

The Role of Dopamine Receptors in the Medial Preoptic Area
on the Sexual Behaviour of Female Rats

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ABSTRACT

The Role of Dopamine Receptors in the Medial Preoptic Area

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M. Dean Graham

The medial preoptic area (mPOA) is critical in the control of male sexual behaviour, and dopamine (DA) plays an important role within it. However, both the roles of DA and the mPOA in female sexual behaviour are not fully understood, with few studies producing consistent data. In the present set of experiments the role of DA within the mPOA on the full cascade of female sexual behaviour is investigated. Ovariectomized female rats were bilaterally cannulated into the mPOA and hormonally primed either fully, with estradiol benzoate (EB) and progesterone (P), or partially with EB-alone. Since it was hypothesized that DA plays a facilitative role in female sexual behaviour, a nonselective DA receptor, and selective DA D1 and D2 receptor agonists (apomorphine, SKF 38393 and quinpirole, respectively) were infused to EB-alone females and nonselective DA receptor, and selective DA D1 and D2 receptor antagonists (flupenthixol, SCH 23390, and raclopride, respectively) were infused to EB+P rats. Copulatory behaviour was then immediately tested over a period of thirty minutes in a bilevel chamber with a sexually experienced male. Precopulatory behaviours were increased in EB-alone females following infusions of a low dose (0.25 µg) of apomorphine and both a low (0.05 µg) and a high dose (0.2 µg) of quinpirole. Hops and/or darts were decreased following infusion of a low dose (0.05 µg) of SKF 38393. In EB+P females, precopulatory behaviours were decreased following infusions of a high dose (4.0 µg) of SCH 23390, but were increased following infusions of a high dose (4.0 µg) of raclopride. Flupenthixol had no effect on sexual behaviour. These results suggest that the ratio of DA D1/D2 activity within the mPOA of female rats is critical for the expression of precopulatory behaviours, and may work within brain areas responsible for stimulating lordosis to control the timing of female sexual behaviour.

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Female sexual behaviour is characterised by three phases of activity described as appetitive, consummatory and precopulatory (Pfaus, Kippin, & Coria-Avila, 2003). Appetitive behaviours serve to bring a female in close proximity to a male, and signal the female's interest in copulation. Consummatory behaviours allow for the copulation to take place, and are usually stereotyped, sexually differentiated, and species-specific (Pfaus et al., 2003). Precopulatory behaviours occur both before and during copulation, and serve to trigger mounting behaviour by the male, which in turn elicit a reflexive posture known as lordosis, the only unambiguous consummatory response (Pfaff & Schwartz-Giblin, 1988; Pfaus, 1999b). Similar to what Beach (1976) called proceptive behaviours, precopulatory behaviours act as a transition from appetitive to consummatory (Pfaus et al., 2003). This occurs through arousal of the male, enticing him to move from distal to proximal in order to complete the goal of copulation (Pfaus et al., 2003); thereby increasing the likelihood that the female will become pregnant (Erskine, Kornberg, & Cherry, 1989). The most commonly studied precopulatory behaviours include solicitations, hops and/or darts, and pacing behaviour.

For copulation to be successful in the rat, the female must first solicit sex from the male, pace the subsequent sexual contact with him, and finally show lordosis when the male mounts. As a copulatory series progresses, the number of solicitations decrease, while lordoses displayed and the time spent pacing increase (Bermant, 1961; Peirce & Nuttall, 1961; Krieger, Orr, & Perper, 1976; McClintock & Adler, 1978; Erskine, 1985; Pfaus, Smith, Byrne, & Stephens, 2000). Thus, while precopulatory behaviours and lordosis frequently occur during a copulatory session, these two sets of behaviours are mutually exclusive and cannot occur simultaneously.

The precopulatory and consummatory aspects of female sexual behaviour are under the control of hypothalamic-limbic circuits, which contain concentrations of receptors for the ovarian steroid hormones estrogen (E) and progesterone (P). These are necessary for the full display of female sexual behaviour (Beach, 1976; Pfaff & Schwartz-Giblin, 1988). For example, it is thought that E activates lordosis through binding in the ventromedial nucleus of the hypothalamus

(VMH), while P further promotes lordosis within that area, while also stimulating precopulatory behaviours (Whalen, 1974). This activation occurs in a complex interplay within these hypothalamic circuits, and P receptors are found within many brain areas including the VMH and medial preoptic area (mPOA; MacLusky & McEwen, 1978), and the ventral tegmental area (VTA; Frye, 2001; Frye & Walf, 2008).

The VMH is the most extensively studied brain area for female sexual behaviour within the hypothalamic circuit. Electrical stimulation facilitates lordosis in E-primed rats (Pfaff & Sakuma, 1979), while lesions abolish it (Mathews & Edwards, 1977; Pfaff & Sakuma, 1979). Implantation of E to the VMH restores lordosis in females that have had VMH lesions (Rubin & Barfield, 1980; 1983a), while implantation of P into the VMH of E-primed rats facilitates female sexual behaviour, including lordosis and solicitations, in a synergistic manner (Rubin & Barfield, 1983b). It has therefore been concluded that the VMH is an area of critical importance for E to promote female sexual behaviour in rats (Pfaff & Schwartz-Giblin, 1988), and is the most important region for the display of lordosis (Kow & Pfaff, 1998).

The VMH shares connections to the mPOA (Conrad & Pfaff, 1975), although the nature of these connections is unknown. The mPOA is an area of critical significance in male sexual behaviour, as efferent projections from the mPOA are critical for the initiation of copulation in male rats (Hull et al., 1999). For example, mPOA-lesioned males are unable to commence mount and thrust patterns, but may still demonstrate appetitive behaviour to be with a female (Hansen, Kohler, Goldstein, & Steinbusch, 1982; Everitt, 1990). The major efferent projections of the mPOA are to hypothalamic, midbrain, and brain stem nuclei that regulate autonomic and somatomotor patterns, as well as motivational states (see Simerly & Swanson, 1988). Specifically in female rats, projections to the VTA in the midbrain (Brackett & Edwards, 1984), nucleus accumbens (NAcc), anterior cingulate cortex (ACC), and main olfactory (piriform) cortex (PirCtx; Gaykema, Luiten, Nyakas, & Traber, 1990), may be critical for the display of solicitations (Coria-Avila & Pfau, 2007).

In contrast to lesions of the VMH, which impair lordosis, lesions to the mPOA appear to affect female precopulatory behaviours; however there is considerable variation in such findings. Originally, studies found that lordosis responses were enhanced by lesions to the mPOA in rats (Law & Meagher, 1958; Powers & Valenstein, 1972) and guinea pigs (Rodriguez-Sierra & Terasawa, 1979). These findings were supported by studies employing electrical stimulation to the mPOA, discovering that the stimulation reduced lordosis frequency in rats (Napoli, Powers, & Valenstein, 1972; Moss, Paloutzian, & Law, 1974) and hamsters (Malsbury, Pfaff, & Malsbury, 1980). The conclusion that the mPOA exerts an inhibitory influence on lordosis was then formed.

Other studies have found that the potentiation of lordosis by mPOA lesion is context-specific, as Whitney (1986) reported that these effects depended on the testing situation. If a female was tested in a chamber where she could not escape, thereby unable to control the amount of stimulation she received from the male, mPOA lesions did potentiate lordosis. Solicitatorial behaviour, measured by Whitney (1986) using pose frequency, did not change from that of the baseline tests. In an exit test, where a female could flee from the male at her preferred interval and the male could not follow, the display of lordosis was no different from that of female rats without mPOA lesions. Lesioned rats did spend less time with the males, receiving fewer mounts, intromissions and ejaculations. Solicitatorial behaviour was reduced however, as mPOA-ablated rats exhibited fewer poses compared to that of controls (Whitney, 1986). Finally, in contrast to the above mentioned studies, lordosis was reported to be disrupted in a no-exit condition by preoptic area (POA) lesions by Bast and collaborators (1987); an area which includes the mPOA.

More recent studies have focused not only on lordosis, but also on precopulatory behaviours. Hoshina and colleagues (1994) lesioned the mPOA using ibotenic acid and found that while it increased lordosis in females, in line with the earliest studies mentioned above, it also resulted in a reduction of precopulatory behaviours, decreasing the number of solicitations,

hops and darts, and disrupting pacing. Hops, darts, ear wiggling and pacing behaviours were also diminished in female rats with mPOA lesions, but not in animals with medial amygdala (MeA) or bed nucleus of the stria terminalis (BNST) lesions (Guarraci, Megroz, & Clark, 2004). In this study however, the sexual receptivity of the female was not affected in any way. These suppressions of precopulatory behaviours are in accordance, and thus extend, the similar finding using pose frequency as the sole measurement (Whitney, 1986). The data are therefore consistent with the idea that the mPOA is critical for the expression of precopulatory behaviours, while its role in regards to consummatory behaviours remains unclear. As noted by Erskine (1989), and based on work by Whitney (1986), the variation in responsiveness of POA-lesioned females may potentially be due to the characteristics of the testing environment, such as the exit versus no-exit conditions Whitney (1986) examined. In addition, the discrepancies in lordosis reported in these studies may be due to an inability to perform precopulatory behaviours due to the testing environment. The female may instead exhibit an increase in lordosis, in order to serve the purpose of triggering male mounting. This suggests that the mPOA may control the motivational aspects of copulation through the management of precopulatory behaviours instead of consummatory behaviours, which are mainly under the control of the VMH.

It is possible that both precopulatory and consummatory behaviours occur in a synchronized manner, as a consequence of activational “leakage” across multiple sets of receptors or brain areas. This may result in one set of behaviours being displayed whilst the other set is inhibited. While much is known about neurotransmission in the VMH in the control of female consummatory behaviours (for review, see Kow, Mobbs, & Pfaff, 1994), far less is known about the role of other brain structures in regards to female sexual behaviour, most importantly the mPOA.

Additional techniques examining sexual behaviour have contributed more credence to the role of the mPOA on female sexual behaviour. For example, mechanical stimulation of the cervix has been shown to excite neurons in the mPOA (Haskins & Moss, 1983). Furthermore, in

the mPOA, expression of c-fos immunoreactivity, a marker of brain activation, has been shown to increase in response to vaginocervical stimulation (VCS) both from a male partner during copulation (Pfaus, Kleopoulos, Mobbs, Gibbs, & Pfaff, 1993) and artificially from a glass rod (Pfaus, Marcangione, Smith, Manitt, & Abillamaa, 1996). Of particular interest is the finding that mPOA lesions influence the temporal pattern of female sexual behaviour by increasing the latency for females to return to the male following intromissions and ejaculations. This increase in time to return was not seen following non-intromissive mounts, as they do not involve VCS (Yang & Clemens, 2000). The authors theorized that the mPOA may play a vital role in processing and responding to VCS, which may be conveyed by pelvic and pudendal nerves to central control mechanisms as part of the supraspinal neural circuit. It is thought that perhaps mPOA lesions disrupt the processing of genital stimulation by interrupting this neural circuit. Since the lesioned animals in this study also spent less time with the male, this lends additional support to the idea that the mPOA influences precopulatory behaviours as well (Yang & Clemens, 2000).

