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The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats

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Abstract. Although it has been demonstrated that many of the behavioral responses to psychomotor stimulants are gender dependent and hormonally sensitive, few studies have examined the possibility that the estrous cycle interacts with drug reinforcement in laboratory animals. The present experiment assessed the effect of the estrous cycle on two aspects of cocaine self-administration behavior: the breaking point on a progressive ratio (PR) schedule and the rate of cocaine intake on a fixed ratio one (FR1) schedule. On the PR schedule, the first lever response produced a drug infusion. Subsequent response requirements escalated with each injection until the behavior extinguished. Breaking points were defined as the final ratio completed. On a FR1 schedule, the estrous cycle had no effect on the rate of drug intake. On a PR schedule, female rats reached higher breaking points during estrus than during other stages of the estrous cycle. Furthermore, female rats displayed higher breaking points than male rats. It appears that the estrous cycle influences an animal's motivation to self-administer cocaine.

Key words: Gender – Estrous cycle – Cocaine self-administration – Fixed ratio one – Progressive ratio schedule

Male and female animals appear to respond differently to psychomotor stimulants. In general, females tend to display more intense behavioral responses to psychostimulants than do males. Amphetamine (AMPH) administration induces greater rotational behavior (Brass and Glick 1981; Robinson et al. 1982a; Camp et al. 1986), elicits more intense and extended periods of stereotypy (Beatty and Holzer 1978; Savageau and Beatty 1981), and increases locomotor activity to a larger extent (Schneider and Norton 1979; Savageau and Beatty 1981) in female rats than in their male counterparts. Female rodents also respond to apomorphine (APO) with more acute stereotypy than do male rats (Hruska et al. 1982).

The magnitude of these stimulant-induced responses varies across the females' estrous cycle. The behavioral response is usually reported to be augmented during estrus as in the cases of AMPH-elicited rotational behavior (Joyce and Van Hartesveldt 1984; Becker and Beer 1986) and bromocriptine-potentiated wheel running behavior (Steiner et al. 1980). However, not all of the responses affected by stimulant administration peak during the estrous stage. APO-induced hypothermia is attenuated and APO-evoked hyperlocomotion is increased during proestrus (Kazandjian et al. 1987). Furthermore, when compared to the levels of stereotypic behavior during the proestrous and diestrous stages, APO-elicited stereotypy reaches a maximum during both estrus and metestrus (Miller 1983). Although the estrous cycle appears to play a decisive role in mediating the intensity of the female animals' responses to stimulant administration, the effect of each stage of the cycle seems to be response specific and difficult to predict.

Few studies examining gender differences and hormonal fluctuations have evaluated the effect of the estrous cycle on the reinforcing properties of psychomotor stimulants. Previous work from our laboratory has shown that the rate of apomorphine (unpublished observations) and cocaine (Dalton et al. 1986; Roberts et al. 1987) self-administration on a fixed ratio (FR) schedule of reinforcement is unaffected by ovariectomy, antiestrogen treatment, or the estrous cycle. These findings need to be re-evaluated. The potentiation of haloperidol's ability to increase the rate of cocaine intake during diestrus (Roberts et al. 1987) suggests that estrous-related fluctuations in an animal's motivation to self-administer psychomotor stimulants may remain undetected. Recently, the use of progressive ratio (PR) schedules of reinforcement has led us to the conclusion that measures of rate and drug intake are poor indicators of a drug's reinforcing properties and are not sensitive to changes in an animal's motivation to self-administer a drug (Roberts 1988; Roberts et al. 1988). Intake and rate cannot quantify a drug's incentive value. By contrast, we have found that "breaking point" analysis on a PR schedule offers a reliable and quantitative method for assessing changes in motivation.

According to a PR schedule of reinforcement, the number of lever responses required to earn an injection (or other reinforcement) is increased systematically until the animal fails to meet the demands of the schedule. The value of the final ratio reached by the animal is defined as the breaking point. We have applied this PR schedule to the question of hormonal involvement in drug reward and now report that, while the rate of cocaine self-administration remains unaffected, breaking points are significantly altered across the estrous cycle.

