ELSEVIER

Pharmacology, Biochemistry and Behavior 73 (2002) 743–752

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Vaginal lavage attenuates cocaine-stimulated activity and establishes place preference in rats

Q. David Walker, Christina J. Nelson, Daegan Smith, Cynthia M. Kuhn*

Department of Pharmacology, 401 Bryan Research Building, Box 3813, Duke University Medical Center, Durham, NC 27710, USA

Received 9 April 2002; accepted 26 April 2002

Abstract

Sex and estrous cycle stage affect psychostimulant responses in animals. Cycle stage is typically monitored by vaginal lavage. The present studies tested the hypothesis that vaginal lavage modifies behavioral responses to acute cocaine. Female rats were restrained by briefly holding the tail for either vaginal lavage or touching the thigh, or were undisturbed, for 7–10 days prior to testing. Although habituation to the open-field test chamber was equal in each group, repeated lavage decreased horizontal activity relative to naive rats following acute cocaine (10 mg/kg ip). Lavage and touch attenuated cocaine-stimulated vertical activity. A single lavage prior to testing did not affect cocaine-stimulated motor behavior. Estrous cycle influenced motor activity only in nonlavaged rats. The high cocaine-induced responding observed in proestrous and estrous nonlavaged rats was completely blocked by vaginal lavage. A separate experiment tested the ability of vaginal lavage to establish a conditioned place preference. Vaginal lavage immediately prior to the conditioning session, but neither lavage after conditioning nor touch before, induced a significant preference. These results suggest that vaginal lavage serves as a reinforcing stimulus and interacts with a neural substrate that mediates enhanced locomotor responses to cocaine during proestrus and estrus.

Keywords: Vaginocervical stimulation; Horizontal activity; Vertical activity

1. Introduction

Numerous behavioral responses vary across the female estrous cycle (Becker et al., 2001; van Hartesveldt and Joyce, 1986) and activational effects of ovarian steroids may partly explain robust sex differences in psychostimulant-induced behavioral responses in rodents (Becker et al., 1982; Camp et al., 1986; Camp and Robinson, 1988; Haney et al., 1994; Savageau and Beatty, 1981; Schneider and Norton, 1979; van Haaren and Meyer, 1991). For instance, cocaine induced more locomotion and stereotypy in estrous females than in other stages of the cycle (Quinones-Jenab et al., 1999). Intrastriatal injections of dopamine and amphetamine induced the most postural deviation and contralateral rotation in estrous females (Joyce and van Hartesveldt, 1984). Electrical stimulation (Robinson et al., 1981; Robinson et al., 1982) and systemic amphetamine (Becker and Beer, 1986) elicited more rotations in estrous females than ovariectomized rats. The intensity of stereotyped behavior following amphetamine was greatest during estrus (Becker and Cha, 1989). These reports suggest that estrous cycle stage, and probably fluctuating estradiol concentrations, modulate a dopaminergic substrate on which psychostimulants act (Becker, 1999). Thus, determining estrous cycle stage is vitally important for interpreting results in most behavioral and neuroendocrine experiments.

This laboratory has also shown that female rats are more sensitive than males to cocaine (Bowman and Kuhn, 1996; Kuhn et al., 2001; Walker et al., 2000; Walker et al., 2001b, 2001a). Our preliminary finding that cocaine-stimulated locomotion was not different across the estrous cycle (Walker et al., 1998) was unexpected in light of the previously discussed literature. Also unexpected was our observation that ovariectomy decreased cocaine-stimulated locomotion only in experiments in which females had not been vaginally lavaged to assess cycle status (Walker et al., 2001a). Cocaine-stimulated locomotion was not different in ovariectomized and sham females that were lavaged. Our use of vaginal lavage to monitor estrous cycle stage (or lack

* Corresponding author. Tel.: +1-919-684-8828; fax: +1-919-681-8609.

E-mail address: ckuhn@acpub.duke.edu (C.M. Kuhn).

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)00883-3

thereof in ovariectomized rats) was a commonality in both of these experiments that yielded contrary results.

Vaginal lavage or ''smearing'' is a routine technique used to determine estrous cycle stage, and thereby fluctuating ovarian steroid effects, on stimulant and stress responses. This procedure involves inserting the tip of a medicine dropper into the vagina and swishing saline in and out to collect cells for histologic analysis (Long and Evans, 1992). Despite the widespread use of this technique, essentially no consideration has been given to possible adverse effects on behavioral parameters. Cooper et al. (1993) summarized the benign nature of lavage as a technique to monitor estrous cyclicity ''easily and noninvasively by observing changes in the vaginal cytology''.

The present studies tested the hypothesis that vaginal lavage alters cocaine-stimulated motor behavior. Female rats were lavaged for 7–10 consecutive days and compared to nonlavaged and restrained females during habituation to a novel open-field test chamber and subsequent injection with 10 mg/kg cocaine ip. Furthermore, we were interested to know if vaginal lavage is a discriminable stimulus and if it is aversive or rewarding. To test this possibility, we conducted a conditioned place preference experiment using vaginal lavage as the unconditioned stimulus. The present results suggest vaginal lavage is more invasive than previously thought and are therefore directly relevant to the many studies of estrous cycle effects on psychostimulant behavioral, neuroendocrine, and neurochemical effects that have used vaginal lavage.

2. Materials and methods

2.1. Subjects

Adult female Sprague–Dawley rats were purchased from Charles River Laboratories (Raleigh, NC, USA). They were housed three per cage in plastic cages under a 12:12-h light–dark cycle with lights on at 0600 h. Food and water were provided ad libitum. Animals were moved to the testing facility and weighed the day before observations. Animal care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 865-23, Bethesda, MD, USA) and approved by the Institutional Animal Care and Use Committee.

2.2. Locomotor behavior methods

Motor activity was determined in photocell devices (San Diego Instruments, San Diego, CA). The devices were comprised of an open Plexiglas arena (18 in. (l) × 18 in. (w) × 14 in. (h)) with wood chip bedding on the floor. Horizontal activity and vertical activity were determined from interruptions of photobeams spaced 1 in. apart. Computer software supplied by the manufacturer recorded data at 5-min intervals. Horizontal activity is reported as the number of inches traveled and vertical activity, as the number of beam interruptions.