By concentrating on identifying different types of neurons in the POA, Kato and Sakuma (2000) found that neurons in the mPOA may have the ability to respond preferentially to varying aspects of both precopulatory and consummatory behaviours in a no-exit environment. Using a bundle of wire electrodes to record single unit activities, the authors were able to record 31 neurons in the POA, identifying four types of neurons overall. Type 1 neurons increased their firing rate when precopulatory behaviours were initiated by the female; these neurons continued firing from the initiation of a solicitation up to when the male intromitted, at which time their firing became suppressed. If the male only mounted without penile insertion, this suppression of activity was not seen. Type 2 neurons showed a brief activation when the female was mounted by the male, while type 3 neurons exhibited activation when the female was intromitted by the male. Finally, type 4 neurons were inhibited immediately before and during any display of lordosis (Kato & Sakuma, 2000). Interestingly, neurons in the type 1-3 category were located in

the transitional region between the medial and lateral POAs, while type 4 neurons were located medially, falling within the mPOA. The authors believe that type 1 neurons signify the motivational state of the rat, represented by solicitations. There may also be a role for the POA in consummatory behaviours following penile intromission. Type 2 and type 3 neurons may be firing in response to visceral or somatosensory input, while type 4 neurons act as though they inhibit the execution of lordosis (Kato & Sakuma, 2000). Therefore it seems that the female mPOA, and the areas surrounding it, have neurons that respond to all types of sexual behaviour. Some are specific for precopulatory behaviours, and some for consummatory behaviours, including the particular components that compose it.

Lesions to the mPOA disrupt male sexual behaviour in practically all species studied to date, including rats, mice, guinea pigs, rhesus monkeys and many others (reviewed in Hart & Leedy, 1985; Meisel & Sachs, 1994). Following mPOA lesions, consummatory aspects of male sexual behaviour normally shown during a copulatory bout are impeded, including initiation and execution; however these lesions may not affect sexual interest (Slimp, Hart, & Goy, 1978; Hansen, 1982). The hypothesis that mPOA lesions fail to affect sexual interest has been raised due to the evidence that lesioned male monkeys continue to masturbate, even though copulation has been attenuated, if not eliminated (Slimp et al., 1978). Lesioned male mice also continue to emit ultrasonic “courtship” vocalizations though mating has ceased (Bean, Nunez, & Conner, 1981), and male rats with mPOA lesions continue to pursue receptive females, and even show inappropriately positioned mounts without pelvic thrusting, though they do not copulate (Hansen, 1982; Brackett & Edwards, 1984). Evidence against this hypothesis comes from a study showing that mPOA-lesioned male rats showed a decreased preference for a receptive female (Edwards & Einhorn, 1986).

More recently there has been a shift in thinking, whereby mPOA lesions are thought to reduce copulation by decreasing sexual motivation (Paredes & Baum, 1997; Paredes, 2003). Support comes from the lack of approach mPOA-lesioned males demonstrate towards a receptive

female in an unconditioned sexual incentive motivation task (Paredes & Baum, 1995; Paredes, Tzschentke, & Nakach, 1998; Agmo, 2003). This effect has even been shown with temporary inactivation of the mPOA through an infusion of lidocaine, causing temporal inhibition of sexual motivation (Hurtazo, Paredes, & Agmo, 2008). Thus, a theory was put forward that the preoptic motor region, formed by the lateral part of the mPOA and the lateral POA (Sinnamon, 1992), works to bring the rat into proximity with stimuli deemed to be sexually incentive, forming the basis of sexual motivation (Sinnamon, 1993; Hurtazo et al., 2008).

The mPOA contains receptors which may allow neurotransmitters to influence sensory processing in regards to sexually relevant stimuli (Hull et al., 1999). Of special importance is dopamine (DA), a catecholamine neurotransmitter associated with the mediation of reward-related appetitive behaviour (i.e. Wise & Rompre, 1989; Salamone, 1994; Ikemoto, Glazier, Murphy, & McBride, 1997; Sutton & Beninger, 1999). A common characteristic of dopaminergic action is augmentation of sensorimotor function, thought to be attained through removal of tonic inhibition (Chevalier & Deniau, 1990). This removal of tonic inhibition allows other steroid hormones to increase the responsiveness of certain neurons. This means that DA may not directly influence behaviour on its own, but instead allows stimuli to have easier access to hormonally-primed output pathways (Hull, Du, Lorrain, & Matuszewich, 1997).

Five different DA receptors have been identified, designated as D1-D5. These make up two subtypes: the D1 family consists of the D1 and D5 receptors, while the D2 family consists of the D2, D3, and D4 receptors. The distinguishing characteristic separating these receptors are that the D1 family stimulate adenylate cyclase, whereas D2 receptors either inhibit, or have no effect on the enzyme (reviewed in Civelli, Bunzow, & Grandy, 1993). Most often these two families of receptors act synergistically, though occasionally the two subtypes produce opposing influences (reviewed in Arnt, 1987).

Dopamine's input to the mPOA comes predominantly from the zona incerta (Wagner, Eaton, Moore, & Lookingland, 1995), which is a subthalamic nucleus found caudal to the

hypothalamus, and which in turn receives diffuse input from the motor cortex and cerebellum (Bjorklund, Lindvall, & Nobin, 1975). Cell bodies are mostly located caudally in the posterior hypothalamus and ventral thalamus, predominately scattered around the mamillothalamic tract. Projections to the mPOA derive from the rostral part of the system, in the anterior periventricular cells (Moore & Bloom, 1978). These areas make up part of the incertohypothalamic DA system that originates in the A13 cell group of the hypothalamus, a system that is separate from the mesolimbic DA system, which originates from the A10 cell group (Bjorklund et al., 1975; Wagner et al., 1995). A smaller DA input comes from the rostral periventricular dopaminergic cell system, which makes up the A14 cell group (Bjorklund et al., 1975).

Rather than having a role in sexual behaviour specifically, the mesolimbic DA system is thought to be more important for attention towards reward-related stimuli (Schultz, 2002). The motivational aspects of appetitive-approach behaviours are then activated through modulation of an animal's overall arousal to both positive and negative emotional conditions (Alcaro, Huber, & Panksepp, 2007; Van den Heuvel & Pasterkamp, 2008). Recently, theories have been put forth stating that the mesolimbic DA system is responsible for translating motivation to action, particularly in the attention paid to incentives, or reward-related, stimuli (i.e. Berridge, 2007). This is in agreement with the idea put forth by Everitt (1990), that mesolimbic DA controls the motivational aspects of copulation, with the mPOA mediating the consummatory aspect.

The mesolimbic and incertohypothalamic DA systems may work in conjunction with respect to sexual behaviour. The largest output from the mPOA goes to the VTA (Conrad & Pfaff, 1976; Swanson, 1976; Brackett & Edwards, 1984) where mesolimbic DA neurons originate. The VTA, in turn, projects to the NAcc. The incorporation of both the hypothalamic and limbic areas could implement a circuit that, through the tonic inhibition of DA and with the modulation of steroid hormones, focuses the attention of the rat toward sexual stimuli and sexual interaction (Pfaus, 2006). Evidence in agreement with this theory comes from the finding that the pattern of DA release during sex in the mPOA and NAcc show nearly identical patterns in both

male rats (Blackburn, Pfaus, & Phillips, 1992) and female rats (Mermelstein & Becker, 1995; Pfaus, Damsma, Wenkstern, & Fibiger, 1995; Matuszewich, Lorrain, & Hull, 2000). The VTA and mPOA connections could also be responsible for synchronizing goal-directed behaviours mediated by mesolimbic DA with the hypothalamic control of sympathetic and parasympathetic activation during sexual behaviour, at least in male rats (Dominguez & Hull, 2005).

Dopamine's effects on sexual behaviour have received considerable attention, especially in males. Dopamine was first linked with sexual behaviour when it was discovered that L-DOPA, a precursor to DA derived from the amino acid tyrosine, induced hypersexuality in men with Parkinson's disease (Barbeau, 1969; Bowers, van Woert, & Davis, 1971). Since then, much more evidence has been found exemplifying dopamine's enhancement of human sexual behaviour, where DA agonists facilitate male sexual behaviour, and DA antagonists impair it (for review, see Melis & Argiolas, 1995; Dominguez & Hull, 2005). The story in male rat models is consistent with this, as apomorphine (APO), a non-selective DA agonist, increases male rat sexual behaviour when injected systemically (Butcher, Butcher, & Larsson, 1969; Tagliamonte, Fratta, Del Fiacco, & Gressa, 1974). Drugs that decrease dopaminergic activity, such as the DA receptor blocker pimozide, inhibit male rat sexual behaviour (Malmnas, 1973; 1977).

The function of DA in regards to female sexual behaviour is much more complex, and research has focused predominantly on lordosis. Pimozide injected systemically increases lordosis (Herndon, Caggiula, Sharp, Ellis, & Redgate, 1978), while the DA receptor stimulant piribedil (ET495) decreases the behaviour, which originally implied an inhibitory role for DA (Everitt, Fuxe, & Hokfelt, 1974; Everitt & Fuxe, 1977). These findings that DA played an inhibitory role for lordosis were replicated with nonspecific DA antagonists such as α -flupenthixol (FLU; Everitt, 1990) and spiroperidol (Caggiula et al., 1979). Other nonspecific DA agonists have been found to inhibit the lordosis reflex, including APO (Eliasson & Meyerson, 1976; Everitt & Fuxe, 1977), bromocriptine (CB154; Everitt & Fuxe, 1977) and lergotril (Everitt & Fuxe, 1977).

Contradictory results have been reported demonstrating a stimulatory effect of APO on lordosis using doses comparable to the other APO studies mentioned above (Hamburger-Bar & Rigter, 1975). APO was also found to increase lordosis in female rats primed with low levels of E, but only when microinjected into the mPOA and arcuate-ventromedial area (ARC-VM); it had no effect when injected into the lateral hypothalamus (Foreman & Moss, 1979). In rats primed with very high levels of E, and thus very sexually receptive, DA receptor blockage caused by infusion of FLU and haloperidol (HAL) into the mPOA and ARC-VM resulted in a decreased lordosis response (Foreman & Moss, 1979).

In regards to specific DA receptors, administration of both APO and SKF 38393, a selective D1 agonist, directly into the 3rd ventricle were found to facilitate lordosis in EB-alone rats, at a response rate equivalent to progesterone. The D2 agonist quinpirole had no effect on lordosis when administered intracerebroventricularly (icv; Mani, Allen, Clark, Blaustein, & O'Malley, 1994). Perhaps more puzzling, D2 receptor stimulation by subcutaneous or oral administration of medium doses of the specific D2 agonist LY163502 increased lordosis in low receptive females primed with 200 µg of estrone; very high and very low doses of the same agonist decreased lordosis in sexually receptive females primed with 250 µg of estrone and 2.5 mg of P (Foreman & Hall, 1987). It was suggested that high doses caused marked stereotyped behaviours that disrupted the sexual response pattern, while low doses were thought to stimulate DA autoreceptors (Foreman & Hall, 1987). The effect of sulpiride, a D2 antagonist, on female sexual behaviour has been consistent with this, exhibiting dual effects at different doses. It was found to inhibit lordosis in small doses in EB+P females, but enhanced lordosis in EB-alone females. Drugs affecting D1 receptors meanwhile had no effect (Grierson, James, Pearson, & Wilson, 1988).

