A Selective Serotonin$_{1B}$ Receptor Agonist Modulates Cocaine Self-Administration in Female Rats Regardless of Estrous Cycle Phase

by

Samantha Nicola Scott

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Graduate Supervisory Committee:

Janet Neisewander, Chair
M. Foster Olive
Miles Orchinik

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ABSTRACT

Greater than 11% of the total population of Americans age 12 and older were illicit drug users with close to 1 million suffering from cocaine use disorder in 2017 alone (SAMHSA, 2017), yet there are no effective pharmacological treatments for this disorder. Previous research from the Neisewander Laboratory in male rats found that administration of a 5-HT_{1B}R agonist facilitates cocaine intake when given prior to a daily self-administration session, while inhibiting cocaine intake and attenuating drug-seeking behavior following 21 days of protracted abstinence, yet it is not known whether such effects are observed in female rats. Women face unique challenges in all phases of the drug addiction cycle. With respect to active drug-taking (i.e., the maintenance phase), women tend to increase their rate of consumption more rapidly than men, and female rats acquire cocaine self-administration faster than males. In part, this is due to ovarian hormone influences on the reinforcing properties of cocaine, where peak levels of endogenous estrogen hormones correspond to an increase in cocaine intake. In this study, we investigated the effects of CP94253, a selective $5HT_{1B}$R agonist, on cocaine intake across all phases of the estrous cycle in female rats. The rats were trained to self-administer cocaine (0.75 mg/kg, IV) on a fixed ratio (FR) 5 schedule of reinforcement and daily vaginal smears were taken after each session to monitor the estrous cycle. Rats were pretreated with CP 94,253 (5.6 mg/kg, IP) or vehicle prior to separate tests during each estrous cycle phase and were then either given 1-h access to 0.75 mg/kg cocaine followed by 1-h access to 0.375 mg/kg cocaine or 1-h access to 0.1875 mg/kg cocaine followed by 1-h access to 0.075 mg/kg cocaine. Similar to males, CP 94,253 decreased

cocaine intake in females at intermediate doses, however, the estrous cycle phase did not alter this effect.

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CHAPTER 1

INTRODUCTION

40 percent of drug abuse-related emergency department visits in 2011 involved cocaine (Drug Abuse Warning Network, DAWN) and despite substantial research investigating psychostimulant abuse, there are no effective, FDA-approved treatments for cocaine dependence. Though there are contrasting reports on the use of antidepressants for the treatment of cocaine dependence, it seems that they may be selectively effective in individuals comorbid with depression (Pollack & Rosenbaum, 1991; Batki, Manfredi, Jacob, & Jones, 1993; Pollack, Brotman, & Rosenbaum, 1989). Drugs that alter 5-HT neurotransmission were first recognized for their use in treating affective disorders, such as anxiety and depression (Grahame-Smith, 1989, 1992). However, affective and other psychiatric disorders are often comorbid with substance abuse, suggesting that these disorders may increase the risk of substance abuse (Regier, et al., 1990; Kessler, et al., 1994). Specifically, the evidence indicates that up to 56 percent of individuals diagnosed with an affective disorder had a co-occurring lifetime substance abuse. In some cases, treating affective disorders can diminish substance abuse and craving (Cornelius, 1997). There is strong clinical evidence that serotonin neurotransmission plays a role not only in affective disorders but also cocaine effects, suggesting a role in cocaine use disorders.

The serotonergic system is complex as there are 14 receptor subtypes differentially distributed throughout the brain (Peroutka, 1995; Mohammad-Zadeh, Moses, & Gwaltney-Brant, 2008). Among the 14 receptor subtypes are the 5-HT_{1B} receptors (5-HT_{1B}Rs), which are located on the terminals of presynaptic neurons where they act to inhibit neurotransmitter release. 5-HT_{1B}Rs are autoreceptors on serotonergic neuron terminals

(Miszkiel, Filip, & Przegaliñski, 2011; Sharp, Bramwell, Hjorth, & Grahame-Smith, 1989; Hjorth & Tao, 1991) and heteroreceptors on non-serotonergic neuron terminals (Sari, 2004; Yan, Zheng, & Yan, 2004). Localization studies indicate that 5-HT_{1B}R binding sites are highly concentrated in the mesocorticolimbic and nigrostriatal dopamine systems, which include the substantia nigra, nucleus accumbens, ventral tegmental area, and caudate putamen (Bonaventure, Langlois, & Leysen, 1998; Bonaventure, Schotte, Cras, & Leysen, 1997; Varnas, Hall, Bonaventure, & Sedvall, 2001; Sari, 2004). These systems have been implicated in mediating the rewarding effects of cocaine (Sari, 2004).

The 5-HT_{1B}R is a potential target for novel pharmacological medications for cocaine abuse (Rocha, et al., 1998; Miszkiel, Filip, & Przegaliñski, 2011; Neisewander, Cheung, & Pentkowski, 2014). For example, 5-HT_{1B}R knockout mice display a greater tendency to self-administer cocaine (Castanon, Scearce-Levie, Lucas, Rocha, & Hen, 2000), and 5-HT_{1B}R agonists decrease psychostimulant intake and seeking behavior after a period of abstinence in male rats (Neisewander et al., 2014; Garcia, Cotter, Leslie, Foster Olive, & Neisewander, 2017). Evidence from our lab suggests that in male rats that self-administer cocaine, a switch in the effects of a 5-HT_{1B}R agonist occurs as a result of abstinence. Prior to experiencing a prolonged abstinence period, pretreating male rats with the selective 5-HT_{1B}R agonist, CP 94,253, prior to daily cocaine self-administration sessions produces a leftward shift in the dose-response curve, indicating an increase in the reinforcing value of cocaine. However, following 21 days of forced abstinence, CP 94,253 decreases cocaine intake, flattening the inverted U-shaped dose-response curve (Pentkowski et al., 2014), suggesting attenuation of cocaine reinforcement. In addition, CP 94,253 attenuates both cue-induced and cocaine-primed reinstatement of cocaine-seeking

behavior after daily extinction training during which the rats are abstinent (Pentkowski, Acosta, Browning, Hamilton, & Neisewander, 2009). These findings suggest that 5-HT_{1B}R agonists attenuate motivation to seek cocaine. Collectively, this research suggests that 5-HT_{1B}R agonist may be useful in decreasing cocaine intake in males.

Serotonergic neurotransmission, as well as 5-HT_{1B}Rs in particular, are affected by ovarian hormones. Early studies by Fludder and Tonge (1975) show that the concentration of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), significantly increases across brain regions during the proestrus phase when blood estrogen levels are highest, but decreases in all brain regions examined, except the hypothalamus, during the estrus phase when peak estrogen levels begin to decline. This same study revealed that serotonin concentration significantly decreases in the amygdala during proestrus and only decreases in areas such as the striatum and thalamus during estrus (Fludder & Tonge, 1975). These complex changes in serotonin during the estrous cycle may contribute to the effects of 5-HT_{1B}R agonists on cocaine self-administration. Later studies by Biegon and McEwen (1982) assessed how exogenous hormones, such as estradiol, modulate serotonin 1 receptors in the brain. Their findings suggest that estradiol produces a biphasic effect on serotonin 1 receptor density in female rat brains. Specifically, estradiol pretreatment decreases serotonin 1 receptor density throughout the brain initially, followed by a selective increase in the hypothalamus, preoptic area, and amygdala 48-72 hours after initial estradiol pretreatment (Biegon & McEwen, 1982). The brain regions showing a selective increase in serotonin 1 receptor density are also areas known to be rich in estrogen receptors (Liu & Shi, 2015; Li, Blaustein, De Vries, & Wade, 1993; Yokosuka, Okamura, & Hayashi, 1998).