Repeated lavage began 7–10 consecutive days prior to behavioral testing. Rats were held by the base of the tail and lifted slightly. The tip of a medicine dropper (3 mm outside diameter) was gently inserted into the vagina and 0.25-ml saline at room temperature was washed in and out several times. This lavage fluid was then placed on a microscope slide and allowed to dry. Other females, serving as controls for the vaginal lavage, were also restrained by holding the base of the tail but were stroked gently on the outside of the thigh with a cotton-tip wood applicator for about 5 s (the time required for lavage). All lavage and touch rats received their respective treatments before the habituation session on the morning of testing. A naive third group was undisturbed prior to behavioral testing and lavaged afterward. A separate experiment determined the effect of a single, acute lavage prior to testing. This experiment utilized only lavage and naive groups because maximal differences were expected. Half of the rats were lavaged prior to habituation and the others were lavaged after the cocaine challenge. Estrous cycle stage was determined post-hoc by analysis of cell types in vaginal smears. Cells were fixed with ethanol and stained with 1% toluidine blue. Identification of cell types was made microscopically according to published methods (Cooper et al., 1993; Long and Evans, 1992).

Assignment to test chambers was counterbalanced across days with respect to treatment condition (lavaged, touched, or no treatment). Habituation test sessions began when rats were placed in the open arena without injection. After this 1-h session (40 min in the acute lavage experiment), all rats were injected with 10 mg/kg cocaine ip and another 1-h activity recording session was started. Behavioral testing occurred between 0800 and 1600 h.

2.3. Estrous cycle effects in lavaged and nonlavaged rats

The effects of estrous cycle on cocaine-stimulated locomotion were compared in female rats that had and had not been vaginally lavaged repeatedly prior to testing. Nonlavaged rats could not be preselected for cycle stage and repeated lavage rats were not staged prior to testing. To have a sufficient number of rats in each cycle stage to enable this analysis, data from the acute and repeated lavage experiments were combined. All data from the touch and naive rats from the repeated lavage experiment were combined with those from the acute lavage experiment to form a nonlavaged group. All the rats in the acute experiment are termed ''nonlavaged'' because the results indicated a single acute lavage did not affect cocaine-stimulated activity.

2.4. Conditioned place preference methods

For place preference studies a dark Plexiglas box was inserted into the clear arena described above. The insert

(11 in. high) occupied exactly one-half of the floor area of the open arena and fit snugly to one side of the clear Plexiglas. An opening (4.5 in. (h) × 4 in. (w)) was located in the center of the wall of the dark insert that separated the dark chamber from the rest of the open arena. A dark lid was placed on the dark box during behavioral testing. A white background was placed under the clear Plexiglas floor of the dark compartment, while a blue background was placed under the floor of the white compartment. White paper bordered the outside walls of the open chamber that had no lid.

On the first day of testing, each rat was placed in the white compartment and allowed to explore both sides for 15 min. The computer software reported the amount of time spent in each compartment and the side in which each rat spent the most time will be referred to as the preferred side. The following morning each rat was confined on its preferred side for 15 min by placing a guillotine door in the opening between compartments. Four to six hours later in the day, each rat was confined on the nonpreferred side for 15 min. One group of rats was vaginally lavaged immediately prior to placement on the nonpreferred side. Another group was touched with a cotton applicator (as described above), and a third group was placed without any treatment but these rats were lavaged immediately after the session. A total of 4 conditioning days preceded another 15-min choice session. This final choice session began mid-way between the morning and afternoon conditioning times. The time spent on each side on the final choice day was determined. A delta time on each rat's initially nonpreferred side was determined by subtracting the time on the nonpreferred side on the first day from the time spent on the same side on the final day.

2.5. Cocaine

Cocaine HCl was obtained from NIDA and the Research Triangle Institute (Research Triangle Park, NC). Cocaine