Materials and methods

Sexually mature male and female Wistar rats (Charles River Canada Inc., Quebec), weighing approximately 300 g and

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230 g, respectively, at the beginning of the experiment, were used. The animals were first trained to press a lever for food reward. Once the lever-press response had been acquired, rats were anaesthetized with sodium pentobarbital and permanently implanted with jugular cannulae based on the procedure described in detail by Roberts and Goeders (1988). The cannulae emerged from the animals' backs at the mid-scapular level and were suspended, through a protective spring, above the cage by a counterbalance/swivel assembly allowing the subjects freedom of movement. The rats were housed individually in test boxes (25 cm × 30 cm × 30 cm high) resembling the food training chambers for the remainder of the experiment. Food and water were freely available. A 12 h light/dark cycle (lights off at 10 a.m.) was maintained. After a 2-day recovery period, daily 4-h sessions began approximately 1 h after light offset (11 a.m.). A lever, similar to the one used in the food training session, was introduced into the cage. Each lever-press response activated a 20 s stimulus light and resulted in the delivery of a single intravenous injection of cocaine HCl (0.50 mg/0.1 ml saline/4 s delivery). Each animal, regardless of sex, received the same dose of cocaine.

The female rats were vaginally lavaged daily at light offset. In order to accustom the rat to the procedure, the lavages were begun 6–8 days before the animals were cannulated. Vaginal lavages were placed on slides, stained with cresyl violet, and cover-slipped. Each sample was examined under light microscopy to determine the stage of the cycle (metestrus, diestrus, proestrus, estrus) on the basis of the predominant cell type (leukocyte, nucleated epithelial or cornified epithelial). Animals showing 3-day cycles seldom exhibited a clear metestrous stage throughout the experiment. In order to include these females in the final analysis, the cocaine self-administration responses made by all the subjects during metestrus and diestrus were categorized together (metestrus/diestrus).

Self-administration behavior was initially reinforced on a continuous reinforcement schedule (FR1) and the number of infusions for each animal was recorded during a 4-h testing period. Male rats were required to maintain a stable pattern of drug intake (range of less than 10%) over 3 baseline days before being transferred to a PR schedule of reinforcement. Female rats were required to exhibit a similar pattern of intake, on the FR1 schedule, over one complete estrous cycle before proceeding to the PR schedule. Approximately one third of the cannulated animals failed to learn the lever-press response, showed erratic patterns of self-administration behavior, or developed problems with the cannula implant and were dropped from the study. The remaining rats (11 male and 11 female) were assigned to the PR schedule.

The PR response requirement escalated through the series: 1, 2, 4, 6, 9, 12, 16, 20, 28, 36, 48, 63, 83, 110, 145, 191, 251, 331, 437, 575, 759, 999. The number of bar presses made every minute, the total number of lever-presses, and the time each injection (0.6 mg/0.12 ml saline/5 s delivery) was received were recorded on a 6809 based laboratory microcomputer. Failure to earn an injection over the course of 1 h was defined as behavioral extinction. Breaking points were defined as the final ratio the rat completed prior to behavioral extinction. Only those female rats that continued to show complete estrous cycles during PR testing and only those female and male rats that remained untroubled by cannula problems were included in the final analyses. A total of 12 animals, 6 male and 6 female rats, fulfilled the requirements.

All subjects self-administered cocaine for 12–18 days. In order to evaluate the effect of the estrous cycle on the self-administration behavior, data from the female animals were collapsed into three groups (metestrous/diestrous, proestrus, or estrus). A single score for each stage of the cycle was determined for every female by calculating the mean breaking point score for the 2–6 days the animal spent in each particular state. Mean metestrous/diestrous, proestrous, or estrous breaking point scores were subsequently subjected to a repeated measures analysis of variance. The effect of gender was evaluated by collapsing the female animals' data across all stages of the estrous cycle and comparing the overall mean male and female breaking points using a Student's *t*-test for unpaired groups.

Results

On a PR schedule, the estrous cycle was found to produce a statistically significant effect on cocaine self-administration. A repeated measures analysis of variance ($F=6.58$; $df=2,10$; $P<0.05$) revealed that breaking points varied systematically across the estrous cycle (see Fig. 1). A Scheffe *F*-test indicated that the estrous breaking points were significantly higher than the metestrous/diestrous or proestrous breaking points. On a FR1 schedule, however, the number of infusions earned by the same female rats was not found to vary with the stages of their estrous cycle ($F<1$; $df=2$, 10).