Two possible reasons for the disparities between all these studies have been suggested (Melis & Argiolas, 1995). The first is that the level of baseline receptivity to which these rats were being compared differed from study to study. Sexual behaviours are easier to increase from

a low baseline level and to decrease from a high baseline level. The second factor mentioned is that of the dose of DA agonist and antagonist used. In general, it seems that low levels of DA agonists enhance lordosis in partially-primed (EB-alone) females, while higher doses of the same agonists decrease fully-primed (EB+P or very high levels of EB-alone) females. In fact, following OVX or steroid treatment different brain areas have shown changes in the affinity of DA receptors for DA agonists and antagonists (Levesque & Di Paolo, 1988), the density of DA receptors (Hruska, 1986; 1988), and DA content and turnover (i.e. Alderson & Baum, 1981; Lookingland & Moore, 1984). For example, in hypophysectomised male rats, systemic injections of estradiol and prolactin caused a decrease in DA turnover in medial preoptic, rostral periventricular, and preoptico-suprachiasmatic nuclei, while increasing DA turnover in the dorsomedial nucleus, all components of the incertohypothalamic DA system (Lookingland & Moore, 1984). It is possible that the different effects of DA on female sexual behaviour are due to these secondary neurochemical changes (Melis & Argiolas, 1995). A third, potential factor appears to be method of DA administration, with differing roles on female sexual behaviour following central infusions and peripheral injections. Many of the studies mentioned above had different methods for DA manipulation, resulting in difficulties with direct comparisons between studies.

Though much effort has been put forth to elucidate the roles of the mPOA and DA separately on female sexual behaviour, there has not been much focus on DA within the mPOA of female rats. This may be due to the inconclusive results found for the specific effects of the brain area and neurotransmitter thus far. The first study reported to directly test this was by Foreman and Moss (1979); as mentioned above they observed an increase in lordosis following infusions of DA agonists and a decrease following DA antagonists into the mPOA and ARC-VM. In contrary, no change in lordosis was found for EB-alone females infused with SKF38393 into the POA (Apostolakis et al., 1996). A more recent study looked at amphetamine (AMPH), a DA agonist, infused into the mPOA of female rats. The results demonstrated that AMPH altered

paced mating behaviour by increasing the time it takes the female to return to the male following mounts and ejaculations (Guarraci et al., 2008). This is similar to the disruption in pacing and reductions in precopulatory behaviours discovered following ibotenic acid lesions in the mPOA mentioned above (Hoshina et al., 1994; Yang & Clemens, 2000). This has been postulated to be the result of increased sensitivity to the sensory input provided by male sexual stimulation (Yang & Clemens, 2000). Thus, Guarraci and colleagues (2008) have suggested that dopamine in the mPOA, despite it causing a disruption in paced mating, may potentiate female sexual behaviour by enhancing the female's sensitivity to copulation.

Although DA has been shown to play a role in many brain areas in female sexual behaviour, its role in male sexual behaviour is much more restricted. Infusion of DA has been shown to be ineffective in many brain areas including the NAcc, BNST, VTA, caudate nucleus, paraventricular nucleus and lateral septum (Hull et al., 1986; 1989); though it has been reported that infusion of HAL into the NAcc reduced anticipatory level changes by the male (Pfaus & Phillips, 1991). Nevertheless, the effect of DA within the mPOA on male sexual behaviour has been established, predominantly by the work of Hull and colleagues, and its effect is critical in facilitating both erection and ejaculation. For example, infusion of APO into the mPOA was found to increase the copulatory rate and number of ejaculations males experienced per test in a dose-dependent manner (Hull et al., 1986). This suggested that, similar to females, the mPOA is insensitive to low doses of DA, but higher doses are enough to trigger its facilitative effects (Pehek, Thompson, & Hull, 1989).

The nonspecific DA antagonist, FLU, when administered into the mPOA produced opposite results to that of APO, resulting in a reduced ejaculatory frequency; however this only occurred at high doses. FLU also decreased the number of males that mounted and intromitted, perhaps implying a role of mPOA DA receptors in sexual arousal, indicated by initiation of copulation (Pehek et al., 1988). HAL infused into the mPOA had comparable effects, resulting in increased mount and intromission latency, and a decrease in number of intromissions,

ejaculations and level changes (Pfaus & Phillips, 1991). In addition to decreasing copulatory behaviour, FLU also decreased ex-copula penile reflexes and sexual motivation, leading to the conclusion that these effects on copulation were DA receptor mediated, and that the decrease in male copulation by DA antagonists might occur through inhibition of penile reflexes. This could cause a decrease in sexual motivation, or could occur simultaneously through separate but interactive systems (Warner et al., 1991). In all of these studies, there were no effects of APO or FLU into the mPOA on locomotion, eating or drinking, suggesting that alteration of a general motivational state is not responsible for the effects of these dopaminergic drugs on copulation (Hull et al., 1986; Pehek et al., 1988; 1989).

After the facilitating effect of DA in the mPOA on male sexual behaviour was determined, research shifted toward understanding the roles of specific DA receptor subtypes. Findings indicate that the two DA receptor families both affected male copulatory behaviour, but in different ways. Although D1 stimulation had no effect, blocking D1 receptors and stimulating D2 receptors impair the onset of copulation. This happens through the increase in latency of first mount and first intromission. As well, the threshold for ejaculation also is decreased, as a result of a reduction in the number of intromissions required to ejaculate (Hull et al., 1989). The effects of D1 blockage and D2 stimulation were additive as well, suggesting that the ratio between the two subtypes may play an important role. Later studies found that stimulation of D1 receptors promoted erection in male rats, while decreasing the latency to ejaculate (Markowski, Eaton, Lumley, Moses, & Hull, 1994). Thus, D1 receptors are thought to serve to facilitate the early stage of copulation through promotion of penile erection, while acting with D2 receptors synergistically to promote sexual motivation and decrease the latency to ejaculate (Moses et al., 1995). The conclusion put forth was that D2 receptors enhance sympathetically mediated ejaculatory mechanisms, while inhibiting the parasympathetically controlled erectile responses. Stimulation of both receptor subtypes, by APO for example, primarily facilitates erectile responses; shifting to D2 stimulation through higher levels of DA selectively results in a switch

towards ejaculatory mechanisms (Hull et al., 1989). It may be then, that different levels of extracellular DA, acting through the two subtypes of receptors, may aid in controlling the timing of copulatory events (Hull et al., 1999).

Dopamine levels have been found to increase not only when the male rat copulates, but also in the presence of a sexually exciting stimulus (Hull, Du, Lorrain, & Matuszewich, 1995; Dominguez, Riolo, Xu, & Hull, 2001). Interestingly, following medial amygdala (MeA) lesions, this DA increase is not seen, implicating DA activity in the mPOA in mediating male sexual behaviour, aided by inputs from the MeA (Dominguez et al., 2001). The effects are equivalent in females. Increases in DA in the mPOA are seen not only in response to circulating gonadal hormones, but also in concurrence with copulation with a male (Matuszewich et al., 2000). Since there was no increase in extracellular DA in females primed with high doses of EB, only low doses, it could be that DA in the mPOA is critical for the facilitating effect of P on female sexual behaviour. Dopamine's rise also was not influenced by type of sexual stimulation, as increases observed following intromissions and ejaculations were no different than those following mounts only. All of these acts contribute perineal stimulation to the female, while only intromissions and ejaculations add VCS (Matuszewich et al., 2000). Oddly, the release of extracellular DA in the mPOA was not seen in females tested in a pacing chamber, though dopamine's metabolites were observed to rise during copulation. This effect is in direct contrast to the increase in DA levels seen in the NAcc of female rats in response to copulation (Pfaus et al., 1995), as well as the striatum, although these increases are seen only if the female is pacing (Mermelstein & Becker, 1995). Dopamine was also found to show no change in the mPOA in response to the presence of a male alone (Matuszewich et al., 2000); this is opposite to dopaminergic activity observed in the NAcc (Pfaus et al., 1995). The authors concluded that hormonal state, copulatory environment, and perineal stimulation contribute to alter extracellular DA levels within the female mPOA (Matuszewich et al., 2000).

Given that the pattern of behaviour in response to dopaminergic alteration in the female mPOA is not only unclear, but also predominately focused on consummatory behaviours, the present set of experiments investigate the effects of dopaminergic agents infused into the mPOA of female rats on the complete cascade of sexual behaviour. To determine whether there is any discrepancy in the effects of specific DA receptor subtypes, or if the subtypes have a synergistic effect, nonspecific and selective receptor agonists and antagonists were microinfused bilaterally into the mPOA of ovariectomized, hormonally-primed rats, and the entire paradigm of female sexual behaviour was analyzed. Since it was hypothesized that DA agonists would increase female sexual behaviour, female rats were tested under partial hormonal-priming, being tested under EB-alone conditions. On the other hand, fully-primed EB+P females received microinfusions of DA antagonists, since it was hypothesized these would decrease sexual behaviour. Finally, in line with the dual effects of the receptor subtypes seen in male sexual behaviour, it was hypothesized that D1 receptors would play a role in precopulatory behaviours, while D2 receptors would have an effect on lordosis. This study is the first to look in-depth at the role of dopaminergic activity in the female mPOA in regards to precopulatory behaviours, as well as examining specific receptor roles.

EXPERIMENT 1

The purpose of this experiment was to determine whether the effects of apomorphine (a non-specific dopamine agonist), SKF 38393 (a selective DA D1 receptor agonist), and Quinpirole (a selective DA D2 receptor agonist) infused into the mPOA would increase female sexual behaviour, either in terms of increased precopulatory behaviours, increased consummatory behaviours, or both. All drugs were infused to EB-alone primed females, as their lower receptivity levels were hypothesized to increase following infusions of the nonspecific and selective DA receptor agonists.

Method

Subjects and procedure

Female Long-Evans rats, weighing 150-200g, and male Long-Evans rats, weighing 200-250g, all six weeks old, were obtained from Charles River Canada, Inc. (St. Constant, Quebec). Female rats were pair-housed in plastic cages (36 x 26 x 19 cm) leading up until cannulation, after which they were housed individually, while males were housed either in groups of three in hanging wire gang cages, or pair-housed in plastic cages similar to those housing the females. All rats were kept in the same room, maintained on a reversed 12 hour light/dark cycle, with lights off at 0800. Regular rat chow and tap water was available ad libitum, and the room temperature was kept constant at 21°C. Male rats remained in their cages except for when they were used as a stimulus partner during the sex testing (see below). Each male was tested only once a day randomized among the females receiving the same drug infusion.

Ovariectomies

One week after arrival, females were ovariectomized bilaterally in order to prevent impregnation and allow for the control of hormone levels throughout testing. Females were anaesthetized with a cocktail of four parts ketamine hydrochloride (100 mg/ml) to three parts xylazine hydrochloride (20 mg/ml), given intraperitoneally at a dose of 1 ml/kg. Ovaries were then removed via a lumbar incision, and rats were allowed one week recovery period.

Sexual experience

To ensure that all rats, both male and female, were adequate at copulation, animals received four sessions of sexual experience prior to any experimental testing. Five days after ovariectomy, female rats were injected subcutaneously with estradiol benzoate (EB) in a dosage of 10 µg per 0.1 ml of sesame oil. On day seven post-recovery, 44 hours after the EB injection, females received progesterone (P) in a dosage of 500 µg in 0.1 ml of sesame oil via another subcutaneous injection. This would establish full receptivity in the female four hours later, at which time the female was paired with a male in a bilevel chamber and given thirty minutes to copulate. This procedure was repeated for three more copulatory sessions, spaced four days apart, with ejaculations noted so that all males and females experienced the full range of sexual behaviours on the last two trials at least.