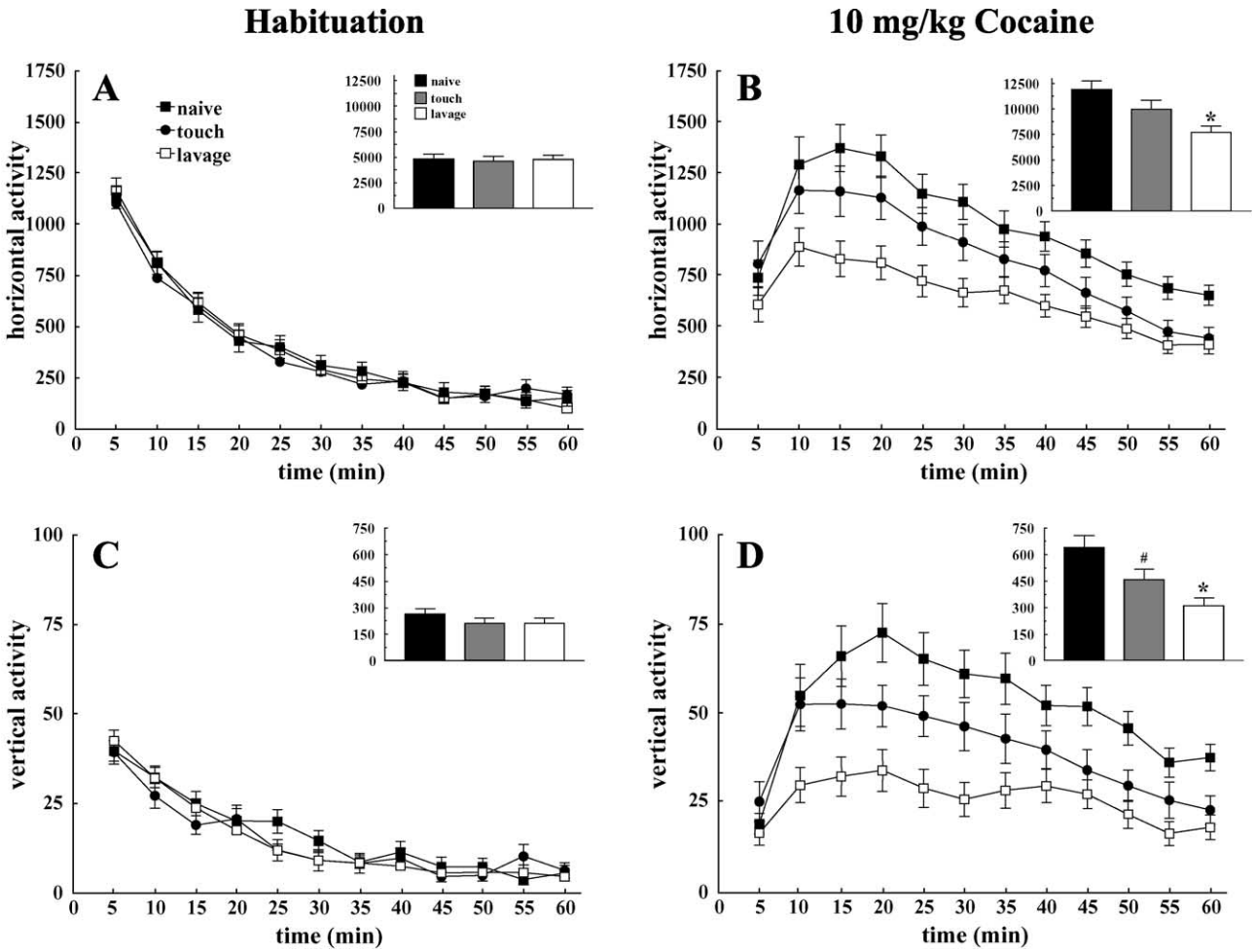

Fig. 1. Activity time courses in female rats repeatedly lavaged, touched, or naive (n = 30–33/group) in an open-field. Horizontal activity in inches traveled is shown during habituation (A) and following injection of 10 mg/kg cocaine (B). Vertical activity or rearing was measured by the number of photobeam interruptions during habituation (C) and following cocaine (D). Group means ± standard errors are shown in this and all figures. Error bars are subsumed by the symbols in some cases. The inset graphs show session totals for each group. * Significantly different than naive and touch (P < .05); # significantly different than naive and lavage (P < .05).

HCl was prepared fresh in 0.9% saline and either the saline vehicle (1 ml/kg) or cocaine was injected intraperitoneally.

2.6. Data analysis

The time courses of horizontal and vertical activity in 5-min intervals (mean + S.E.M.) beginning immediately after animals were placed in the chamber for habituation or injected with cocaine are shown. All 5-min intervals were summed and session totals are also shown in Figs. 1–3. Activity induced by cocaine was compared to the activity of each rat during the habituation session using repeated measures ANOVA. The effect of repeated treatment (touch, lavage, or none) on cocaine-stimulated activity was analyzed using one-way ANOVA. The effect of estrous cycle on cocaine-stimulated activity was analyzed in lavaged and nonlavaged rats using one-way ANOVAs. The effect of repeated treatment on conditioned place preference was analyzed with one-way ANOVA. When significant main effects were found ($P < .05$), post-hoc analysis was employed using Newman–Keul's multiple-comparison test to determine differences between treatment groups. Statistical analyses used NCSS 2000 software (NCSS, Kaysville, UT).

3. Results

3.1. The effect of repeated lavage and touch in female rats

Fig. 1A and B shows activity time courses and session totals for horizontal motor behavior or locomotion during habituation to a novel environment and following injection of 10 mg/kg cocaine, respectively ($n = 30$ or 33/group). Locomotion (Fig. 1A) gradually decreased throughout the first 1-h exposure to the novel test chamber and no group differences were observed. Subsequent injection of 10 mg/kg cocaine ip (Fig. 1B) increased horizontal activity in all rats relative to the habituation period ($P < .0001$). Cocaine-stimulated horizontal activity was greatest in naive female rats that were not handled before testing and lowest in the