To date, no experiments to our knowledge have investigated effects of 5-HT_{1B}R agonists on cocaine self-administration and cocaine seeking in female rats. However, ovarian hormones are known to modulate the reinforcing properties of cocaine, suggesting that males and females may differ in their response to a treatment that is aimed at reducing drug intake (Carroll, Lynch, Roth, Morgan, & Cosgrove, 2004; Becker & Hu, 2008; Lynch, 2006). Furthermore, in female rats, the response to test treatments may vary depending on the estrous cycle phase. In women, smoking cocaine during the follicular phase when estradiol level is elevated results in higher subjective ratings, such as higher self-confidence, increased drug potency and a greater high compared to ratings during the luteal phase of the menstrual cycle (Sofuoglu, Dudish-Poulsen, Nelson, Pentel, & Hatsukami, 1999; Evans, Haney, & Foltin, 2002). The higher subjective effects observed during the follicular phase are attenuated by the administration of progesterone (Evans & Foltin, 2006). Additionally, women with high levels of progesterone display lower stress-induced and cue-induced cocaine craving when compared to women with lower levels of progesterone alone or in combination with estradiol (Sinha, et al., 2007). The progesterone metabolite, allopregnanolone, blocks the escalation of cocaine self-administration in rats (Anker, Zlebnik, & Carroll, 2010). These findings suggest that estradiol and progesterone have opposing effects on cocaine reinforcement in females.

Cocaine self-administration studies with female rodents are generally consistent with the findings observed in women. Female rats acquire cocaine self-administration at a faster rate and consume a greater amount of cocaine than male rats (Lynch & Carroll, 1999). Estrogen may play a role in this sex difference as the exogenous administration of estradiol increases the acquisition of cocaine self-administration in ovariectomized female

rats when compared to castrated male rats (Jackson, Robinson, & Becker, 2006) or ovariectomized vehicle control females and gonad-intact females receiving the estrogen antagonist, tamoxifen (Lynch, Roth, Mickelberg, & Carroll, 2001). These effects extend to other phases of the drug addiction cycle, such as maintenance of use and relapse. During the maintenance of daily cocaine self-administration, female rats in estrus display a preference for higher doses of cocaine compared to females in other phases (Lynch, Arizzi, & Carroll, 2000; Lynch, 2006). Additional research using the discrete trial procedure suggests that females escalate cocaine intake more than males. In this procedure, rats housed in operant self-administration chambers for 24 hours per day are given intermittent, discrete trials during which they have access to cocaine. Using this procedure, female rats take more cocaine over a 7-day period and display a greater initial binge period than males (Lynch & Taylor, 2004). Furthermore, administration of estradiol benzoate significantly increases binge length and the amount of cocaine self-administered (Lynch & Taylor, 2005). Female rats tested on a progressive ratio (PR) schedule of reinforcement, which requires an exponential increase in the number of responses required to earn successive reinforcers, demonstrate higher breakpoints during estrus compared to other cycle phases. Breakpoint is the final schedule completed at which responding stops on the PR schedule, suggesting that motivation for cocaine is higher during the estrus phase (Roberts, Bennett, & Vickers, 1989; Hecht, Spear, & Spear, 1999).

Other animal models of motivation for cocaine also suggest sex differences and the role of ovarian hormones in cocaine-seeking behavior. For example, Kippin and colleagues (2005) extinguished drug-seeking behavior of gonad-intact male and female rats trained to self-administer cocaine. They then found that females in estrus showed higher

reinstatement of drug-seeking behavior when primed with a high dose of cocaine compared to non-estrus females and males. The females' sensitivity to the reinstatement of drug-seeking behavior was further supported when female rats self-administered more saline than male rats after a cocaine-priming injection (Lynch & Carroll, 2000). Again, this sex difference may involve ovarian hormones as ovariectomized females given estradiol administration during the reinstatement test showed facilitated responding for cocaine compared to female rats treated with an oil control (Becker & Hu, 2008).

Given that there are sex differences in acquisition, maintenance, and reinstatement of cocaine self-administration and cocaine-seeking behaviors with evidence that ovarian hormones are mediating these sex differences, it is important to examine both sexes when assessing potential pharmacological treatments for cocaine use disorders. I hypothesize that freely cycling female rats are more sensitive to the effects of the 5-HT_{1B}R agonist CP 94,253 on cocaine self-administration during the estrus and proestrus phases when blood estrogen levels are relatively higher and progesterone is low. I predict that CP 94,253 will produce a leftward shift in the dose-effect function in the estrus phase. We examined the effects of the 5-HT_{1B}R agonist CP 94,253 on cocaine self-administration during the proestrus, estrus, metestrus, and diestrus stages of the estrous cycle using a fixed-ratio, low effort schedule of reinforcement.

CHAPTER 2

METHODS

Animals

Female Sprague-Dawley rats weighing 200-225 g arrived from Charles River (San Diego, CA) and were single-housed in a climate-controlled facility on a reversed light/dark cycle (10 h light/ 14 h dark cycle). Animals were handled for 1 week prior to any procedures. All rats had access to *ad libitum* food and water unless specified otherwise. Animal husbandry and experimental procedures were approved by the Institutional Animal Care and Use Committee at Arizona State University.

Surgery

Rats underwent surgery to implant a chronic indwelling catheter into the jugular vein according to methods previously described (Pockros, Pentkowski, Swinford, & Neisewander, 2011). Briefly, rats were anesthetized with isoflurane (1-4%) and after implanting the catheter into the jugular vein, the opposite end which was attached to a metal cannula was anchored to the skull with dental acrylic cement. Rats were subsequently given 5-6 days of recovery before starting cocaine self-administration training. Catheters were flushed daily with 0.1 mL (i.v.) saline containing heparin sodium (70 USP/1 mL; SAGENT pharmaceuticals, Schaumburg, IL) and cefazolin (10 mg/0.1 ml; WG Critical Care, LLC) to maintain patency and prevent infection. Also, catheters were periodically tested for patency by administering 0.05 mL methohexital sodium (Jones Pharma Inc) at a dose that produces anesthetic effects only when administered i.v. (16.7 mg/ml).

Drugs

Cocaine hydrochloride (RTI International, Research Triangle Park, NC) was dissolved in 0.9% bacteriostatic saline (Hospira Inc, Lake Forest, IL) and filtered through 0.2 µm membrane Acrodisc syringe filters (PALL Corporation, Ann Arbor, MI). CP 94,253 hydrochloride (5.6 mg/kg s.c.; Tocris Bioscience, MN) was made fresh daily and dissolved in 0.9% bacteriostatic saline. The volume of i.v. injections were 0.1 ml and all other injections were given at a volume of 1 ml/kg.

Vaginal Swabs

As previously described by Byers et al. (2012), estrous cycle phases were determined by vaginal cytology. Rats were held firmly while a cotton-tipped swab (Puritan Medical Products Company, LLC, Guilford, ME) soaked in 0.9% bacteriostatic saline (Hospira Inc, Lake Forest, IL) was inserted into the vaginal opening and gently swiped in a circular motion. Cells were then transferred onto a glass slide and immediately viewed under a microscope (Nikon Eclipse E6000) at 10x magnification. A still image was captured and the stage of the estrous cycle was determined based on the presence or absence of different cell types as previously detailed (Marcondes, Bianchi, & Tanno, 2002) and shown in Figure 1.

Apparatus

Operant conditioning chambers (30 x 25 x 25 cm^3; Med Associates, St Albans, VT) were equipped with an active and inactive lever, a cue light above the active lever, a house light, and a tone generator mounted on the outside of the chamber near the active lever. Infusion pumps were located outside of a sound attenuating outer chamber. Polyethylene tubing attached to the drug syringe in the infusion pump entered through a port in the outer chamber wall and attached to liquid swivels (Instech, Plymouth, PA)

suspended above the operant conditioning chambers. The outlet line from the liquid swivel was encased inside a metal spring leash with a plastic connector that fastened to the cannula end of the catheter (PlasticsOne, Roanoke, VA) on top of the rats' head.