Cannulations

After becoming sexually experienced, females were anaesthetized using sodium pentobarbital (60 mg/ml) at a dose of 1 ml/kg. Using a stereotaxic instrument, rats were implanted with a stainless steel, 22 gauge, bilateral guide cannulae aimed 1 mm above the mPOA (AP -0.6, ML ±0.5, DV -7.0 mm from bregma, incision bar set at 0; Paxinos & Watson, 1986), with 28 gauge cannula blockers in place, cut 0.5 mm below the cannulae. Infusion cannulae, also 28-gauge, were cut 1 mm longer than the guide cannulae. All cannulae equipment was obtained from Plastics One (Roanoke, Virginia). Females were given seven days recovery time before any infusions or testing.

Hormonal priming

Females were tested partially hormonally primed in order to avoid any ceiling effects. To accomplish this, females were injected subcutaneously with EB in a dosage of 10 µg per 0.1 ml of sesame oil 48 hours before each experimental sex test.

Sex testing

After the one week postsurgical recovery period, rats were primed and received a baseline test in the bilevel chamber. Four randomized drug tests followed at four day intervals, resulting in each experimental rat receiving a low, medium, and high dose of the drug, as well as a vehicle trial, in a Latin squares randomized order. Four days after the fourth drug test, a second baseline test was given, resulting in each rat undergoing six testing sessions. All rats were primed in the same manner before every test, either fully or partially, depending on the experiment, and each test was thirty minutes in length. Females were introduced to the chamber immediately following the drug infusion, where a sexually vigorous male was awaiting her following a five minute acclimation period. Following the last baseline test, females were perfused and their brains extracted for placement confirmation.

Drugs and infusions

Physiological saline was infused before every baseline test, and it was also used as the vehicle for each drug in the experiment. Doses of each drug infused into the mPOA were: Apomorphine (n = 8): High: 1.0 µg; Medium: 0.5 µg; Low: 0.25 µg; SKF 38393 (n = 10): High: 0.2 µg; Medium: 0.1 µg; Low: 0.05 µg; Quinpirole (n = 11): High: 0.2 µg; Medium: 0.1 µg; Low: 0.05 µg. Drugs were purchased from Sigma Chemical Co. (St. Louis, MO). All infusions were done at a rate of 0.5 µl/min per side for one minute using an infusion pump (Harvard Apparatus, Pump 22), giving a total volume of 1 µl. Infusion cannulae were left in place for one minute following infusion to allow for absorption, after which testing proceeded.

Perfusions and histology

Following completion of testing, animals were anaesthetized with 1 ml of sodium pentobarbital injected intraperitoneally, and perfused using PBS and 4% Paraformaldehyde solutions. This was done so that correct cannulae placement could be confirmed. Following perfusion, the extracted brains were placed in the 4% Paraformaldehyde solution overnight, and then transferred to a 30% sucrose solution until they were sliced. Coronal slicing was done at 30

microns, and cannulae placement was confirmed by a blind, third-party experimenter and placement was marked in an atlas.

The criterion for exclusion from statistical analyses was set so that rats with both injector cannulae ending outside the boundaries of the mPOA were exempt from the study. The end result of this was that only animals that had correct unilateral or bilateral cannulations to the mPOA were included in the analyses. Cannulae placement data from subjects included in the statistical analyses of the three parts of Experiment 1 are shown in Figure 1.

Behavioural analyses

All experimental testing sessions were captured onto DVD via camcorder, and viewed at a later time on a laptop allowing for behaviours to be scored using a computerized event recorder (Cabilio, 1996). Frequencies of both female and male behaviours were scored. Male behaviours consisted of the number of mounts, intromissions and ejaculations occurring during each thirty minute test session. Female behaviours consisted of the number of solicitations (characterized by a head-wise orientation to the male, followed by a 180 degree turn and runaway), hops and/or darts (characterized by either a hopping motion, or a burst of speed away from the male and sudden stop, without head-wise orientation; these could occur with or without each other), defensive behaviours (characterized by kicks, sideways takedowns, boxing postures, and prone positions in response to the male (Barnett, 1967)), level changes (going from one level of the chamber to the other), and magnitude of reflexive lordosis posture (ranging from 0, no lordosis posture, to 3, full lordosis posture).

Statistical analyses

A non-parametric equivalent of a one-sample repeated measures ANOVA, the Friedman Test (Friedman, 1937), was performed on all sexual behaviours, both male and female, for each drug independently. A non-parametric test was used as the homogeneity assumption necessary for using an ANOVA was not satisfied, as partially primed female rats show reduced (if not abolished) components of sexual behaviour. Analyses of behaviours included solicitations

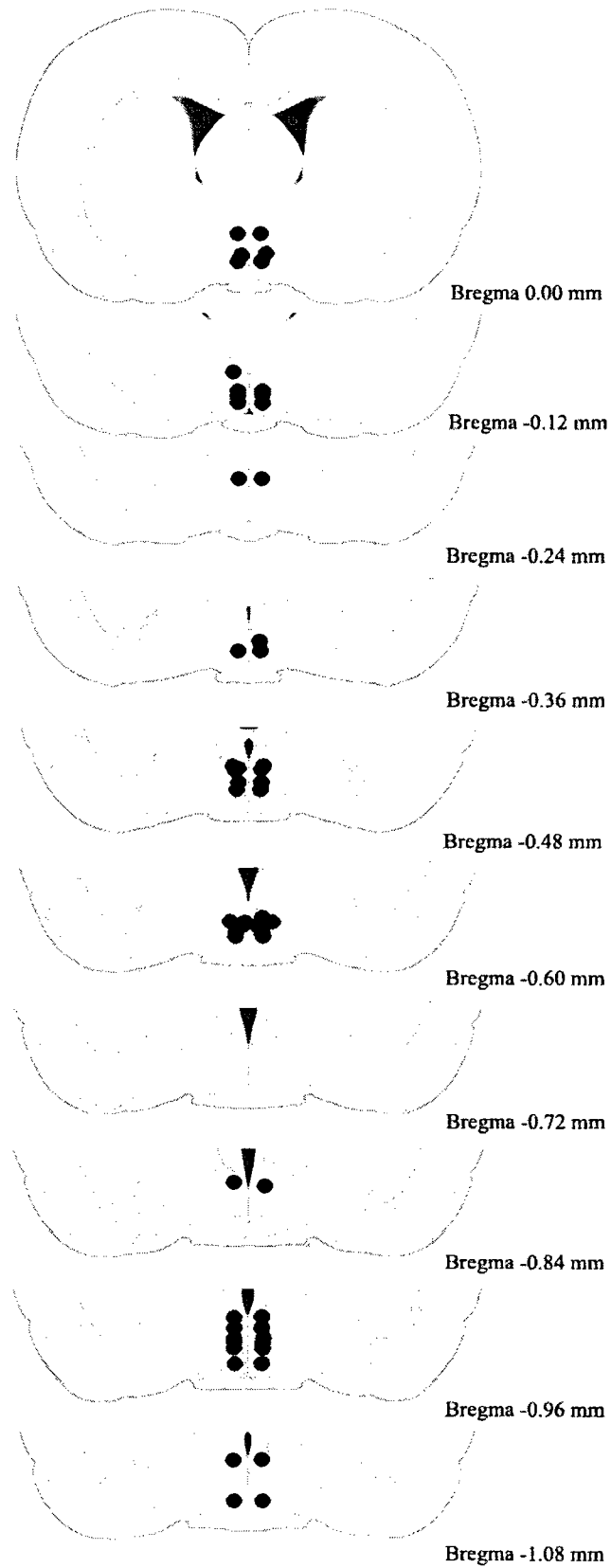


Figure 1. Placement data for subjects in Experiment 1.

(SOL), hops and/or darts (H/D), defensive behaviours (DEF), lordosis magnitudes 0-3 (LM), lordosis quotient (LQ; the number of lordosis postures taken divided by the number of mounts, intromission, and ejaculations), pacing (as indicated by level changes), mounts, intromissions (INTRO), and ejaculations (EJAC). In each case, the vehicle trial and the three drug infusion doses were compared. Any significant results were then further analyzed using a Wilcoxon signed-rank test to determine which two trials differed.

Results

Behavioural observations

No unusual behaviours were observed in animals following any of the doses of any of the DA agonists. Animals were easy to handle, and behaved normally before and after testing.

Apomorphine

The effects that infusions of saline and three doses of apomorphine directly into the mPOA of EB-alone female rats had on sexual behaviour can be seen in Figure 2, and include that of SOL, H/D, pacing (level changes), DEF, LQ, LM, INTRO and EJAC. Compared to the saline vehicle control and high dose (1.0 µg), low doses (0.25 µg) of APO significantly increased the number of H/D females displayed during the copulation test. Solicitations showed a trend in the same direction, with the low dose trial showing more solicitations during the test than the vehicle and high dose trials.

Solicitations. A trend of a main effect of dose of APO on the number of SOL was found (Friedman Test, $\chi^2 = 7.130$, $df = 3$, $p < 0.068$). *Post hoc* analyses using Wilcoxon Signed Ranks Test revealed that the low dose of APO showed a trend towards an increase of SOL compared to the saline vehicle control and the high dose of APO.

Hops and/or Darts. A significant main effect of dose was detected on the number of H/D displayed during the copulation test (Friedman Test, $\chi^2 = 7.831$, $df = 3$, $p < 0.050$). *Post hoc* analyses using Wilcoxon Signed Ranks Test revealed that the low dose of APO showed a