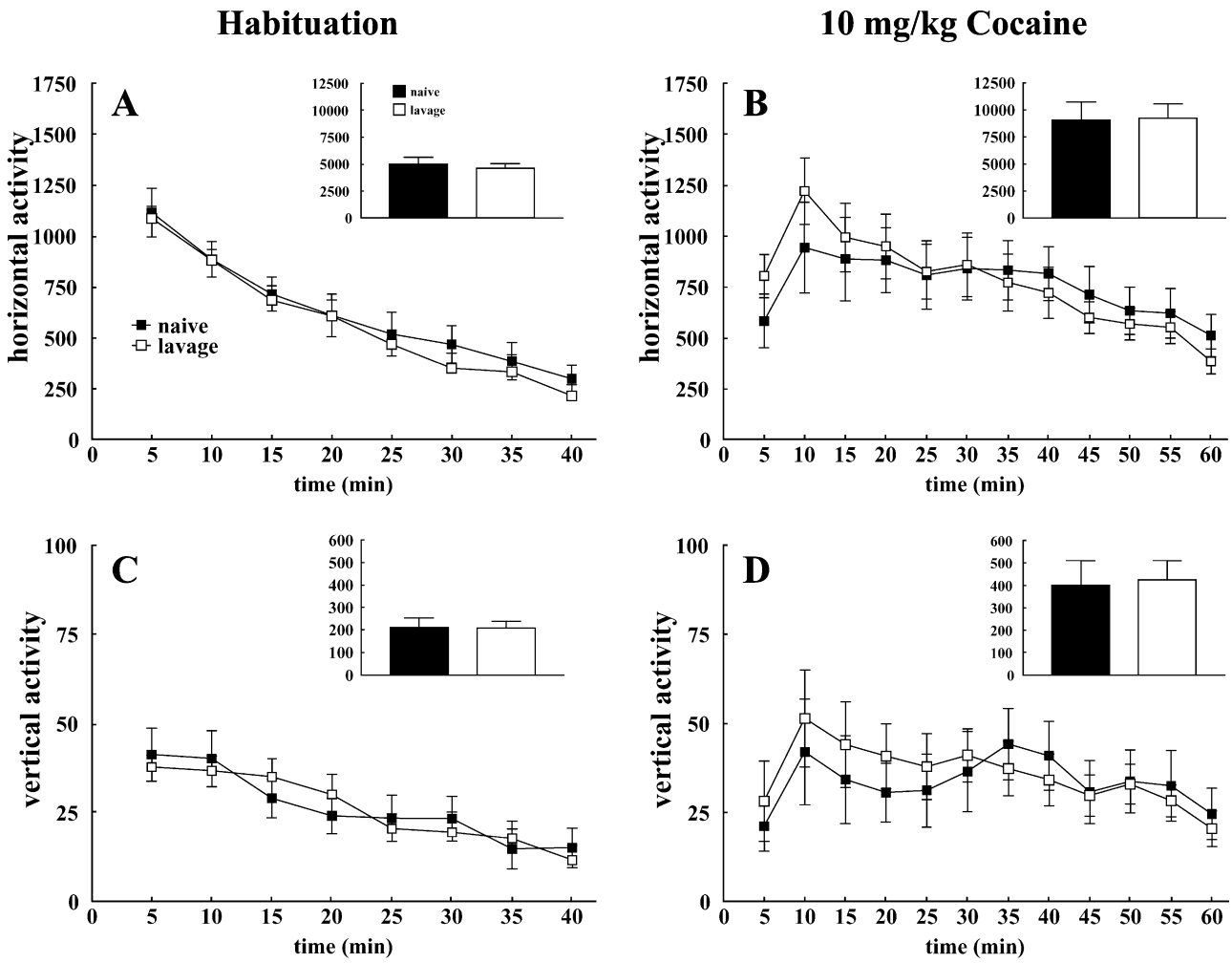

Fig. 2. Effect of a single, acute vaginal lavage on habituation and total horizontal and vertical activity. Female rats were lavaged prior to the habituation test session ($n = 25$) or received no treatment ($n = 28$). Panels A–D and inset graphs are analogous to those in Fig. 1. In contrast to repeated lavage, acute lavage did not affect behavior.

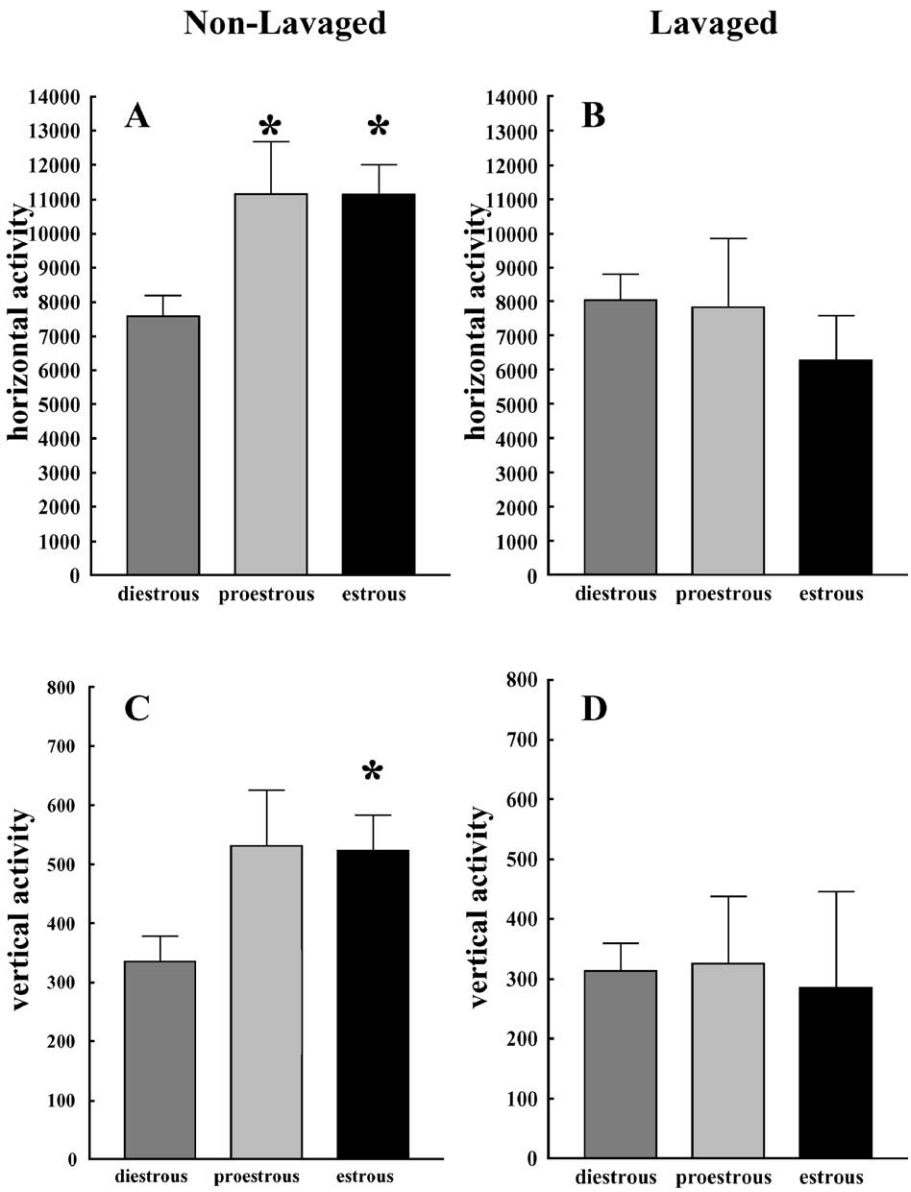

Fig. 3. Effect of estrous cycle on cocaine-stimulated activity in repeatedly lavaged vs. a composite group of singly lavaged, touched, and control female rats (here called nonlavage group). All data from the acute lavage experiment (Fig. 2—no effect of acute lavage) were combined with the control and touched rats from the repeated lavage experiment (Fig. 1). Nonlavage and lavage groups sizes are, respectively, diestrus (41, 21), proestrus (14, 6), and estrus (30, 6). * Significantly different than diestrus ($P < .05$).

rats that had been lavaged repeatedly. Cocaine-stimulated horizontal activity increased rapidly to a peak at about 15 min in the naive and touch rats. The time course in the lavage rats differed most from the other two groups as activity neither rose nor declined steeply. Activity in the touch rats was most similar to the naive rats at early time points but activity fell rapidly after 40 min to levels more similar to those of lavaged rats. One-way ANOVA confirmed that repeated treatment significantly affected cocaine-stimulated horizontal activity [$F(2,96) = 7.08$, $P = .002$]. Post-hoc analysis showed that nonlavaged females locomoted more than lavaged females ($P < .05$). The main effect of trial was highly significant ($P < .0001$) and a significant interaction of treatment group and trial was also found [$F(22,1152) = 2.05$, $P = .003$].