Self-Administration Training

Self-administration sessions occurred at the same time of day, 6 days per week for 2 hours per session. Initially, rats were trained to self-administer 0.75 mg/kg/0.1 mL i.v. cocaine on a fixed ratio (FR) 1 schedule of reinforcement. Based on individual performance, rats progressed from an FR1 through an FR5. Schedule advancement automatically occurred once rats received at least 7 reinforcers within one hour during the session. To encourage exploration of operant chambers, rats were initially food restricted to 16 g of food/day three days prior to the first self-administration session and continuing until the FR5 schedule was achieved. In conjunction with advancing to the FR5, all rats were given *ad libitum* access to food. Daily vaginal swabs were conducted up to 1 hour before and 30 minutes after the sessions to track the estrous cycle phases. Testing began once rats displayed stable cocaine intake, defined as <15% variability in the number of infusions obtained across 3 consecutive sessions.

Experimental Testing Procedure

Rats were tested for the effects of CP 94,253 on cocaine self-administration across metestrus, diestrus, proestrus and estrus cycle phases. We attempted to test each rat in each estrous cycle phase, however, because cocaine and the swabbing procedure appeared to interfere with normal estrous cycling, several rats did not complete all of the tests due to catheter failure. Furthermore, because cycle phase was not verified until after testing in order to avoid confounding effects of the swabbing procedure in close

approximation to the self-administration session, some rats were tested more than once under the same condition due to incorrect prediction of cycle phase, which was verified after the test. During each cycle phase, rats were tested twice, receiving pretreatment with vehicle (1 mL/kg, s.c) prior to one test and with CP 94,253 (5.6 mg/mL, s.c) prior to the other test. Fifteen min after the pretreatment injection, rats were placed into the operant chambers for a 2-hour test session. The 5.6 mg/kg CP 94,253 (CP) dose used in this experiment was reported as the minimally effective dose capable of inducing changes in cocaine self-administration in male rats (Przegalinski, Gołda, Frankowska, Zaniewska, & Filip, 2008; Pentowski et al. 2009). Separate groups of rats were tested with 0.075 and 0.1875 mg/kg, i.v. (low doses) cocaine available or with 0.375 and 0.75 mg/kg, i.v. (high doses) cocaine available. During a given test, the highest dose for each group was available first for one hour followed by the lower dose available for one hour. Rats were given a 5-minute time out in the home cage between the hour-long test intervals. Throughout testing, cocaine reinforcement was available on an FR5 schedule of reinforcement. To re-establish the stability criteria described above, rats were given at least 3 additional sessions between tests.

Statistical Analysis

Statistical analyses were conducted with IBM SPSS Statistics 23.0. Infusions, active and inactive lever responses were analyzed as the dependent variables in separate ANOVAs with estrous cycle phase, cocaine dose, and CP 94,253 or vehicle pretreatment as between subjects' factors. Although we had designed the experiment with CP 94253 vs. vehicle pretreatment as a repeated measure, there was too much missing data to use the repeated measures ANOVA. The data from rats that had been tested multiple times

under the same condition due to mistaken predictions of estrous cycle phase were averaged and the average value was used in the ANOVA. Additionally, two-way ANOVAs with low dose/high dose groups and estrous cycle phase as between subjects' factors were used to analyze effects of these variables on cocaine self-administration at the training dose prior to testing for effects of CP 94,253. Tukey post-hoc tests for multiple comparisons were used to identify the source of significant effects and interactions. All significant effects are reported and descriptive statistics are reported as the mean ± SEM.

CHAPTER 3

RESULTS

Cocaine self-administration during estrous cycle phases

Infusion rates, active and inactive lever responses, and cocaine intake once rats exhibited stable infusion rates on an FR5 schedule of reinforcement are shown for each cycle phase in Figure 2. There were no main effects of cycle phase for infusion rates, $F(3, 195) = 0.61$, n.s. (Figure 2A), active lever responses, $F(3, 195) = 0.87$, n.s. (Figure 2C), or cocaine intake (Figure 2B), nor were there any interactions between estrous cycle phase and groups (i.e., high dose and low dose groups). However, there was a main effect of cocaine dose group for infusion rate, $F(1, 195) = 36.22$, $p < 0.05$, active lever responses, $F(1, 195) = 42.53$, $p < 0.05$, and cocaine intake, $F(1, 205) = 38.57$, $p < 0.05$, during these sessions. In each case, values were higher in rats who were tested at higher doses compared to rats who were tested at lower doses (Figure 2a-c). There were no effects of estrous cycle phase or differences between the high and low cocaine dose groups for inactive lever responses (Figure 2C). Note, the group differences are not related to the cocaine test doses that establish the groups, but rather appear to be due to cohort differences as these groups were run at different times.

Effects of CP 94,253 on cocaine self-administration during estrous cycle phases

Effects of pretreatment with CP 94,253 or vehicle on reinforcement rates across doses of cocaine during each estrous cycle phase are shown in Figure 3A-D. The ANOVA of infusions revealed a main effect of pretreatment, $F(1, 357) = 6.40$, $p < 0.05$ (Figure 4A), indicating that CP 94,253 decreased cocaine infusions when compared to vehicle regardless of estrous cycle phase or dose of cocaine available. There was also a

main effect of cocaine dose group $F(3, 357) = 23.48$, $p < 0.05$ (Figure 4B). Post hoc comparisons showed that rats took fewer infusions when the higher 0.75 and 0.375 mg/kg doses were available compared to when the lower 0.075 and 0.1875 mg/kg cocaine doses were available (Tukey HSD, $p < 0.05$). An interaction between pretreatment and cocaine dose, $F(3, 357) = 3.70$, $p < 0.05$, was observed (Figure 4C). Post hoc analysis showed that CP 94,253 decreased infusions at the middle 0.1875 and 0.375 mg/kg doses. There was no effect of estrous cycle phase on infusions nor were there interactions with the estrous cycle phase.

Cocaine intake (mg/kg, i.v.) across estrous cycle phases, cocaine doses, and pretreatments are shown in Figure 5A-D. Estrous cycle phase did not affect cocaine intake regardless of pretreatment or cocaine dose (Figure 5A-D) $F(9, 358) = 1.29$, n.s. Main effects of pretreatment, $F(1, 358) = 7.13$, $p < 0.05$ (Figure 5E), and cocaine dose, $F(3, 358) = 132.59$, $p < 0.05$ were observed (Figure 5F). CP94253 attenuated cocaine intake compared to the vehicle pretreatment (Figure 5E). Rats also showed a dose-dependent increase in cocaine intake, where intake significantly increased at each successive dose of cocaine (Figure 5F) (Tukey HSD, $p < 0.05$).

Lever responses across cycle phases, cocaine doses, and pretreatments are shown in Figure 6A-D. Although there was no main effect of CP 94,253 versus vehicle pretreatment on active lever responses, $F(3, 104) = 0.96$, n.s., there was a main effect of cocaine dose, $F(3, 104) = 8.99$, $p < 0.05$ (Figure 6E), as well as an interaction between pretreatment and cocaine dose, $F(3, 104) = 3.22$, $p < 0.05$ (Figure 6F). Post hoc analysis of the cocaine dose main effect showed that rats made fewer responses at the 0.75 and 0.375 mg/kg dose compared to both the 0.075 and 0.1875 mg/kg doses (Tukey HSD, $p <$

0.05). This pattern of effects was expected as the lower doses used are on the ascending limb of the typical inverted U-shaped dose-response curve for cocaine self-administration. Post hoc analysis of the interaction between pretreatment and cocaine dose showed that response rate at the intermediate doses of cocaine (0.1875 and 0.375 mg/kg) was attenuated after CP 94,253 pretreatment compared to vehicle pretreatment (Tukey HSD, $p<0.05$). The increase in response rate at the lowest dose of cocaine (0.075 mg/kg) did not differ with CP 94,253 pretreatment compared to vehicle pretreatment. There was no effect of estrous cycle phase nor interactions with cycle phase. There was also no significant main effects or interactions in the ANOVA of inactive lever responses.