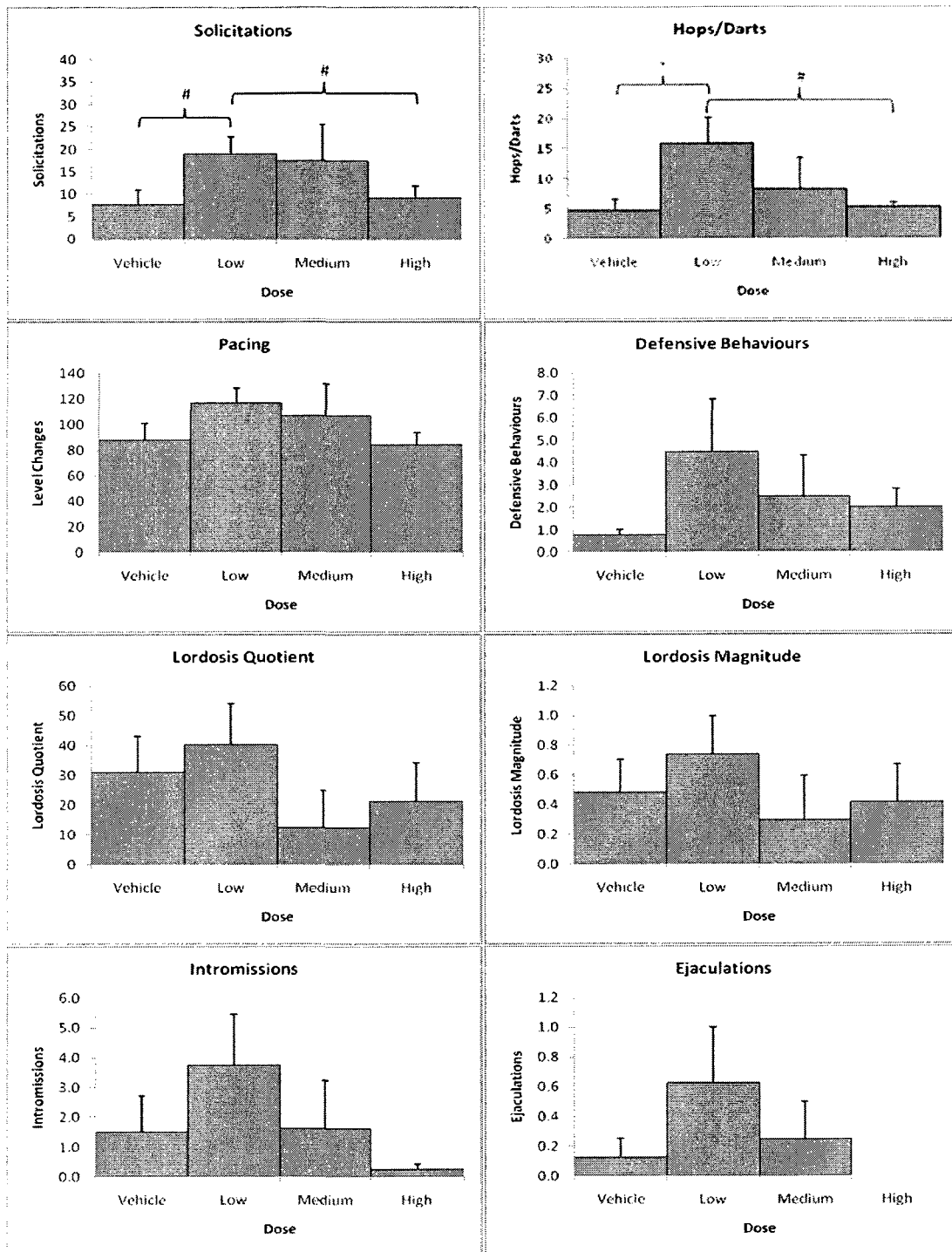


Figure 2. The effect of three doses of apomorphine or saline vehicle infusions on the mean number of solicitations, hops and/or darts, level changes, defensive behaviours, mean lordosis quotient, mean lordosis magnitude, male intromissions and male ejaculations. Error bars represent the standard errors. (* = $p < 0.05$; # = $p < 0.10$)

significant increase of solicitations compared to the saline vehicle control and a trend of an increase when compared to the high dose of APO.

Pacing. No significant main effect of dose was found (Friedman Test, $\chi^2 = 5.550$, $df = 3$, $p < 0.136$).

Defensive Behaviours. No significant main effect of dose was found (Friedman Test, $\chi^2 = 0.696$, $df = 3$, $p < 0.874$).

Lordosis Quotient. No significant main effect of dose was found (Friedman Test, $\chi^2 = 4.850$, $df = 3$, $p < 0.183$).

Lordosis Magnitude. No significant main effect of dose was found (Friedman Test, $\chi^2 = 4.131$, $df = 3$, $p < 0.248$).

Intromissions. A trend of a main effect of dose was found (Friedman Test, $\chi^2 = 7.000$, $df = 3$, $p < 0.072$), however *post hoc* analyses using Wilcoxon Signed Ranks Test failed to reveal any significant differences between two doses.

Ejaculations. No significant main effect of dose was found (Friedman Test, $\chi^2 = 5.222$, $df = 3$, $p < 0.156$).

SKF 38393

The effects that infusions of saline and three doses of SKF 38393 directly into the mPOA of EB-alone female rats had on sexual behaviour can be seen in Figure 3, and include that of SOL, H/D, pacing (level changes), DEF, LQ, LM, INTRO and EJAC. Compared to the saline vehicle control, the low dose (0.05 μg) of SKF showed a significantly reduced number of hops and/or darts; a trend which is also seen when comparing the low dose to the medium dose (0.1 μg). The low dose trial also showed a significantly reduced number of male ejaculations received when compared to the vehicle group. A trend of reduced ejaculations received is seen when comparing the low dose trial to the medium and high (0.2 μg) dose trials.

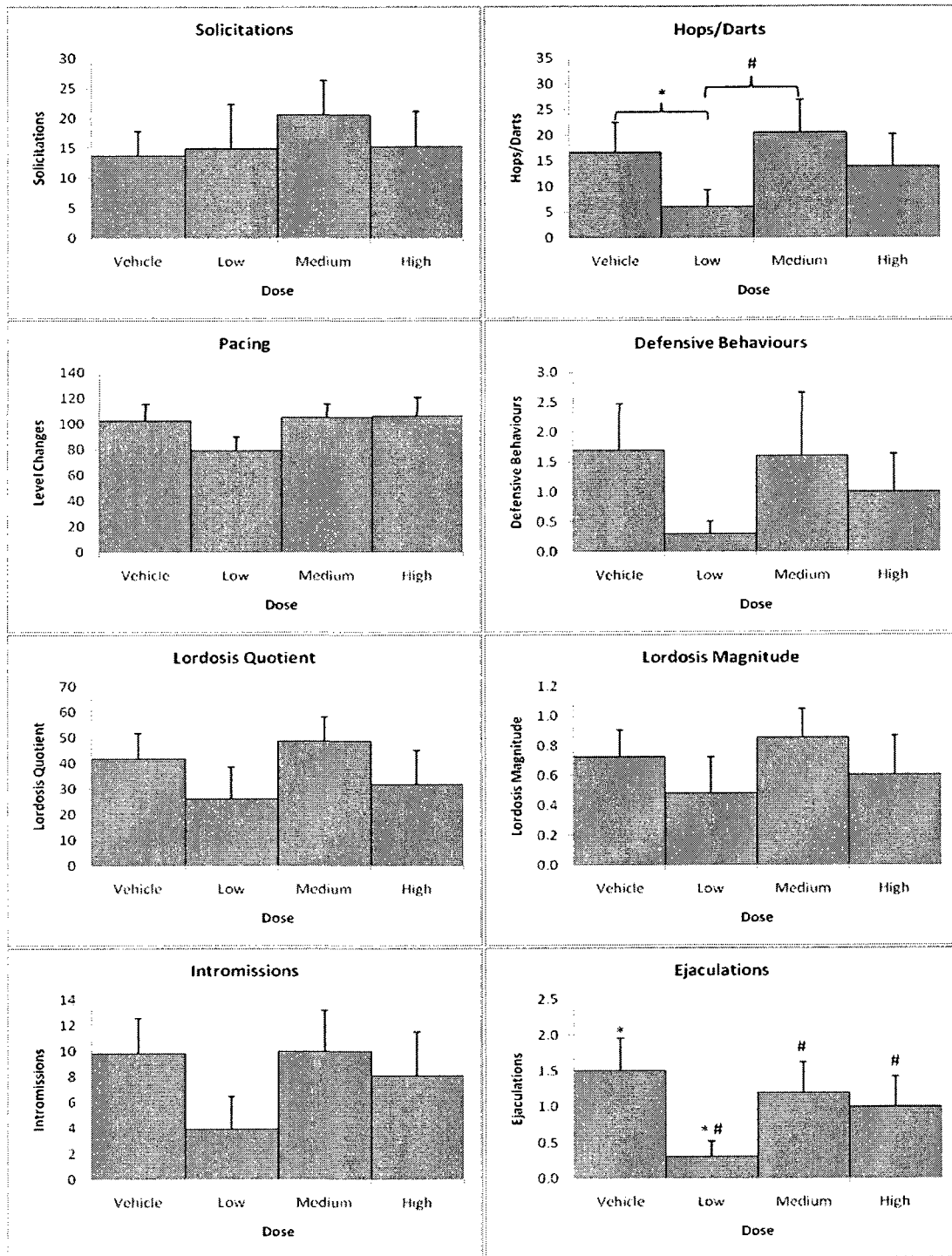


Figure 3. The effect of three doses of SKF 38393 or saline vehicle infusions on the mean number of solicitations, hops and/or darts, level changes, defensive behaviours, mean lordosis quotient, mean lordosis magnitude, male intrusions and male ejaculations. Error bars represent the standard errors. (* = $p < 0.05$; # = $p < 0.10$)

Solicitations. No significant main effect of dose of SKF 38393 was found (Friedman Test, $\chi^2 = 1.226$, $df = 3$, $p < 0.747$).

Hops and/or Darts. A significant main effect of dose was detected on the number of H/D displayed during the copulation test (Friedman Test, $\chi^2 = 7.941$, $df = 3$, $p < 0.047$). *Post hoc* analyses using Wilcoxon Signed Ranks Test revealed that the low dose of SKF showed a significant decrease of H/D compared to the saline vehicle control and a trend of a decrease when compared to the medium dose of SKF.

Pacing. No significant main effect of dose was found (Friedman Test, $\chi^2 = 4.133$, $df = 3$, $p < 0.247$).

Defensive Behaviours. No significant main effect of dose was found (Friedman Test, $\chi^2 = 2.411$, $df = 3$, $p < 0.492$).

Lordosis Quotient. No significant main effect of dose was found (Friedman Test, $\chi^2 = 2.517$, $df = 3$, $p < 0.472$).

Lordosis Magnitude. No significant main effect of dose was found (Friedman Test, $\chi^2 = 1.276$, $df = 3$, $p < 0.735$).

Intromissions. No significant main effect of dose was found (Friedman Test, $\chi^2 = 4.479$, $df = 3$, $p < 0.214$).

Ejaculations. A significant main effect of dose was detected on the number of male EJAC received during the copulation test (Friedman Test, $\chi^2 = 8.357$, $df = 3$, $p < 0.039$). *Post hoc* analyses using Wilcoxon Signed Ranks Test revealed a significant decrease in EJAC for the low dose of SKF compared to the saline vehicle control, and a trend towards a decrease for the low dose trial compared to the medium and high dose trials.

Quinpirole

The effects that infusions of saline and three doses of quinpirole directly into the mPOA of EB-alone female rats had on sexual behaviour can be seen in Figure 4, and include that of SOL, H/D, pacing (level changes), DEF, LQ, LM, INTRO and EJAC. Compared to the saline