Rearing behavior or vertical activity of female rats also habituated during the initial 1-h session (Fig. 1C). The three groups did not differ during this habituation period. Fig. 1D shows that cocaine increased vertical activity relative to the habituation period ($P < .0001$). Similar to the pattern seen for horizontal activity, rats that were lavaged exhibited the lowest levels of cocaine-stimulated vertical activity and naive rats, the most. Touched rats exhibited an intermediate level of cocaine-stimulated rearing behavior. Vertical activ-

ity increased rapidly from 5 to 20 min postinjection in the naive group and then declined steeply to the end of the session. The time course of cocaine-stimulated vertical activity in the lavaged rats was much shallower than the other two groups. ANOVA confirmed a significant difference between treatment groups [$F(2,95) = 9.44$, $P < .0002$]. Post-hoc analysis indicated that the naive group reared significantly more than both the touched and lavaged groups and that the touched group reared more than the lavaged group ($P < .05$). The main effect of trial was highly significant ($P < .0001$) and a significant interaction of treatment group and trial was also found [$F(1,86) = 8.2$, $P = .005$].

Estrous cycle stage was assessed in this experiment to determine if one of the three treatment groups had a skewed distribution of cycle stages. Cycle stage was known on the day of testing for all the rats except for about half of the naive rats because they were not checked. Repeated lavage did not induce pseudopregnancy in any rat and 26 of 33 rats in this group exhibited normal 4- or 5-day estrous cycles. The percentages of rats in diestrus were for each group: lavaged (63%), touched (57%), and naive (75%). Thus, the lavaged group was not disproportionately represented by low-responding diestrous females (see Section 3.2 for estrous cycle effects).

3.2. The effect of acute lavage in intact female rats

The effect of a single vaginal lavage performed prior to behavioral testing on motor behavior of naive female rats is shown in Fig. 2. Acute lavage had no effect on horizontal or vertical activity during habituation or following injection of 10 mg/kg cocaine. Cocaine significantly increased both horizontal and vertical activity (P's $< .0001$).

3.3. The effect of estrous cycle

Fig. 3 shows the effect of estrous cycle on cocaine-stimulated activity in repeatedly lavaged (from Fig. 1) and nonlavaged (from Figs. 1 and 2) female rats (see Section 2). Estrous cycle did not significantly affect horizontal or vertical activity during habituation and those data are not shown. Levels of horizontal and vertical activity in diestrous females were virtually identical in lavaged and nonlavaged groups. However, activity levels in proestrus and estrous females were markedly lower in lavaged than nonlavaged rats. Estrous cycle stage significantly affected cocaine-stimulated horizontal activity in nonlavaged rats [$F(2,84) = 6.63$, $P = .002$]. Fig. 3A shows that nonlavaged rats in proestrus and estrus locomoted significantly more than diestrous females after injection with 10 mg/kg cocaine. A similar effect of estrous cycle stage on vertical activity was observed [$F(2,83) = 4.04$, $P = .02$]. Fig. 3C shows that nonlavaged rats in estrus reared significantly more than females in diestrus. In contrast, no estrous cycle-related differences in cocaine-stimulated horizontal and vertical activity were observed in females vaginally lavaged repeatedly.

3.4. The ability of vaginal lavage to establish conditioned place preference in female rats

The average initial preference for the dark compartment was 65% (data not shown) and the three treatment groups were not significantly different in this regard ($P = .36$). Fig. 4 shows the difference (delta) in time spent on the initially nonpreferred side subtracted from the time spent on that side after four conditioning sessions associated with either vaginal lavage before the session, touch before, or lavage after the session. Rats that were lavaged immediately prior to placement on the nonpreferred side increased the amount of time spent on that side as a result of the conditioning by an average of 104 s. In contrast, the initial side bias of rats that were touched before or lavaged after the session did not change as a result of conditioning. One-way ANOVA indicated a significant main effect of treatment [$F(2,29) = 9.8$, $P = .002$]. Post-hoc analysis showed that the delta for the lavage before rats was significantly greater than both the touched and lavage after groups ($P < .05$).

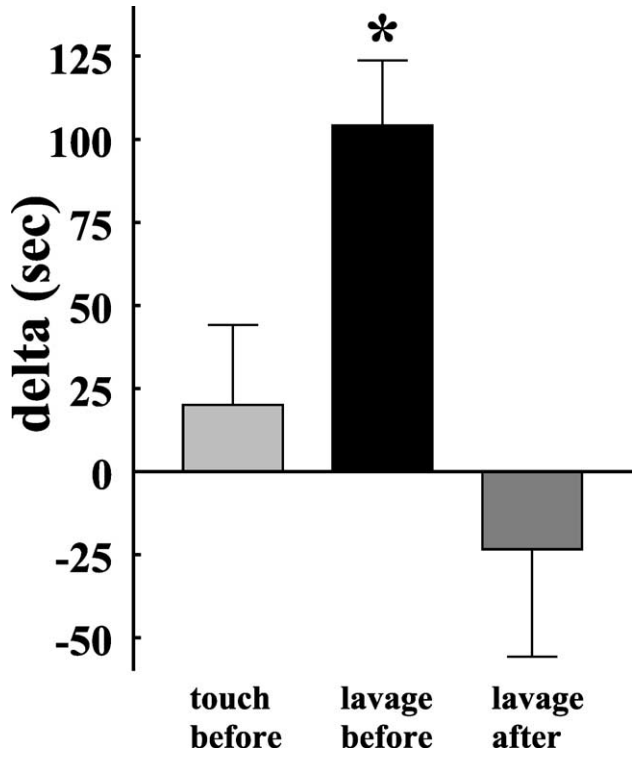

Fig. 4. Ability of vaginal lavage and touch to establish a conditioned place preference. Female rats were either touched on the thigh before ($n = 9$), vaginally lavaged before ($n = 10$), or lavaged after ($n = 10$) placement on the initially nonpreferred side using a two-compartment design (see Section 2). * Significantly different than touched and naive rats ($P < .05$).

