaction between sex and sucrose amount. Despite the significant main effects of sex ($F_{1,71} = 14.55$, $P < 0.001$) and value ($F_{1,71} = 21.83$, $P < 0.0001$), the interaction was not significant ($F_{1,71} = 1.46$, $P = 0.23$) as shown in Figure (1). To further tackle this issue, we calculated the motivational sensitivity after adjusting the sucrose amount with the body weight and determined whether a difference in the sensitivity exists between sexes. The Mann-Whitney test failed to reveal any significant difference between sexes ($P = 0.21$). Together, these data suggest no intrinsic sex differences in the motivational sensitivity to sucrose.

There is evidence that sex differences in the motivational response to cocaine exist. For example, female rats show significantly higher BPs at a unit dose of 0.6 mg [17] and such a difference cannot be fully explained by the difference in the body weight [20]. Consistent with these results, 0.2 mg/kg of cocaine produces significantly higher BPs in female rats selectively bred for either high or low intake of saccharin compared with male rats bred for high intake [18]. Note, however, that either no sex differences [25,29,30] or higher BP values in male rats [21] have also been reported. The reasons for these inconsistent results are unclear. The different doses used in these studies make it difficult to compare the results. The dose adjusted by body weight is often thought to be equivalent between sexes. There are, however, other sex differences in duding blood volume, body fat percentage, and metabolism [31,32] that also affect the cocaine concentrations at the action sites in the brain. Therefore, it is difficult to compare the motivational effects of different doses of cocaine between sexes even after the body weight is taken into consideration. The difficulty, however, does not prevent us from investigating the motivational responsiveness or sensitivity in response to cocaine. It can be argued that the sensitivity likely plays as much if not more a role as the magnitude of response in the vulnerability to cocaine abuse and addiction. As shown in Figure (2), despite the significant effects of sex (male = 21, female=20; $F_{1,39} = 36.09$, $P< 0.0001$), no significant interaction was found ($F_{1,39} = 1.63$, $P = 0.21$).
The Mann-Whitney test revealed no significant sex differences in the motivational sensitivity. Together, these data suggest that the males and females have similar motivational sensitivity to cocaine.

Cocaine addiction is not only characterized by the pathological motivation for cocaine but the diminished motivation for natural rewards [15,16]. The latter probably exacerbates cocaine-seeking and cocaine-taking behaviors. Thus, the enhanced contrast of the motivational effects between cocaine and natural rewards likely plays a functional role in the vulnerability to cocaine abuse and addiction. To this end, we determined the differences between the BPs for sucrose and cocaine obtained from the same rats. As shown in the top left panel of Figure (3), the differences between the BPs for one sucrose pellet and 0.125 mg of cocaine were significantly higher in the males than the females indicating that the motivational contrast was significantly greater in the males. To determine whether the reward size may affect the conclusion, we calculated the differences from the other three possible combinations of cocaine doses and sucrose amounts. In each case, the motivational contrast was significantly higher in the males (for 3-pellet and 0.25 mg, male = 20 and female = 19) supporting the original conclusion.

The menstrual cycle alters the intensity of the subjective effects of cocaine [33,34]. Preclinical studies also demonstrate that female rats have higher motivation for cocaine during the estrus than metestrus/diestrus [17,26], which are consistent with the current findings. It is, however, unclear whether the motivational sensitivity was also influenced by the cycle. To this end, two groups of female rats (proestrus/estrus = 11; diestrus = 9) were tested for their motivation for 0.125 and 0.25 mg/infusion, respectively. Consistent with previous results [17,26], the estrous cycle had significant effects on the BPs for cocaine ($F_{1, 18} = 5.59, P < 0.05$) but no significant interaction was found ($F_{1, 18} = 0.12, P = 0.73$) as shown in Figure (4). The Mann-Whitney test revealed no significant differences in the motivational sensitivity between the estrous phases.

To compare the effects of the estrous cycle between sucrose and cocaine, we also investigated how the motivational sensitivity to sucrose might be affected by the estrous cycle in two groups of female rats (proestrus/estrus = 8; diestrus = 9). As shown in Figure (5), despite a significant effect of value ($F_{1, 15} = 5.92, P < 0.05$), no significant interaction ($F_{1, 15} = 3.19, P = 0.09$) or cycle effect ($F_{1, 15} = 2.18, P = 0.16$) was found. The Mann-Whitney test revealed no significant differences in the motivational sensitivity between the estrous phases.

CONCLUSION

The prevalence rates of cocaine abuse and addiction are significantly higher in men than in women with the age of 12 and older [6]. The difference cannot be explained by drug availability or other environmental factors because the difference has been consistently reported over decades despite increased drug availability to women and significant social and cultural changes during the period [5,6]. These observations strongly support the biological role in sex differences in the vulnerability. Our results showed that the motivational contrast between cocaine and sucrose was amplified in the male rats suggesting that cocaine may be more motivationally salient to the males than females compared with other natural rewards. The enhanced saliency likely increases the chance to continue using the drug and subsequently, increases the vulnerability to cocaine addiction.
Figure 4 Impact of the estrous cycle on the motivational sensitivity to cocaine. Upper panel: Impact of estrous phase and cocaine dose on the BP values (mean ± SEM). Lower panel: boxplot of the motivational sensitivity to cocaine during the different estrous phases and Pro indicates proestrus.

Figure 5 Impact of the estrous cycle on the motivational sensitivity to sucrose. Upper panel: Impact of estrous phase and sucrose amount on the BP values (mean ± SEM). Lower panel: boxplot of the motivational sensitivity to sucrose during the different estrous phases.

ACKNOWLEDGEMENTS

The project described was supported by Grant Number DA034776 (WLS) from the National Institute on Drug Abuse and its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIDA or NIH. Drs. Datta and Martini equally contributed to the paper.

REFERENCES


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