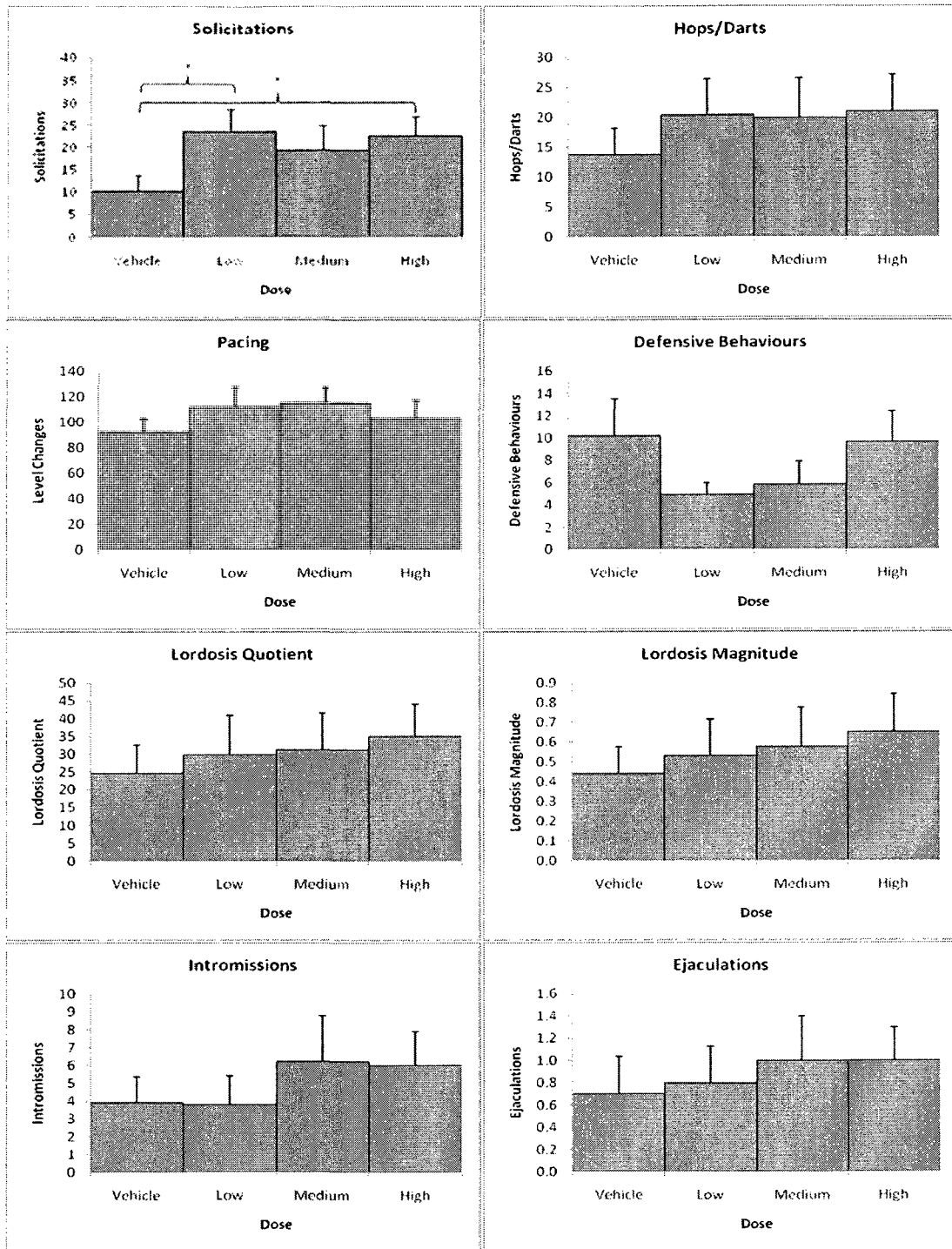


Figure 4. The effect of three doses of quinpirole or saline vehicle infusions on the mean number of solicitations, hops and/or darts, level changes, defensive behaviours, mean lordosis quotient, mean lordosis magnitude, male intromissions and male ejaculations. Error bars represent the standard errors. (* = $p < 0.05$; # = $p < 0.10$)

vehicle control, the low dose (0.05 µg) and high dose (0.2 µg) trials showed an increased number of solicitations.

Solicitations. A significant main effect of dose of quinpirole on the number of SOL was found (Friedman Test, $\chi^2 = 10.273$, $df = 3$, $p < 0.016$). *Post hoc* analyses using Wilcoxon Signed Ranks Test revealed that the low and high doses of QUIN had an increased number of solicitations when compared to the saline vehicle control.

Hops and/or Darts. No significant main effect of dose was found (Friedman Test, $\chi^2 = 1.806$, $df = 3$, $p < 0.614$).

Pacing. No significant main effect of dose was found (Friedman Test, $\chi^2 = 5.400$, $df = 3$, $p < 0.145$).

Defensive Behaviours. No significant main effect of dose was found (Friedman Test, $\chi^2 = 2.563$, $df = 3$, $p < 0.464$).

Lordosis Quotient. No significant main effect of dose was found (Friedman Test, $\chi^2 = 0.393$, $df = 3$, $p < 0.942$).

Lordosis Magnitude. No significant main effect of dose was found (Friedman Test, $\chi^2 = 0.321$, $df = 3$, $p < 0.956$).

Intromissions. No significant main effect of dose was found (Friedman Test, $\chi^2 = 1.259$, $df = 3$, $p < 0.739$).

Ejaculations. No significant main effect of dose was found (Friedman Test, $\chi^2 = 0.563$, $df = 3$, $p < 0.905$).

EXPERIMENT 2

The goal of the second experiment was to test whether the effects of Flupenthixol (FLU; a non-specific DA antagonist), SCH 23390 (a selective DA D1 receptor antagonist), and Raclopride (a selective DA D2 receptor antagonist) infused into the mPOA would decrease female sexual behaviour, either through reduced appetitive behaviours, reduced copulatory behaviours, or both. All females tested were primed with EB+P, as their higher receptivity levels were hypothesized to decrease following infusions of the nonspecific and selective dopamine antagonists.

Method

Subjects and procedures

All conditions were identical to that of Experiment 1. Females and males were housed the same way, and sexual experience trials, ovariectomies, cannulations, sex testing sessions, histological procedures and behavioural analyses were carried out in the same manner. In this experiment non-flank stimulated lordosis postures (NFSLP) were analyzed. These are lordosis postures the female exhibited that were not preceded by a male stimulation. In other words, they were lordosis postures undertaken that were not reflexively caused by the male touching the females flanks.

Cannulae verification resulted in rats with correct unilateral and bilateral cannulations to the mPOA included in the analyses. Exclusion criteria explained in Experiment 1 stipulated that rats with incorrect placement were excluded from statistical analyses. Cannulae placement data from subjects included in the statistical analyses of the three parts of Experiment 2 are shown in Figure 5.

Hormonal priming

Females were tested fully hormonally primed, in an identical manner to how females were primed prior to sexual experience training, in order to avoid any floor effects. Females were subcutaneously injected with EB in a dosage of 10 μ g per 0.1 ml of sesame oil 48 hours before

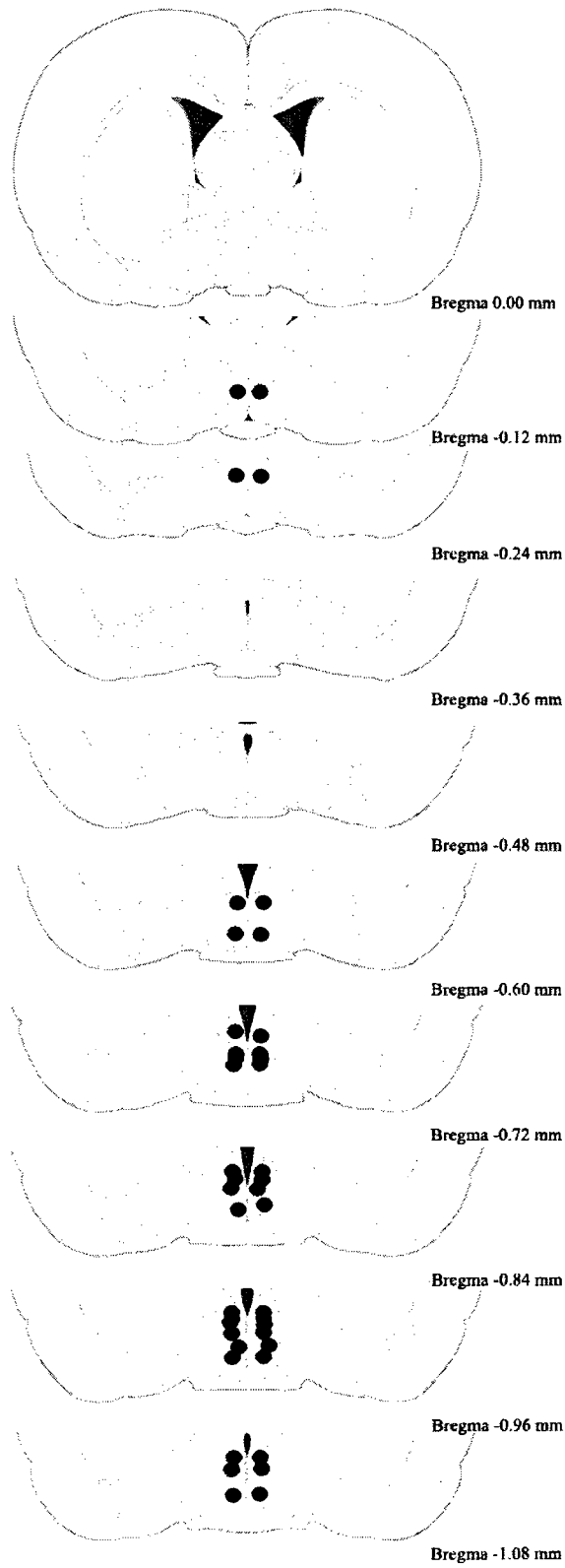


Figure 5. Placement data for subjects in Experiment 2.

each experimental sex test. This was followed by a subcutaneous injection of P four hours before the test in a dosage of 500 µg in 0.1 ml of sesame oil.

Drugs and infusions

Physiological saline was infused before every baseline test, and it was also used as the vehicle for each drug in the experiment. Doses of each drug infused into the mPOA were: Flupenthixol (n = 6): High: 20.0 µg; Medium: 2.0 µg; Low: 0.2 µg; SCH 23390 (n = 12): High: 4.0 µg; Medium: 1.0 µg; Low: 0.25 µg; Raclopride (n = 8): High: 4.0 µg; Medium: 1.0 µg; Low: 0.25 µg. Drugs were purchased from Sigma Chemical Co. (St. Louis, MO). All infusions were done at a rate of 0.5 µl/min per side for one minute using an infusion pump (Harvard Apparatus, Pump 22), giving a total volume of 1 µl. Infusion cannulae were left in place for one minute following infusion to allow for absorption, after which testing proceeded.

Statistical analyses

A one-sample repeated measures analyses of variance (ANOVA) was performed on the same sexual behaviours as in Experiment 1, for each drug independently, as the homogeneity assumption was met since EB+P primed females show a full display of sexual behaviours. Every behaviour analyzed had four levels examined: the vehicle trial and the three drug infusion doses. Pair-wise comparisons examining main effects between the means were made using the Least Significant Difference (LSD) pair-wise multiple comparison test.

Results

Behavioural observations

All rats were fully receptive, showing LQs over 90%. No significant differences were observed in LQs between any of the drug doses for any of the dopaminergic agents (data not shown). Additionally, no unusual behaviours were observed in animals following any of the doses of any of the dopamine agonists. Animals were easy to handle, and behaved normally before and after testing.

Flupenthixol

The effects that infusions of saline and three doses of FLU directly into the mPOA of EB+P female rats had on sexual behaviour can be seen in Figure 6, and include that of SOL, H/D, pacing (level changes), DEF, NFSLP, LM, INTRO and EJAC. No differences were found comparing the three doses of FLU to the saline vehicle control on any of the measures.

Solicitations. No significant main effect of dose of FLU was found (ANOVA, $F(3,15) = 0.486$, $p < 0.697$).

Hops and/or Darts. No significant main effect of dose was found (ANOVA, $F(3,15) = 1.750$, $p < 0.200$).

Pacing. No significant main effect of dose was found (ANOVA, $F(3,15) = 0.704$, $p < 0.565$).

Defensive Behaviours. No significant main effect of dose was found (ANOVA, $F(3,15) = 2.025$, $p < 0.154$).

Non-Flank Stimulated Lordosis Postures. No significant main effect of dose was found (ANOVA, $F(3,15) = 1.296$, $p < 0.312$).

Lordosis Magnitude. No significant main effect of dose was found (ANOVA, $F(3,15) = 1.262$, $p < 0.323$).