CHAPTER 4

DISCUSSION

We hypothesized that freely cycling female rats would be more sensitive to the effects of the 5-HT_{1B}R agonist on cocaine self-administration during the estrus and proestrus phases when blood estrogen levels are relatively higher and progesterone is low. First, we predicted that female rats will have higher cocaine intake during proestrus and estrus compared to all other phases of the estrous cycle. We also predict that CP 94,253 will facilitate a more robust cocaine intake during estrus and proestrus phases of the estrus cycle. Surprisingly, the results did not support the hypothesis and predictions. Cocaine infusion rates, intake, or responses either prior to testing or during tests for CP 94,253 effects were not different between estrous cycle phases, in contrast to previous research demonstrating increased intake during estrus (Becker & Hu, 2008). We previously reported that the selective 5-HT_{1B}R agonist CP 94,253 (5.6 mg/kg, s.c.) enhanced cocaine infusion rates during the maintenance phase of self-administration in male rats (Pentkowski, et al., 2014). The findings from this study indicated that CP 94,253 decreased cocaine intake and active lever response rates at moderate doses (Figure 4C, 6F). The CP 94,253-dependent decreases in cocaine self-administration observed at these particular doses of cocaine in female rats are the same as CP 94,253-dependent decreases observed in male rats. In our study in males, we observed a leftward shift in the dose-response curve after CP 94,253 pretreatment, such that behavior when lower doses of cocaine were available appeared similar to the behavior of vehicle-pretreated controls when higher doses were available. Thus, we interpreted these findings as enhanced reinforcing value of cocaine not only based on this dose-response data on the FR5 schedule of reinforcement, but also the corroborative

findings of an increase in infusions on a high effort progressive ratio schedule. In the present study, we observed a nonsignificant increase in infusions (Figure 3A) and active lever presses (Figures 6A, 6F) at the lowest dose of cocaine, suggestive of a leftward shift in the dose-response function. However, we are unable to definitively conclude that CP 94,253 produced a leftward shift in the FR5 dose-response function as these effects were not significant and we have no other data in female rats at this point to confirm a consistent pattern of changes indicative of enhancement in the value of cocaine reinforcement. The addition of a lower dose of cocaine on the ascending limb is needed because an increase in cocaine intake at a subthreshold dose after the administration of the 5-HT_{1B}R agonist is one of the hallmarks of a leftward shift and this would show that female rats respond to cocaine self-administration in the same manner as male rats when CP 94,253 is on board.

There is an apparent discrepancy between our findings and others implicating the role of estrogen in cocaine intake (Lynch & Taylor, 2004) and preference for higher doses of cocaine (Lynch, Arizzi, & Carroll, 2000) during the estrus phase in rats. Human studies parallel these findings with evidence suggesting that relative to the luteal phase of the menstrual cycle, women report more positive subject effects of psychostimulant use during the follicular phase when estradiol levels are high (Justice & De Wit, 2000; Justice & De Wit, 1999). The reason for the discrepancy is unclear, but may be related to the use of a low effort schedule in the present study because estrous cycle effects are more reliably observed under high effort schedules, such as the PR schedule (Lynch, 2006; Lynch & Carroll, 1999; Roberts drug-taking, 1989; Carroll, Morgan, Lynch, Campbell, & Dess, 2002). For example, Roberts and colleagues (1989) studied how ovarian hormones influence the maintenance of cocaine self-administration on an FR1 schedule of

reinforcement, and found no hormonal effect. Under a PR schedule, these same researchers found that females obtained higher breakpoints during the estrus phase. These findings suggest that FR schedules may not be sensitive enough to detect changes in the estrous cycle-dependent motivation to self-administer cocaine. The PR schedule is likely more sensitive to the motivational effects of a drug because the work demand increases after each reinforcer obtained, whereas low effort FR schedules may result in ceiling effects (Lynch, Roth, & Carroll, 2002). In addition, the relatively long duration of cocaine self-administration prior to monitoring the estrous cycle in the present study may have a reduced sensitivity to detect estrous cycle effects. Consistent with this idea, chronic cocaine exposure disrupts the menstrual cycle in rhesus monkeys (Mello et al, 1997) and the estrous cycle in rats (King et al, 1990). After 18 days of cocaine self-administration, female rats often develop irregular cycles (Roberts et al, 1989) characterized by prolonged diestrus, multiple days of estrus, and an absence of proestrus (King et al, 1990).

The estrous cycle did not appear to influence the effects of CP 94,253 on the cocaine dose-effect functions. The typical psychostimulant dose-effect function under low ratio schedules of reinforcement, such as an FR5, is an inverted U-shaped function (Neisewander et al, 2014; Pentowski et al, 2014; Garcia et al, 2017). As the dose increases within the low range of doses, intake and responses increase forming the ascending limb of the curve; however, at intermediate doses to high doses these measures decrease as inter-infusion intervals increase with increases in cocaine dose, forming the descending limb of the curve. As described in Mello et al. (1986), a shift to the left of the dose-response function suggests that the treatment facilitates drug reinforcement, while a downward shift suggests attenuated drug reinforcement. Previous research in our laboratory, as well as

others, in male rats demonstrates that CP 94,253 shifts the dose-effect function for psychostimulant self-administration to the left (Pentowski et al, 2014; Parsons, Weiss, & Koob, 1996; Parsons, Weiss, & Koob, 1998; Garcia et al, 2017). It is possible that CP 94,253 agonistic action on 5-HT_{1B}Rs has opposing effects across sexes, but as discussed above, it is difficult to definitively conclude how CP 94,253 altered the female dose-effect functions in this study, yet the effects observed at the intermediate cocaine doses did not interact with estrous cycle phase.

The ability of ovarian hormones to regulate 5-HT_{1B}Rs may have played a role in the findings from this study. Biegon and McEwen (1982) showed that exogenous estradiol modulates 5-HT1 receptors (5-HT_1R) in a biphasic manner in ovariectomized (OVX) rats. The initial decrease of 5-HT_1R density observed in these OVX rats may be restricted to brain regions concentrated with estrogen receptors in the intact female rats that are freely cycling. Estrogen affects 5-HT receptor density but not affinity, suggesting that estradiol is not competing for the serotonin receptor but may cause regulatory changes (Biegon & McEwen, 1982). Considerable research has been devoted to investigating the mechanisms through which estrogen regulates the 5-HT transporter (SERT), 5-HT_{1A} and 5-HT_{2A} receptors, but not 5-HT_{1B}R. We know that estrogen exert its effects on estrogen receptors (ERs) α and β, activating transcription factors for proteins that increase SERT (Hildebrandt, Alfano, Tricamo, & Pfaff, 2010). The activation of ERs deactivates 5-HT_{1A}Rs through the uncoupling of its G proteins (Mize & Alper, 2000; Mize & Alper, 2002; Mize, Poisner, & Alper, 2001), yet ER activation increases post-synaptic 5-HT_{2A}R mRNA and amplifies sensitivity to pre-synaptic serotonin release (Sumner et al., 1999; Sumner et al., 2007). Estrogen is also known to down-regulate 5-HT_{1B} autoreceptor mRNA in the dorsal raphe

nuclei (Hiroi & Neumaier, 2009). The mechanisms underlying estrogen effect on 5-HT_{1B}R and its subsequent functional effects are still unclear. However, the evidence suggests that estrogen may down-regulate the density of 5-HT_{1B}R, which may reduce the effect of CP 94,253 on 5-HT_{1B}R-mediated behavior.