4. Discussion

The present results show that vaginal lavage, a technique previously considered a "noninvasive" method to determine estrous cycle stage, significantly decreased cocaine-stimulated behavior. Repeated lavage attenuated cocaine-stimulated horizontal and vertical activity relative to both nonlavaged and touched rats. Our novel finding that vaginal lavage established a place preference in female rats further suggests that lavage is a profound signal to the animal because it serves as a reinforcing stimulus. Thus, this supposedly benign manipulation, through an as yet unknown physiologic mechanism, proved a reinforcing stimulus in female rats that substantially altered cocaine-stimulated activity.

Cocaine-stimulated motor behavior was greatest in female rats that had been handled least (naive group) before testing. The effects of lavage and/or touch were specific to cocaine-stimulated activity, as activity levels of all three groups were identical during habituation to a novel environment. Touch induced disparate effects on the two behavioral topographies. Cocaine-stimulated horizontal activity in touched rats was not significantly different than that of naive rats; however, touch induced intermediate levels of vertical activity. Thus, vertical activity was more sensitive than horizontal activity by detecting differences in magnitude of the effects of touch and vaginal lavage. Similarly, our previous report indicated that vertical activity was more sensitive than horizontal activity for detecting ovariectomy-induced decreases in cocaine-stimulated motor behavior (Walker et al., 2001a).

The conditioned place preference paradigm has been used to determine motivational properties of both drug and nondrug reinforcers (Tzschentke, 1998). The present studies used this paradigm to demonstrate that vaginal lavage established a place preference in female rats. This preference was seen in the group that was lavaged prior to, but not after, the conditioning session. Similar strategies have been used in contextual sensitization experiments with cocaine (Post et al., 1981, 1987). Thus, the rats associated the lavage effects experienced during confinement to one particular side and reported an increased preference for the conditioned side in the choice test session. This implies that neural sequelae from the stimulation endured and thereby enabled conditioning to the paired compartment. Touching the rats before conditioning had no effect on place preference. Thus, the effect of touching was behavioral topography-specific as it significantly decreased cocaine-stimulated vertical activity, but did not affect conditioned place preference or horizontal activity.

The present study appears to be the first demonstration that conditioned place preference can be acquired by manual vaginocervical stimulation. Prior reports have shown place preference can be induced in females by mating and related stimulation from males. For instance, Meisel and Joppa (1994) showed that both sexual and aggressive activity with male hamsters induced a place preference in ovariectomized, hormone-primed female hamsters. Also, in ovariectomized, hormone-primed female hamsters, Paredes and Alonso (1997) demonstrated specifically that paced mating produced a place preference to a separate compartment in which the hamsters were placed after mating. Oldenburger et al. (1992) conducted a similar place preference study with ovariectomized, hormone-primed female rats that showed a place preference to the compartment in which sexual interactions with a male rat occurred. The preference was only seen during the final 5-min period of the 15-min choice session, however. Our study, which has the advantage of being conducted in intact, cycling females, suggests that this brief insertion of the dropper into the vagina represents a sexual signal that is reinforcing like the stimulation from mating in the studies discussed above.

The physiologic mechanism through which vaginal lavage decreased cocaine-stimulated motor activity and produced a place preference is unclear. Previous literature shows that afferents from the vagina and cervix influence neural functions and behavior. Vaginocervical stimulation induced an increase in basal firing rate of units in the mitral cell layer of the main olfactory bulb when rats were in proestrus or estrus, but decreased firing rate when rats were in metestrus or diestrus (Guevara-Guzman et al., 1997). This report and another (Estrada-Palma et al., 1993) indicate that an afferent pathway from the vagina and/or cervix influences the olfactory bulb, a brain region known to affect female reproductive behavior (McGinnis et al., 1985).

Vaginocervical stimulation has also been reported to affect dopaminergic neurotransmission. Such reports are particularly relevant to the present findings because psychostimulant drugs like cocaine induce behavioral and reinforcing effects by binding to the dopamine transporter and inhibiting the uptake of dopamine (Heikkila et al., 1979; Ritz et al., 1987; Wise and Rompre, 1989). Mermelstein and Becker (1995) have shown that dopamine in striatal dialysates increases in female rats during mating. Pfaus et al. (1995) have shown that extracellular dopamine increases more in the nucleus accumbens than in the striatum of female rats during mating. Kohlert et al. (1997) have further shown that intromission by the male is the essential part of mating that increases nucleus accumbens dopamine in female hamsters. Meredith et al. (1998) found that vaginocervical stimulation increased phosphorylation of DAARPP-32, a postsynaptic D_1 receptor mediated response likely evoked by increased extracellular dopamine. Thus, these reports indicate that vaginocervical stimulation increases extracellular dopamine and thereby may alter the dopaminergic systems that mediate the locomotor responses to cocaine.

The ability of vaginocervical stimulation to increase dopamine release could be a mechanism for establishing a conditioned place preference. However, the present finding that vaginocervical stimulation depresses subsequent cocaine responses seems counterintuitive since

prior exposure to stimulants (and thereby increased dopamine stimulation) typically sensitizes subsequent behavioral responses to stimulants. Two reports offer limited support for the possibility of diminished dopaminergic response following mating and parturition. One study found decreased dopamine metabolite concentrations in dialysate from primiparous relative to nulliparous rats in response to onset of dark-cycle and haloperidol (Hucke et al., 1998). The same group found attenuated dopamine-related behavioral responses in primiparous relative to nulliparous rats (Hucke et al., 2001). Because the effects of vaginocervical stimulation induced by mating in these primiparous rats are confounded by parturition, etc., additional studies are needed to support diminished dopaminergic activity due to vaginocervical stimulation.

A nondopaminergic mechanism for vaginocervical stimulation inducing oxytocin release and thereby attenuating cocaine-stimulated activity is possible but speculative. Vaginal distension induces oxytocin release (Dreifuss et al., 1976; Kendrick et al., 1993; Negoro et al., 1987). Systemic oxytocin administration has been shown to decrease cocaine-stimulated exploratory activity in rats and mice (Kovacs et al., 1998). Both subcutaneous and intracerebroventricular oxytocin administration inhibited cocaine-induced sniffing (Sarnyai et al., 1991). Moreover, injections of oxytocin into the nucleus accumbens and olfactory tubercles also inhibited cocaine-induced sniffing (Sarnyai et al., 1991). Thus, oxytocin is another potential mediator between vaginocervical stimulation and cocaine-stimulated activity.