Intromissions. No significant main effect of dose was found (ANOVA, $F(3,15) = 0.884$, $p < 0.471$).

Ejaculations. No significant main effect of dose was found (ANOVA, $F(3,15) = 0.261$, $p < 0.853$).

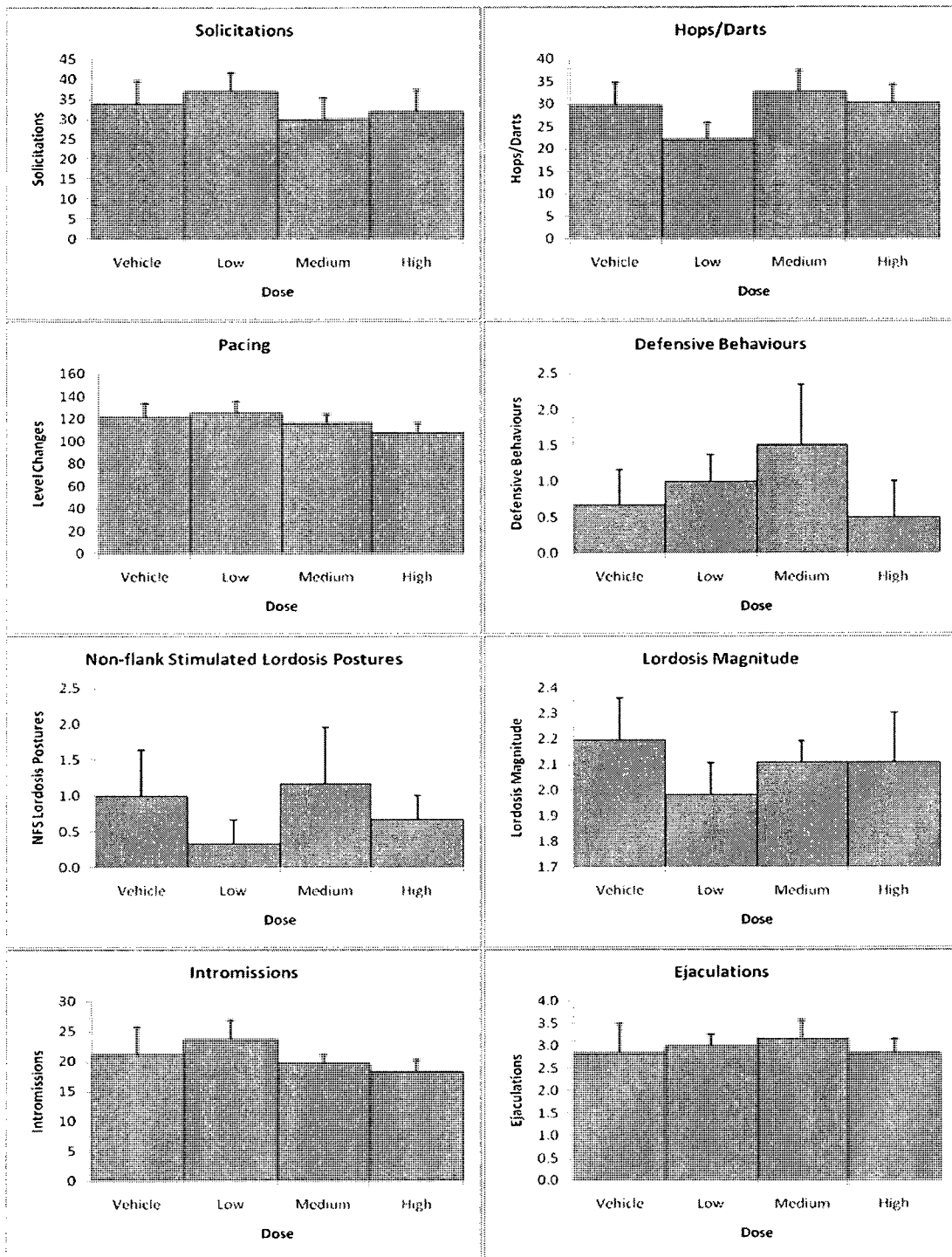


Figure 6. The effect of three doses of flupenthixol or saline vehicle infusions on the mean number of solicitations, hops and/or darts, level changes, defensive behaviours, non-flank stimulated lordosis postures, mean lordosis magnitude, male intromissions and male ejaculations. Error bars represent the standard errors. (* = $p < 0.05$; # = $p < 0.10$)

SCH 23390

The effects that infusions of saline and three doses of SCH 23390 directly into the mPOA of EB+P female rats had on sexual behaviour can be seen in Figure 7, and include that of SOL, H/D, pacing (level changes), DEF, NFSLP, LM, INTRO and EJAC. Compared to the other three trials, the high dose (4.0 µg) of SCH significantly decreased solicitations and number of level changes. There was also a trend seen in an increase of NFSLP during the high dose trial, when compared to the saline vehicle control and the low dose (0.25 µg) trials.

Solicitations. A significant main effect of dose of SCH 23390 on the number of SOL was found (ANOVA, $F(3,33) = 7.168$, $p < 0.001$). *Post hoc* analyses using LSD revealed that the high dose of SCH showed a significantly decreased number of SOL when compared to the other three dose trials.

Hops and/or Darts. No significant main effect of dose was found (ANOVA, $F(3,33) = 0.674$, $p < 0.574$).

Pacing. A significant main effect of dose on the number of level changes was found (ANOVA, $F(3,33) = 9.917$, $p < 0.000$). *Post hoc* analyses using LSD revealed that the high dose of SCH showed a significantly decreased number of level changes when compared to the other three dose trials.

Defensive Behaviours. No significant main effect of dose was found (ANOVA, $F(3,33) = 0.786$, $p < 0.510$).

Non-Flank Stimulated Lordosis Postures. A trend towards a main effect of dose on the number of NFSLPs was found (ANOVA, $F(3,33) = 2.442$, $p < 0.082$). *Post hoc* analyses using LSD revealed that the high dose of SCH showed a trend towards an increased number of NFSLPs when compared to the saline vehicle control and the low dose trials.

Lordosis Magnitude. No significant main effect of dose was found (ANOVA, $F(3,33) = 1.973$, $p < 0.137$).

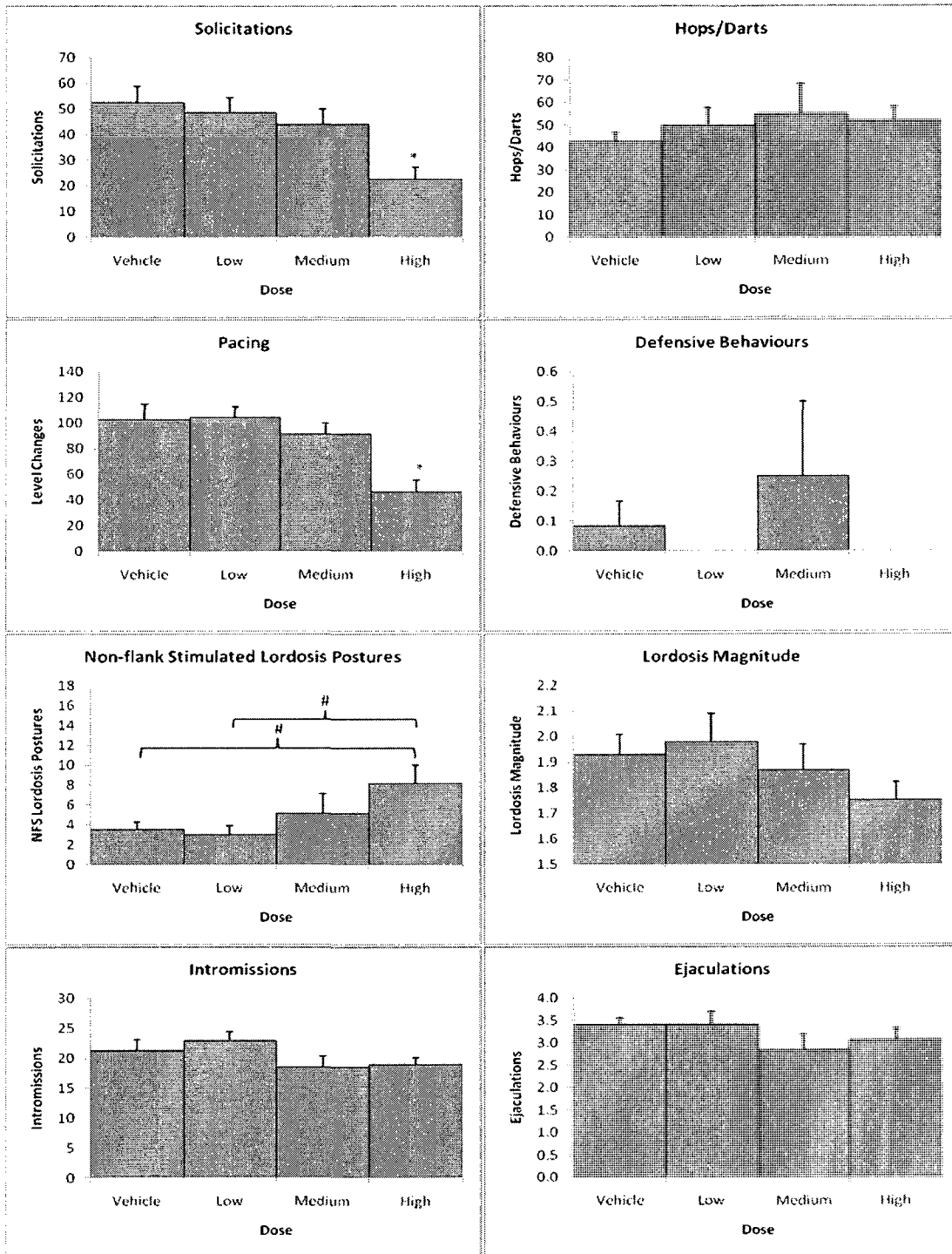


Figure 7. The effect of three doses of SCH 23390 or saline vehicle infusions on the mean number of solicitations, hops and/or darts, level changes, defensive behaviours, non-flank stimulated lordosis postures, mean lordosis magnitude, male intromissions and male ejaculations. Error bars represent the standard errors. (* = $p < 0.05$; # = $p < 0.10$)

Intromissions. No significant main effect of dose was found (ANOVA, $F(3,33) = 1.743$, $p < 0.177$).

Ejaculations. No significant main effect of dose was found (ANOVA, $F(3,33) = 1.104$, $p < 0.361$).

Raclopride

The effects that infusions of saline and three doses of raclopride directly into the mPOA of EB+P female rats had on sexual behaviour can be seen in Figure 8, and include that of SOL, H/D, pacing (level changes), DEF, NFLSP, LM, INTRO and EJAC. Compared to low (0.25 µg) and medium (1.0 µg) doses, the high (4.0 µg) dose of RAC significantly increased the number of SOL seen during the copulation test. A similar trend was found when the high dose was compared to the saline vehicle control. The high dose also showed a significantly increased number of level changes when compared to the vehicle and low dose trials, and a trend when compared to the medium dose.

Solicitations. A significant main effect of dose of RAC on the number of SOL was found (ANOVA, $F(3,21) = 3.381$, $p < 0.037$). *Post hoc* analyses using LSD revealed that the high dose of RAC showed a significantly increased number of SOL when compared to the low and medium dose trials, and a trend towards an increase when compared to the saline vehicle control.