The mesocorticolimbic system is a major pathway through which 5-HT_{1B}Rs exert their influence on cocaine reinforcement. Sari (2004) have showed that 5-HT nerve fibers project to the mesocorticolimbic system, where they regulate dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) (O'Dell, Manzardo, Polis, Stouffer, & Parsons, 2006; O'Dell & Parsons, 2004; Yan et al, 2004). In particular, potentiation of cocaine effects in male rats is thought to be due to 5-HT_{1B} heteroreceptors on medium spiny GABAergic neurons in the VTA that inhibit GABA release, which results in the disinhibition of dopaminergic neurons (O'Dell et al., 2006; O'Dell and Parsons, 2004; Yan et al, 2004).

It is important to mention that difficulty in identifying the estrous cycle phase resulted in repeated exposure to CP 94,253 in an aim to test during the desired cycle phase. We cannot rule out the possibility that our effects may have been dampened by tolerance from the repeated exposure to CP 94,253. This seems unlikely in light of Garcia et al. (2017) who found that repeated administration of the 5-$HT_{1B/1A}$ agonist zolmitriptan attenuated methamphetamine intake similarly across repeated injections. Nonetheless, it is important to determine if chronic or multiple exposures to CP 94,253 results in the development of tolerance.

This study followed a similar within-session design described previously with the exception of the testing order of cocaine doses (Pentowski et al., 2012; Pentowski et al.,

2014). While Pentowski et al., (2012, 2014) tested cocaine doses in pseudorandom order, we tested cocaine doses in descending order in both groups (i.e., low vs. high dose groups). Studies have reported that ascending dosing schedule produces a greater condition place preference in mice, suggesting that the pattern of drug exposure can influence the magnitude of CPP (Conrad, Louderback, Milano, & Winder, 2013). Thereby, it is not far-reaching to suggest that the pattern of our cocaine exposure may have had an impact on the magnitude of our dose-response function. Another limitation that may have complicated our findings were the cohort differences in cocaine intake prior to the 5-HT_{1B}R agonist exposure.

Cocaine dependence is a chronic relapsing disorder with evidence suggesting that 5-HT_{1B}Rs are novel therapeutic targets for developing treatments for cocaine abuse. Previous research from our lab found that the 5-HT_{1B}R agonist modulates cocaine abuse related-behavior depending on whether or not male rats have experienced a period of abstinence. Specifically, either viral overexpression of 5-HT_{1B}Rs or the administration of the 5-HT_{1B}R agonist CP 94,253 facilitates cocaine intake during maintenance of self-administration but attenuates cocaine intake after a period of abstinence (Pentowski et al., 2009; Pentowski et al., 2014). Further research using lower doses of cocaine and higher schedules of cocaine reinforcement is needed to elucidate whether or not our results are due to a leftward shift at intermediate doses indicative of reinforcement enhancement or a true downward shift indicative of reinforcement attenuation during maintenance of cocaine self-administration in female rats. It is also necessary to determine if the administration of CP 94,253 will attenuate cocaine intake in female rats after a period of abstinence as observed in males. It is of utmost importance to examine whether the

abstinence-dependent switch in CP 94,253-induced attenuation of cocaine intake in male rats will persist after the resumption of cocaine self-administration. Therefore, it is important to test the effects of CP 94,253 on the resumption of cocaine self-administration after a period of abstinence in male and female rats.

Lastly, I propose further testing using a behavioral economics paradigm as it is designed to provide a greater translational approach to the study of addiction-like behaviors in humans and animals (Cox et al., 2016). This paradigm is amenable to using a within-session dose-response testing procedure, which would improve the efficiency of testing. The approach measures the demand for a drug and elasticity in its consumption. Demand (Q0), elasticity (α), and motivation to self-administer the reinforcer under varying unit prices (i.e., effort requirement) are estimated from the dose-response curve parameters (Bentzley, Fender, & Aston-Jones, 2013; Bentzley, Fender, & Aston-Jones, 2014). The variables measured above have been shown to predict addiction behaviors in humans (Petry, 2001; Gray & Mackillop, 2014; Murphy, Mackillop, Skidmore, & Pederson, 2009) and animals (Powell et al., 2019; Bentzley et al., 2013; Bentzley et al., 2014; Galuska, Banna, Willse, Yahyavi-Firouz-Abadi, & See, 2011). This would be an ideal model for translational purposes because it is modeled after behavioral economic procedures used to assess motivation for a drug in humans with existing drug dependence.

In conclusion, this study showed that the selective 5-HT_{1B}R agonist, CP 94,253, attenuates cocaine intake during maintenance of self-administration when intermediate doses of cocaine are available. Unfortunately, the limited number of doses of cocaine examined preclude drawing firm conclusions on whether the decrease in intake is due to enhancement or inhibition of cocaine reinforcing value due to the inverted U-shape of the

dose-response function. Evidence from human studies suggests that single nucleotides polymorphisms (SNPs) in the 5' untranslated region of the HTR1b gene, which encodes the 5-HT_{1B}R protein, is associated with drug dependence and psychiatric disorders (Proudnikov et al., 2006; Cao et al., 2011). These findings along with our study suggest that the 5-HT_{1B}R may be a novel target for the development of therapeutics for the treatment of psychostimulant use disorders.