Gender was also shown to affect cocaine self-administration on a PR schedule of reinforcement but not on a FR1 program of reinforcement. Female rats reached significantly higher overall mean breaking points on the PR than did their male counterparts (mean breakpoints ± SEM; Females 264.1 ± 47.91; Males 48.2 ± 14.6) ($t=-4.31$; $df=10$; $P<0.001$). For both males and females, reception of a sin-

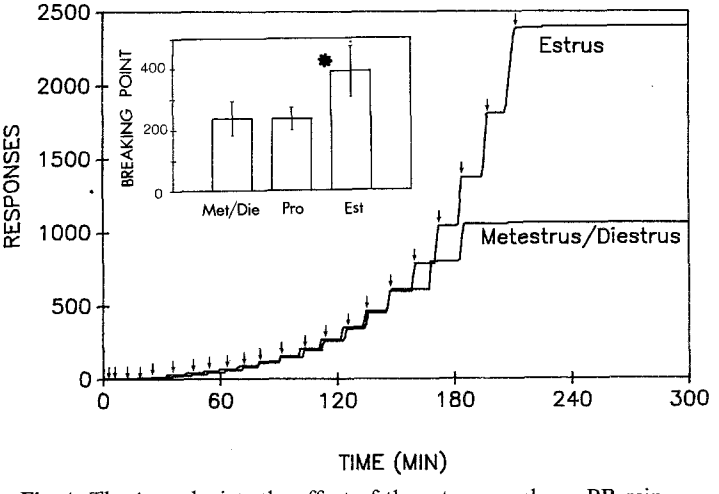

Fig. 1. The *inset* depicts the effect of the estrous cycle on PR reinforced cocaine self-administration. Data represent the mean breaking point (±SEM) of the six female subjects during each stage of the estrous cycle. *Indicates significantly different from other groups ($P<0.05$, Scheffe *F* test). The large graph represents the cumulative responses made by a single animal across the 5-h testing period during metestus/diestrus and estrus. *Arrows* indicate reception of an infusion. The animal was chosen for its clear depiction of the estrous effect on cocaine self-administration

gle injection was followed by a highly regular post-infusion pause and a subsequent resumption in responding. Gender affected the intensity of the self-administration behaviour rather than the pattern of responding. On a FR1 schedule, rate was not found to vary with gender. Male rats tended to self-administer more injections on a FR schedule than the female rats but this difference did not reach statistical significance (mean no. of injections $\pm$ SEM; Males 32.9 ± 2.2; Females 27.7 ± 1.7) ($t = 1.854$; $df = 10$; $P > 0.05$).

Daily lavage demonstrated that the estrous cycle did not retain its regularity with repeated behavioral testing. The disruption was first apparent, on average, on day 18 of behavioral testing. Of the 11 female rats that proceeded to the PR in the first week of the experimental period, five animals continued to exhibit changes in vaginal cell type but daily vaginal lavage was unable to sequentially detect every stage of the estrous cycle. These rats were not included in the statistical analysis. Although the remaining six female rats continued to exhibit changes in vaginal cell type sequentially corresponding to each stage of the estrous cycle, their cycles were extended from either 4–5 days or 3–4 days depending on their original patterns.

Discussion

During estrus, female rats will pay a far greater behavioral price for cocaine reinforcement than during other stages of the estrous cycle. The breaking points reached by females in estrus were higher than those reached by the same animals during the non-estrous stages of the reproductive cycle. This difference was often dramatic. For example, while in estrus, one female would repeatedly reach the second highest final ratio (759 responses/injection) allowed by the schedule. During the other stages of the estrous cycle, the rat would stop responding at final ratios of 331. The data suggest that estrus increases the incentive value of cocaine reinforcement.