The vaginal stimulation employed in the present study is minimal in comparison to that employed in other studies to show neural effects (Guevara-Guzman et al., 1997; Meredith et al., 1998). However, such minimal stimulation has been shown to produce a short-term facilitation of lordosis (Rodriguez-Sierra et al., 1977) and to induce Fos immunoreactivity in the medial preoptic area, lateral septum, bed nucleus of the stria terminalis, and ventromedial hypothalamus (Pfaus et al., 1996). Thus, our finding that vaginal lavage is a reinforcing stimulus is not surprising in light of this literature showing substantial behavioral effects of minimal vaginal stimulation.

The decrease in cocaine-stimulated activity after vaginocervical stimulation required repeated stimulation in contrast to the previously discussed effects of vaginocervical stimulation and oxytocin administration that occurred immediately after a single stimulation. The fact that a single lavage did not affect cocaine-stimulated activity in these studies suggests that one or more of the acute effects of vaginocervical stimulation induce an enduring neural effect(s) that subsequently influences locomotor responses to cocaine. These data show that acute effects of vaginal lavage are sufficient to produce conditioned place preference but require either more time following a single stimulation or multiple stimulations to alter cocaine-stimulated activity.

The current results may explain the variable effect of ovariectomy on cocaine-stimulated locomotion in our previous report (Walker et al., 2001a). That study showed that ovariectomy decreased cocaine-stimulated activity only in rats that had not been vaginally lavaged. The current results indicate that lavage attenuates high behavioral responses in intact nonlavaged rats (in proestrus and estrus) and should thereby attenuate the difference between intact and ovariectomized females.

The current results also clarify another previous report from this laboratory that cocaine-stimulated activity does not vary across the female estrous cycle (Walker et al., 1998). The females in that study were vaginally lavaged prior to testing like the repeated lavage animals in the present report. The present results in nonlavaged rats indicate that estrous cycle does influence cocaine-stimulated locomotion, an effect apparently masked by repeated lavage in our previous studies. Sell et al. (2000) recently reported that horizontal locomotion was greater in estrous and proestrous than diestrous 2 but not diestrous 1 females administered 5 mg/kg cocaine ip. Their study also showed that rearing was greater in proestrous than diestrous 2 females. Why repeated lavage masked estrous cycle effects in this laboratory but not another is unclear. The present finding is fortuitous nonetheless because it points to a putative target for modulating high cocaine sensitivity in females. A most exciting aspect of these studies is the possibility that cocaine effects may be attenuated through a neural site or circuit other than antagonism of the dopamine transporter, where much research is currently directed.

In conclusion, we have shown that repeated but not acute vaginal lavage attenuated cocaine-stimulated horizontal and vertical activity. Repeated lavage decreased the enhanced cocaine-stimulated activity observed in nonlavaged proestrous and estrous females. Furthermore, vaginal lavage induced a conditioned place preference. The implications of these studies are two-fold. First, the use of vaginal lavage in rodent behavioral studies of psychostimulant action may influence the results of these experiments and a less invasive technique like vaginal swabbing might therefore be useful. Lastly, the central nervous system mechanism supporting this lavage effect merits further investigation, in particular, against cocaine self-administration in animals.

Acknowledgements

This work was supported by grant DA09079 to CMK.

References

Becker JB. Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacol, Biochem Behav 1999;64:803–12.
Becker JB, Beer ME. The influence of estrogen on nigrostriatal dopamine