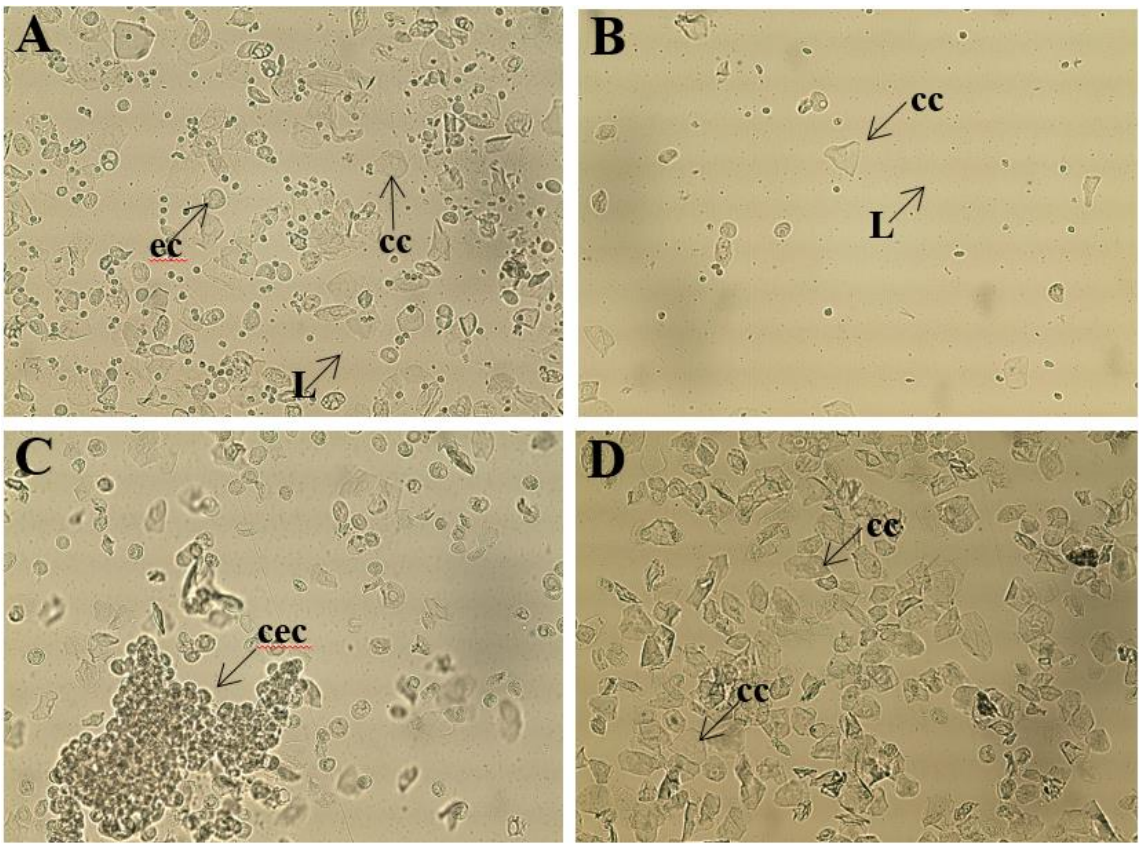

Figure 1. Representative photomicrographs of unstained vaginal smears from female rats in metestrus (A,), diestrus (B), proestrus (C) and estrus (D) phases of the estrous cycle. Cells were observed under a light microscope (Nikon Eclipse E6000) with a 40X objective lens. Estrous cycle phases were determined by the proportion of cell types observed in the vaginal cytology. Cornified cells (cc) are irregular shaped epithelial cells; regular epithelial cells (ec) are round and nucleated, and the smallest round cells are leukocytes (L). Metestrus is characterized by a mixture of all cell types (Figs. 1A). As the rodent transitions to diestrus, the predominant cell types are leukocytes and some cornified cells may still be present (Figs. 1B). Proestrus, the shortest phase of the cycle, will primarily consist of clusters of nucleated epithelial cells (cec) (Figs. 1C). The final phase is estrus, which is characterized by a predominance of cornified epithelial cells (Figs. 1D).

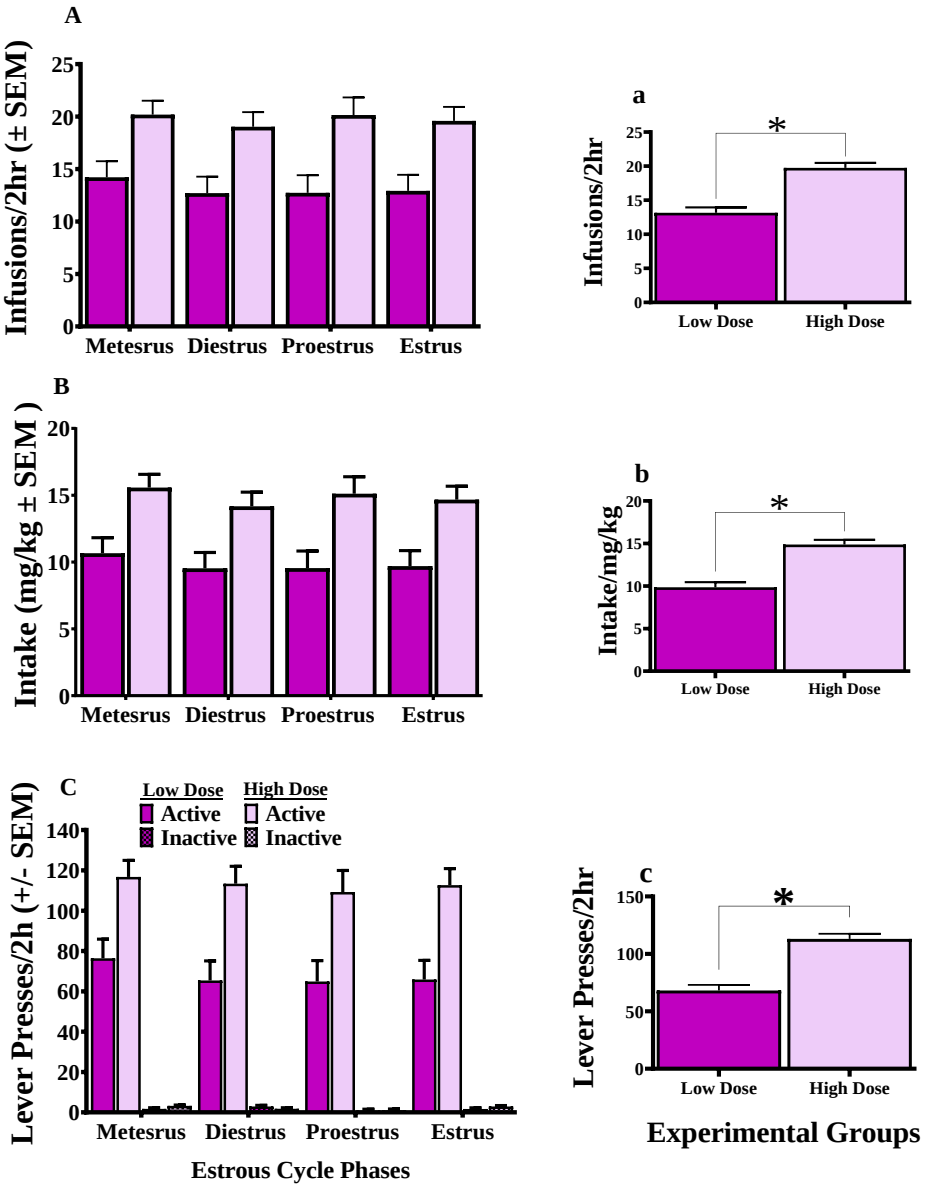

Figure 2. Lack of differences in cocaine self-administration across estrous cycle phases prior to CP94253 testing phase. Graphs represent mean (±SEM) number of infusions (A), mg/kg cocaine intake (B), and active and inactive lever responses (C) during the metestrus, diestrus, proestrus and estrus phases of the estrous cycle in animals tested with the low doses of cocaine available (0.075 and 0.1875 mg/kg, i.v.) or with the high doses of cocaine available (0.375 and 0.75 mg/kg, i.v.). Rats in both dosage groups were given access to 0.75 mg/kg/infusion cocaine on an FR5 schedule of reinforcement. There were no effects of estrous cycle phase for any of the measures, however, the high dose group (n = 20-24/cycle phase) had fewer cocaine infusions (a), active lever presses (b), and cocaine intake (c) when compared to the low dose group (n = 19-33/cycle phase). * indicates a significant difference between Low and High Dose groups ($p < 0.05$, Tukey HSD).