The literature is silent with respect to the effect of the estrous cycle on other behavioral effects of cocaine. Our data are consistent with studies reporting an augmentation of the response elicited by other DA agonists during estrus. For example, AMPH-induced rotational behavior (Joyce and Van Hartesveldt 1984; Becker and Beer 1986), bromocriptine-potentiated wheel running behavior (Steiner et al. 1980), and APO-elicited stereotypy (Miller 1983) are increased during estrus. There are some exceptions. According to Kazandjian et al. (1987), the maximum locomotor and hypothermic response to APO occurs during proestrus. It is suggested that the hormonal fluctuations associated with the estrous cycle differentially affect the anatomical substrates responsible for psychostimulant-induced behaviors.

One of the strengths of the present study is that vaginal lavages were conducted daily throughout the experiment. It has often been assumed that the estrous cycles of animals that appear to be cycling consistently prior to drug administration remain regular throughout behavioral testing (Robinson et al. 1982b; Joyce and Van Hartesveldt 1984). Our results suggest that this assumption may be false. The reasons behind the disruption of the estrous cycle observed in the present study remain unclear. Daily lavage, cannula-related stress and/or chronic administration of cocaine (18 days or more) may each influence the regularity of the estrous cycle. Because of the daily vaginal lavages, however, it can be firmly stated that our augmentation of cocaine self-administration occurred during the estrous state.

The tentative conclusion that cocaine self-administration behavior is affected by gender is based on the fact that female rats in estrus surpassed the male breaking point by approximately 800% and, in metestrus/diestrus or proestrus, by approximately 500%. It must, however, be taken into consideration that all of the animals were tested at 0.6 mg/injection of cocaine. Since the females weighed less than their male counterparts, they were, in effect, responding for a higher dose of drug. (Males were self-injecting approximately 2.0 mg/kg. Females were self-administering approximately 2.6 mg/kg). Had males been responding for an equivalent dose of cocaine (approximately 0.78 mg/injection), our finding would have been easier to interpret. A dose-response relationship has already been generated for male rats responding for 0.3, 0.6, and 0.9 mg/injection (Roberts et al. 1988). It is important to note that the overall female breaking point was 285% higher than the average breaking point for males responding for the highest dose of cocaine (0.9 mg/injection; mean breaking point $\pm$ SEM; 92.5 ± 12.2; $n = 8$). As a result, the effect of gender on cocaine self-administration cannot be dismissed.

In most PR self-administration studies, a fixed ratio is imposed for the duration of each experimental period and is increased during the following session only if the animal meets a certain criterion (Griffiths et al. 1975, 1978, 1979; Hoffmeister 1979; Risner and Silcox 1981; Risner and Goldberg 1983; Risner and Cone 1986). By contrast, our schedule follows the Hodos (1961) model. The response requirements increase after each reinforcement. The data presented in this paper clearly demonstrate that a PR schedule of reinforcement reflects differences in motivation related to the estrous cycle. A continuous reinforcement schedule (FR1) was unable to detect these changes. This finding is consistent with previous results showing that rate, as a dependent variable, failed to reflect changes in the incentive value of apomorphine that were clearly demonstrated using PR breaking points (Roberts 1988). The data force one to conclude that measuring the rate of drug infusion on a FR1 schedule cannot be trusted to accurately reflect changes in an animal's motivation to self-inject cocaine.

The changes in the female animals' incentive to self-administer cocaine during estrus raises some difficult questions. Estrus is a behavioral term that is defined by "sexual receptivity" but has, of course, been correlated with a particular hormonal state and specific vaginal cell types. It is an interesting question whether the observed changes in breaking point are caused by direct hormonal action on dopaminergic systems or are due to an altered motivational state (i.e. sexual receptivity). The fact that others have shown that food deprivation, for example, influences self-administration behavior (Carroll 1981; 1985) leads one to question whether the effect of the estrous cycle on dopaminergic systems is necessarily direct.

It must be stated that the rat estrous cycle cannot be compared to the human menstrual cycle and it is with this reservation that we make any reference at all to human studies. Researchers have suggested that motivational factors vary across the menstrual cycle. For example, Harvey and Beckman (1985) have shown that the alcohol consumption of social drinkers varies across the menstrual cycle. Although we do not intend to link the reported effect of

the estrous cycle with changes in motivation associated with the menstrual cycle, the literature does suggest that a better understanding of all factors influencing the incentive to self-administer drugs of abuse is necessary.

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