activity: behavioral and neurochemical evidence for both pre- and postsynaptic components. Behav Brain Res 1986;19:27–33.
Becker JB, Cha JH. Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. Behav Brain Res 1989;35:117–25.
Becker JB, Robinson TE, Lorenz KA. Sex differences and estrous cycle variations in amphetamine-elicited rotational behavior. Eur J Pharmacol 1982;80:65–72.
Becker JB, Molenda H, Hummer DL. Gender differences in the behavioral responses to cocaine and amphetamine. Implications for mechanisms mediating gender differences in drug abuse. Ann NY Acad Sci 2001; 937:172–87.
Bowman BP, Kuhn CM. Age-related differences in the chronic and acute response to cocaine in the rat. Dev Psychobiol 1996;29:597–611.
Camp DM, Robinson TE. Susceptibility to sensitization: I. Sex differences in the enduring effects of chronic D-amphetamine treatment on locomotion, stereotyped behavior and brain monoamines. Behav Brain Res 1988;30:55–68.
Camp DM, Becker JB, Robinson TE. Sex differences in the effects of gonadectomy on amphetamine-induced rotational behavior in rats. Behav Neural Biol 1986;46:491–5.
Cooper RL, Goldman JM, Vandenbergh JG. Monitoring of estrus cyclicity in the laboratory rodent by vaginal lavage. In: Chapin RE, Heindel JJ, editors. Reproductive toxicology. Methods in toxicology, female reproductive systems vol. 3B. Orlando, FL: Academic Press, 1993. p. 45–56.
Dreifuss JJ, Tribollet E, Baertschi AJ. Excitation of supraoptic neurones by vaginal distention in lactating rats; correlation with neurohypophysial hormone release. Brain Res 1976;113:600–5.
Estrada-Palma LY, Solano-Flores LP, Aldana A, Guevara-Guzman R, Wayner MJ. Olfactory bulb neurons respond to cervicovaginal distension. Brain Res Bull 1993;32:467–9.
Guevara-Guzman R, Barrera-Mera B, Weiss ML. Effect of the estrous cycle on olfactory bulb response to vaginocervical stimulation in the rat: results from electrophysiology and Fos immunocytochemistry experiments. Brain Res Bull 1997;44:141–9.
Haney M, Castanon N, Cador M, Le Moal M, Mormede P. Cocaine sensitivity in Roman high and low avoidance rats is modulated by sex and gonadal hormone status. Brain Res 1994;645:179–85.
Heikkila RE, Cabbat FS, Manzino L, Duvoisin RC. Rotational behavior induced by cocaine analogs in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra: dependence upon dopamine uptake inhibition. J Pharmacol Exp Ther 1979;211:189–94.
Hucke EE, Cruz-Casallas PE, Florio JC, Felicio LF. Reproductive experience reduces striatal dopaminergic responses in freely moving female rats. NeuroReport 1998;9:3589–93.
Hucke EE, Cruz-Casallas PE, Sider LH, Felicio LF. Reproductive experience modulates dopamine-related behavioral responses. Pharmacol, Biochem Behav 2001;68:575–82.
Joyce JN, van Hartesveldt C. Behaviors induced by intrastriatal dopamine vary independently across the estrous cycle. Pharmacol, Biochem Behav 1984;20:551–7.
Kendrick KM, Fabre-Nys C, Blache D, Goode JA, Broad KD. The role of oxytocin release in the mediobasal hypothalamus of the sheep in relation to female sexual receptivity. J Neuroendocrinol 1993;5:13–21.
Kohlert JG, Rowe RK, Meisel RL. Intromissive stimulation from the male increases extracellular dopamine release from fluoro-gold-identified neurons within the midbrain of female hamsters. Horm Behav 1997; 32:143–54.
Kovacs GL, Sarnyai Z, Szabo G. Oxytocin and addiction: a review. Psychoneuroendocrinology 1998;23:945–62.
Kuhn CM, Walker QD, Kaplan KA, Li ST. Sex, steroids, and stimulant sensitivity. Ann NY Acad Sci 2001;937:188–201.
Long J, Evans HM. The oestrous cycle in the rat and its associated phenomena. Berkeley, CA: University of California Press, 1992.
McGinnis MY, Lumia AR, McEwen BS. Increased estrogen receptor binding in amygdala correlates with facilitation of feminine sexual behavior induced by olfactory bulbectomy. Brain Res 1985;334: 19–25.
Meisel RL, Joppa MA. Conditioned place preference in female hamsters following aggressive or sexual encounters. Physiol Behav 1994; 56:1115–8.
Meredith JM, Moffatt CA, Auger AP, Snyder GL, Greengard P, Blaustein JD. Mating-related stimulation induces phosphorylation of dopamine- and cyclic AMP-regulated phosphoprotein-32 in progestin receptor-containing areas in the female rat brain. J Neurosci 1998;18:10189–95.
Mermelstein PG, Becker JB. Increased extracellular dopamine in the nucleus accumbens and striatum of the female rat during paced copulatory behavior. Behav Neurosci 1995;109:354–65.
Negoro H, Uchide K, Tadokoro Y, Honda K, Higuchi T. Vaginal distension induces milk ejection-related burst of oxytocin neurones interacting with suckling stimuli in lactating rats. Brain Res 1987;404:371–4.
Oldenburger WP, Everitt BJ, de Jonge FH. Conditioned place preference induced by sexual interaction in female rats. Horm Behav 1992;26: 214–28.
Paredes RG, Alonso A. Sexual behavior regulated (paced) by the female induces conditioned place preference. Behav Neurosci 1997;111: 123–8.
Pfaus JG, Damsma G, Wenkstern D, Fibiger HC. Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. Brain Res 1995;693:21–30.
Pfaus JG, Marcangione C, Smith WJ, Manitt C, Abillamaa C. Differential induction of Fos in the female rat brain following different amounts of vaginocervical stimulation: modulation by steroid hormones. Brain Res 1996;741:314–30.
Post RM, Lockfeld A, Squillace KM, Contel NR. Drug-environment interaction: context dependency of cocaine-induced behavioral sensitization. Life Sci 1981;28:755–60.
Post RM, Weiss SR, Pert A. The role of context and conditioning in behavioral sensitization to cocaine. Psychopharmacol Bull 1987;23: 425–9.
Quinones-Jenab V, Ho A, Schlussman SD, Franck J, Kreek MJ. Estrous cycle differences in cocaine-induced stereotypic and locomotor behaviors in Fischer rats. Behav Brain Res 1999;101:15–20.
Ritz MC, Lamb R, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 1987;237:1219–23.
Robinson TE, Camp DM, Becker JB. Gonadectomy attenuates turning behavior produced by electrical stimulation of the nigrostriatal dopamine system in female but not male rats. Neurosci Lett 1981;23: 203–8.
Robinson TE, Camp DM, Jacknow DS, Becker JB. Sex differences and estrous cycle dependent variation in rotational behavior elicited by electrical stimulation of the mesostriatal dopamine system. Behav Brain Res 1982;6:273–87.
Rodriguez-Sierra JF, Crowley WR, Komisaruk BR. Induction of lordosis responsiveness by vaginal stimulation rats is independent of anterior or posterior pituitary hormones. Horm Behav 1977;8:348–55.
Sarnyai Z, Babarczy E, Krivan M, Szabo G, Kovacs GL, Barth T, Telegdy G. Selective attenuation of cocaine-induced stereotyped behaviour by oxytocin: putative role of basal forebrain target sites. Neuropeptides 1991;19:51–6.
Savageau MM, Beatty WW. Gonadectomy and sex differences in the behavioral responses to amphetamine and apomorphine of rats. Pharmacol, Biochem Behav 1981;14:17–21.
Schneider BF, Norton S. Circadian and sex differences in hyperactivity produced by amphetamine in rats. Physiol Behav 1979;22:47–51.
Sell SL, Scalzitti JM, Thomas ML, Cunningham KA. Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. J Pharmacol Exp Ther 2000;293:879–86.
Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 1998;56:613–72.
van Haaren F, Meyer ME. Sex differences in locomotor activity after acute

and chronic cocaine administration. Pharmacol, Biochem Behav 1991; 39:923–7.

van Hartesveldt C, Joyce JN. Effects of estrogen on the basal ganglia. Neurosci Biobehav Rev 1986;10:1–14.

Walker QD, Cabassa J, Kuhn CM. Locomotor and neuroendocrine responses to cocaine: sex and estrous cycle differences. Soc Neurosci Abstr 1998;24:495.

Walker QD, Rooney MB, Wightman RM, Kuhn CM. Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. Neuroscience 2000;95:1061–70.

Walker QD, Cabassa J, Kaplan KA, Li S, Haroon J, Spohr HA, Kuhn CM. Sex differences in cocaine-stimulated motor behavior: disparate effects of gonadectomy. Neuropsychopharmacology 2001a;25: 118–30.

Walker QD, Francis R, Cabassa J, Kuhn CM. Effect of ovarian hormones and estrous cycle on stimulation of the hypothalamo–pituitary–adrenal axis by cocaine. J Pharmacol Exp Ther 2001b;297: 291–8.

Wise RA, Rompre PP. Brain dopamine and reward. Annu Rev Psychol 1989;40:191–225.