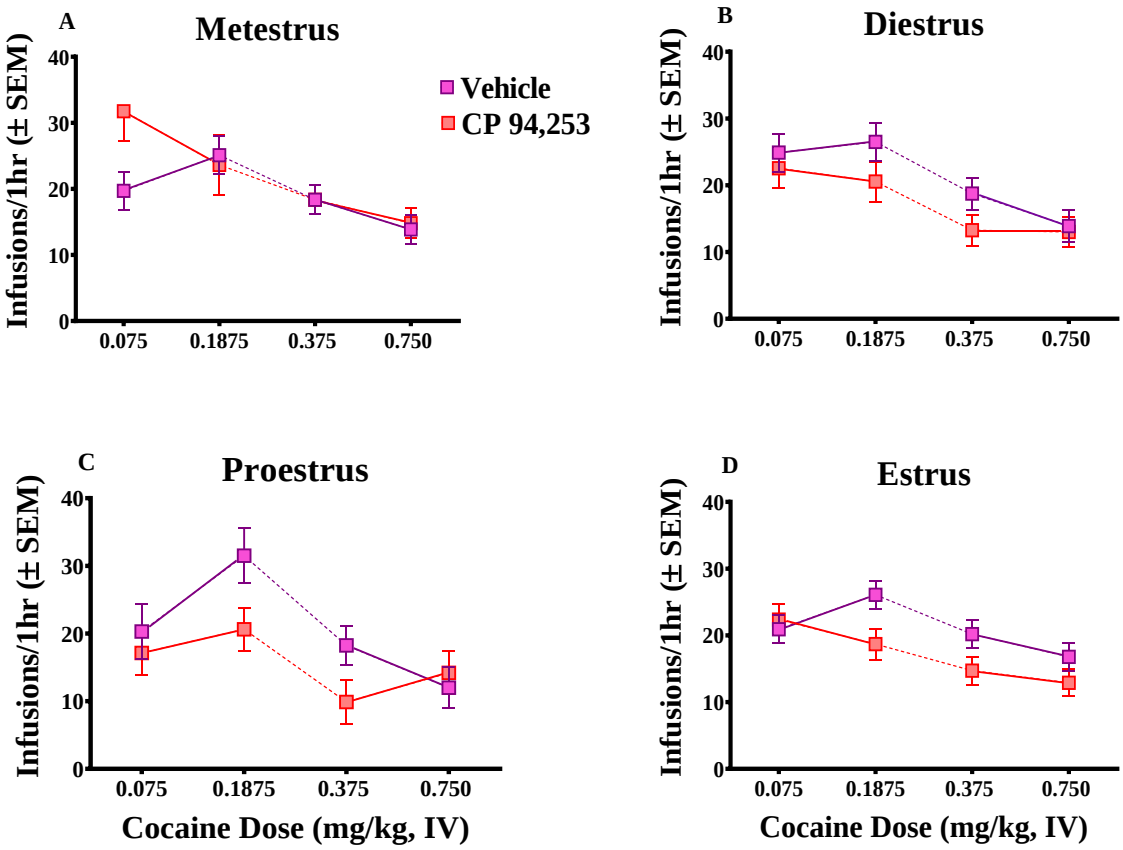

Figure 3. The effects of the 5-HT_{1B}R agonist CP94253 on cocaine infusions during estrous cycle phases. The graphs display the mean (±SEM) number of cocaine infusions. Once infusion rates stabilized on an FR5 schedule, rats were pretreated with CP94253 or vehicle 15 minutes prior to the cocaine self-administration test session. Estrous cycle phases (A-D) did not influence infusion rates of rats pretreated with CP94253 or vehicle.

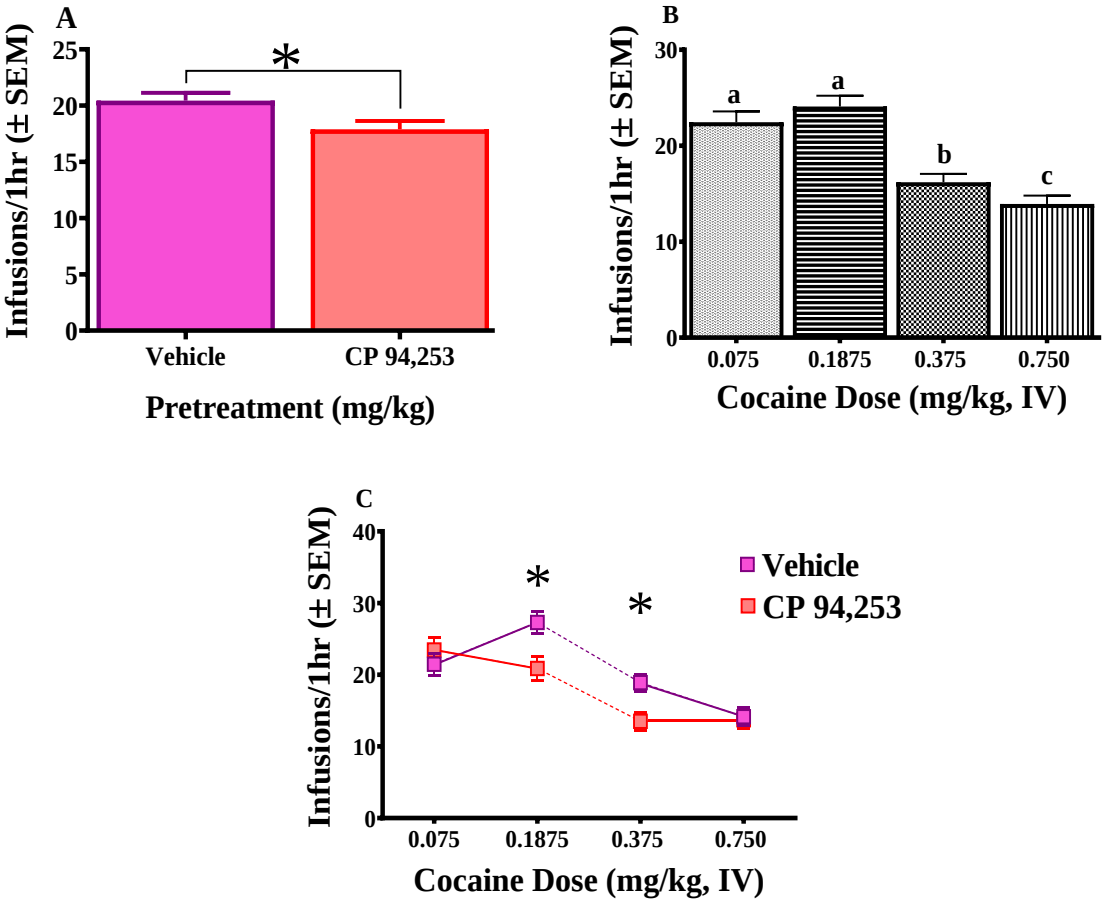

Figure 4. The effects of the 5-HT_{1B}R agonist, CP94253, and dose of cocaine on the number of cocaine infusions obtained. The graphs display the mean (±SEM) number of cocaine infusions (A-C). Once infusion rates stabilized on an FR5 schedule, rats were tested after receiving pretreatment with CP94253 or vehicle 15 minutes prior to the cocaine self-administration test session. A main effect of pretreatment showed that CP94253 decreased cocaine intake regardless of cycle phase (A). A main effect of cocaine dose was also observed where rats consumed fewer infusions on higher doses compared to lower doses (B). When compared to vehicle, CP94253 decreased infusion rate at 0.1875 and 0.375 mg/kg cocaine doses (C). *$p < 0.05$, Tukey HSD. In graph B, bars with different letters are different from each other, $p < 0.05$, Tukey HSD.

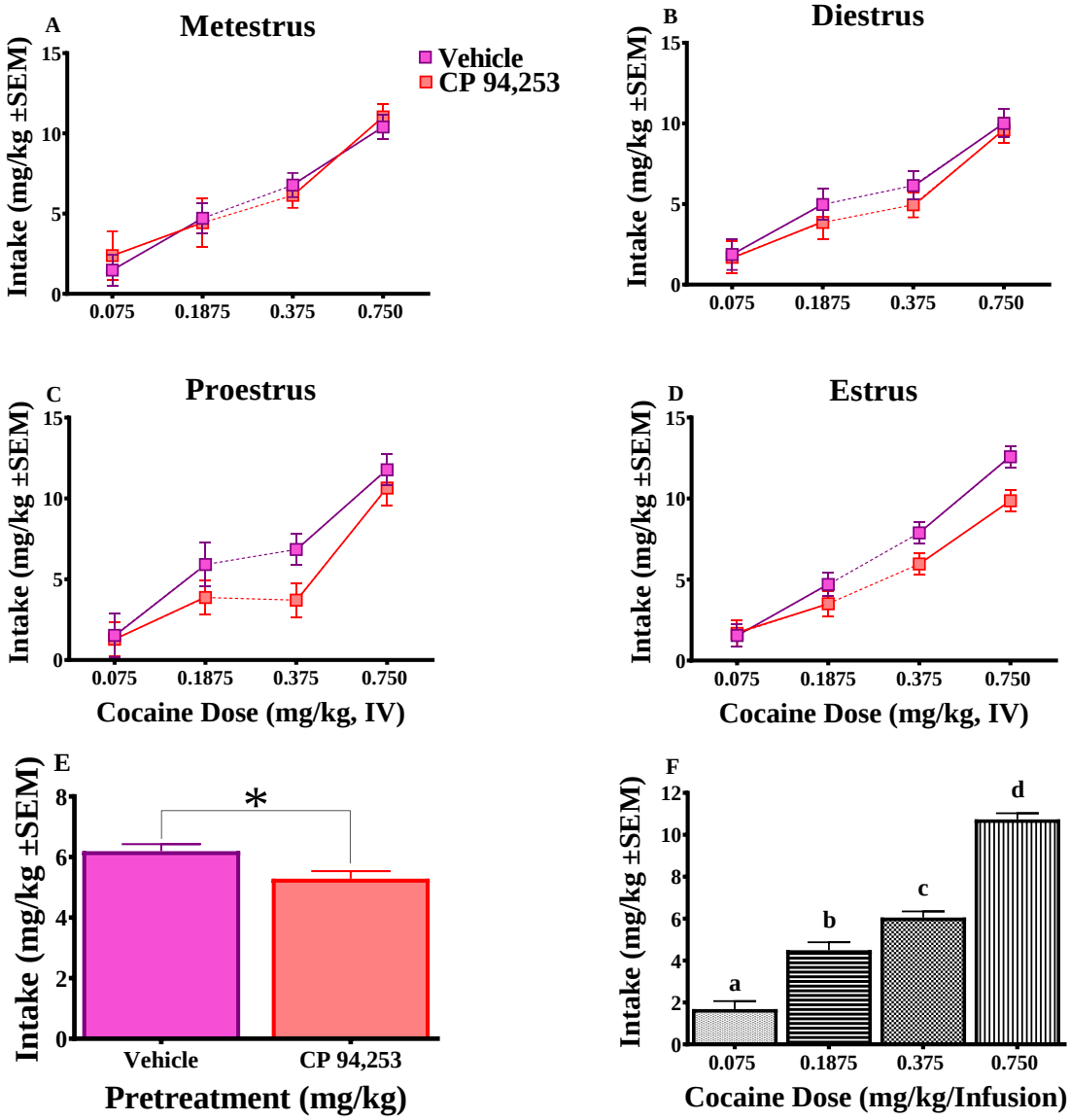

Figure 5. The effects of the 5-HT$_{1B}$R agonist CP94253 on cocaine intake during the metestrus (A), diestrus (B), proestrus (C), and estrus (D) phases. Animals were pretreated with the 5-HT$_{1B}$ agonist CP94253 or vehicle 15 minutes prior to cocaine self-administration tests. CP94253 pretreatment decreased cocaine intake when compared to vehicle (E), ANOVA main effect, $p < 0.05$. As doses increased, cocaine intake increased (F). In graph F, bars with different letters are different from each other, $p < 0.05$, Tukey HSD.

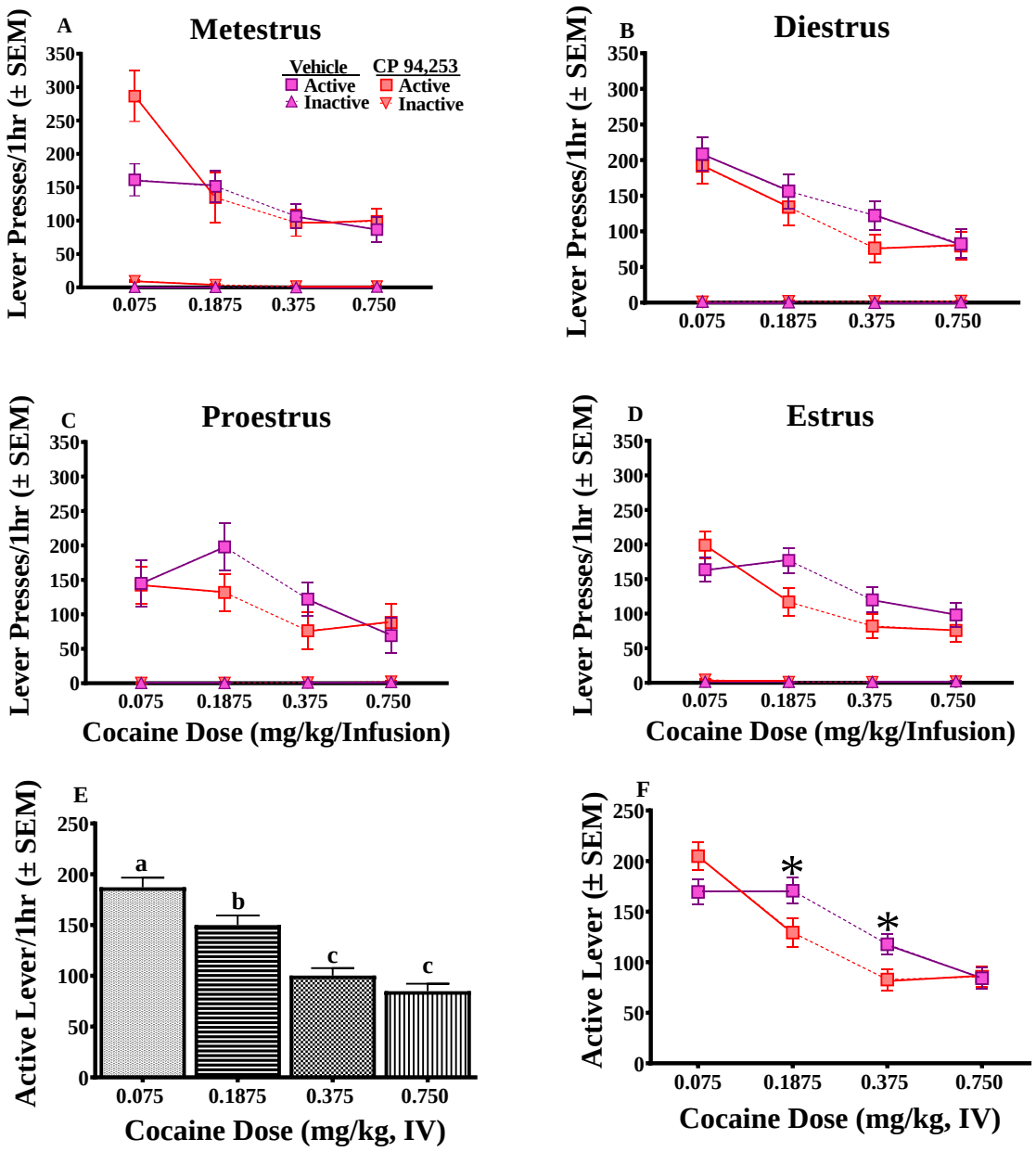

Figure 6. Active and inactive lever responses (mean ±SEM) during each phase of the estrous cycle (A-D) in rats pretreated with CP94253 or vehicle 15 minutes prior to cocaine self-administration tests on an FR5 schedule of reinforcement. Regardless of estrous cycle phase or CP94253 pretreatment, active lever responses dose-dependently decreased as cocaine dose increased (E); bars with different letters are different from each other, $p < 0.05$, Tukey HSD. Compared to vehicle, CP94253 decreased active lever responses at intermediate doses of cocaine (F). *difference from vehicle, Tukey HSD, $p<0.05$.

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