Hops and/or Darts. No significant main effect of dose was found (ANOVA, $F(3,21) = 0.967$, $p < 0.427$).

Pacing. A significant main effect of dose on the number of level changes was found (ANOVA, $F(3,21) = 4.325$, $p < 0.016$). *Post hoc* analyses using LSD revealed that the high dose of RAC showed a significantly increased number of level changes when compared to the saline vehicle control and low dose trials, and a trend towards an increase when compared to the medium dose.

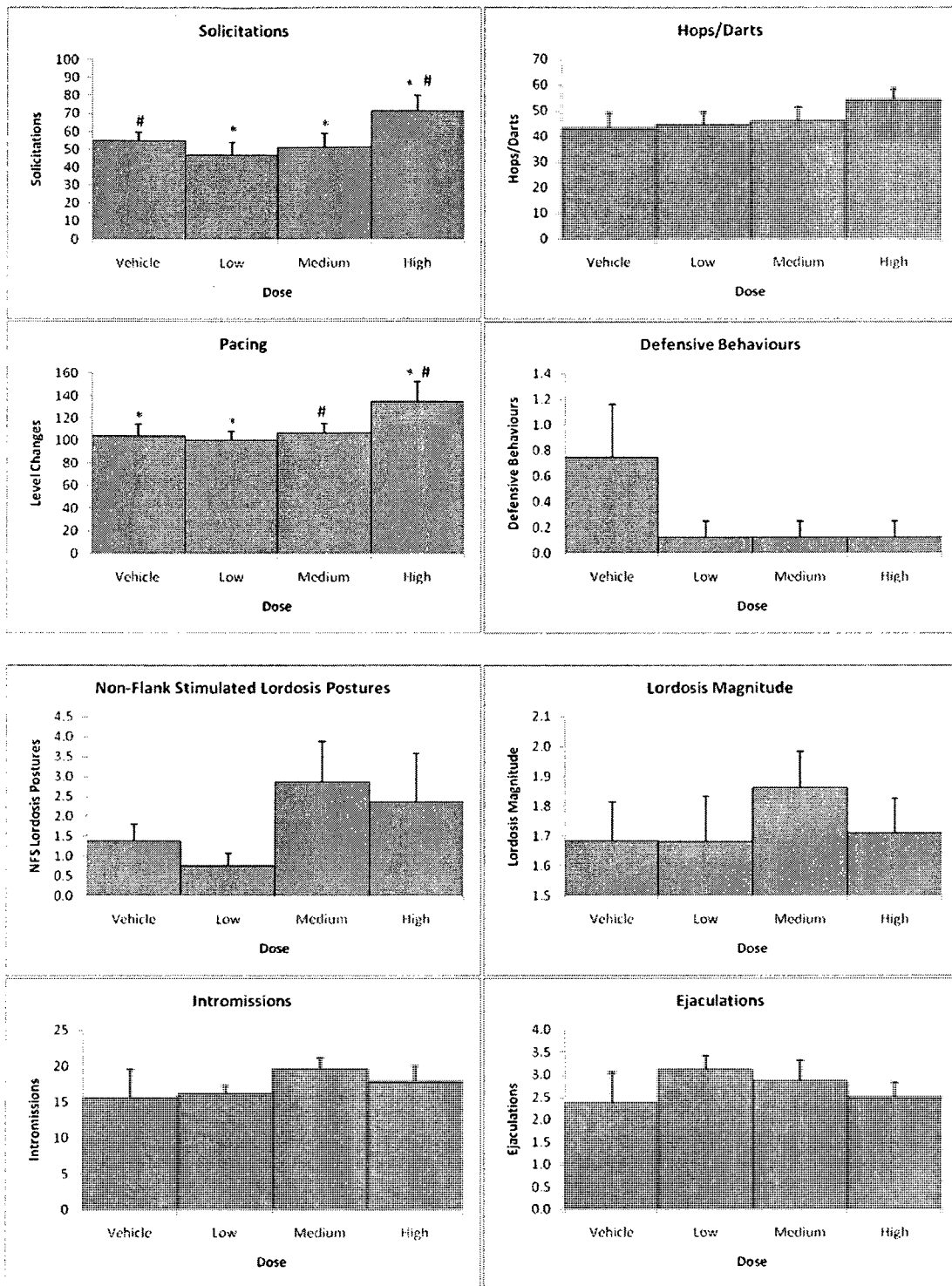


Figure 8. The effect of three doses of raclopride or saline vehicle infusions on the mean number of solicitations, hops and/or darts, level changes, defensive behaviours, non-flank stimulated lordosis postures, mean lordosis magnitude, male intromissions and male ejaculations. Error bars represent the standard errors. (* = $p < 0.05$; # = $p < 0.10$)

Defensive Behaviours. No significant main effect of dose was found (ANOVA, $F(3,21) = 1.842, p < 0.170$).

Non-Flank Stimulated Lordosis Postures. No significant main effect of dose was found (ANOVA, $F(3,21) = 1.398, p < 0.271$).

Lordosis Magnitude. No significant main effect of dose was found (ANOVA, $F(3,21) = 0.916, p < 0.450$).

Intromissions. No significant main effect of dose was found (ANOVA, $F(3,21) = 0.591, p < 0.628$).

Ejaculations. No significant main effect of dose was found (ANOVA, $F(3,21) = 0.816, p < 0.500$).

Discussion

This thesis examined the role of dopamine in the medial preoptic area on both precopulatory and consummatory aspects of female sexual behaviour. In Experiment 1, both a nonspecific DA receptor agonist and specific D1 and D2 receptor agonists were infused bilaterally into the mPOA of female rats hormonally primed with EB-alone. An increase in D2 receptor activity increased precopulatory behaviours, while an increase in D1 receptor activity reduced hops and/or darts. In Experiment 2, a nonspecific DA receptor antagonist and specific D1 and D2 receptor antagonists were infused bilaterally into the mPOA in fully (EB+P) primed females. An opposite pattern of results were found in Experiment 2, where an increase in D1 activity (relative to D2 activity, which was blocked) increased precopulatory behaviours, while an increase in D2 activity over D1 (through D1 receptor blockage) decreased solicitations. These effects signify that DA in the mPOA is important for the control of precopulatory behaviours in female sexual behaviour, and that this control is dependent on the hormonal profile of the female. The ratio of specific DA receptor subtype activity in the mPOA may be important in the timing of behavioural expression during copulation, controlling precopulatory behaviours in order to influence the amount of genital stimulation females receive from males.

Given that DA plays an excitatory role within the mPOA on male sexual behaviour, it was hypothesized that the DA agonists, both general and specific, would promote female sexual behaviour; and thus antagonists would inhibit it. In accordance to the dual excitatory role that DA subtypes play in male sexual behaviour, it was further hypothesized that this same dual role would exist within the mPOA for female sexual behaviour. It was predicted that D1 receptor activation would affect the expression of precopulatory behaviours. Specifically, that stimulating them within the mPOA would increase solicitations and hops and/or darts. Conversely, D2 receptors in the mPOA were hypothesized to affect the expression of consummatory behaviours. Therefore, stimulating D2 receptors in the mPOA was predicted to increase lordosis postures in response to male mounts, both in number and intensity.

The role of DA within the mPOA on female sexual behaviour is more complex; and it goes beyond that of a dual function for male sexual behaviour that found Hull and colleagues (1989) found. Based on the male results, it was hypothesized for the present set of experiments that the two DA subtypes would play different, but complimentary, roles in promoting sexual behaviour in females: one subtype responsible for precopulatory behaviours, and one for consummatory behaviours. However, the current results indicate that each subtype plays contrasting roles in the control of precopulatory behaviours, which are dependent upon the hormonal profile of the female. In partially-primed females, primed with EB-alone, the stimulation of D1 receptors reduced hops and/or darts, and the stimulation of D2 receptors increased solicitations. Stimulating DA receptors overall increased both types of precopulatory behaviours. Since both hops and/or darts and solicitations are two types of precopulatory behaviours, it is possible that instead of having exact effects on these behaviours, the manipulations by the specific receptor agonists instead had an overall influence on precopulatory behaviours that were manifested through one type behaviour or another. It is also possible that other types of precopulatory behaviours that were not measured could have been affected through dopamine alteration. In fully-primed females, the effects of the specific DA receptors contrast those of partially-primed females. Specifically, blocking D1 receptors in fully primed females decreased solicitations, while increasing non-flank stimulated lordosis postures. Blocking D2 receptors increased solicitations. Blocking DA receptors in a non-specific manner had no effect on sexual behaviour.

The contrasting roles of the D1 and D2 receptor subtypes help to explain the inconsistencies seen in the literature regarding the role of both DA and the mPOA on female sexual behaviour. Inconsistencies have been seen in lesion and electrical stimulation studies, where reports have found that the mPOA inhibits lordosis in both conditions (Law & Meagher, 1958; Napoli et al., 1972; Powers & Valenstein, 1972; Moss et al., 1974, Hoshina et al., 1994), as well as in different testing environments, where the mPOA has also been found to enhance

lordosis regardless of testing environment (Bast et al., 1987) or under only specific circumstances (Whitney, 1986). Yet other studies have found no effect of lesioning the mPOA on lordosis, though contact return latencies following intromission and ejaculation were increased (Guarraci et al., 2004). As far as the role of the mPOA in precopulatory behaviours, the data more consistently point to the mPOA being a critical region for the display of these behaviours (Whitney, 1986; Hoshina et al., 1994; Guarraci et al., 2004).

Previous studies that examine the role of DA on female sexual behaviour have generated contrasting results, and most focus solely on lordosis as the main measure of sexual behaviour. Dopamine was initially considered inhibitory for female sexual behaviour, as LQs were reduced following systemic injections of several nonspecific DA agonists (i.e. Everitt et al., 1974; Eliasson & Meyerson, 1976; Everitt & Fuxe, 1977), and increased following nonspecific DA antagonists (i.e. Caggiula et al., 1979; Everitt, 1990). In contrast, LQs have been shown to increase following APO injections (Hamburger-Bar & Rigter, 1975) and infusions into the third ventricle (Mani et al., 1994). Foreman and Moss (1979) infused APO, FLU and HAL into the mPOA and arcuate-ventromedial area and concluded that DA within those areas was critical for display of lordosis responses.

In addition, DA receptor subtypes have been shown to vary in their roles, as a D1 receptor agonist has been reported to facilitate lordosis when infused icv (Mani et al., 1994); though it has also been found to have no effect when injected systemically (Grierson et al., 1988). There is also a disparity surrounding D2 receptors, as stimulating them through systemic injections with medium doses increases lordosis, but high and low doses of the same agonist decrease lordosis (Foreman & Hall, 1987). The D2 antagonist sulpiride also showed dual effects when injected systemically, inhibiting lordosis in fully-primed females, and enhancing it in EB-alone females (Grierson et al., 1988). Yet others have reported that D2 receptor stimulation has no effect on female sexual behaviour when infused icv (Mani et al., 1994).

Although two explanations have been put forth to explain such disparities (Melis & Argiolas, 1995), that of differing levels of baseline receptivity and doses administered, the present thesis offers a third reason. This can be thought of as a double dissociation where the effect of DA within the mPOA on female sexual behaviour is dependant on the ratio of specific DA subtype activation, and upon the specific hormonal profile of the female. Specifically, females in one hormonal state (EB-alone and thus partially receptive) have a D2-biased system, whereas females in the other state (EB+P and thus fully receptive) have a D1-biased system.

The findings of the present thesis show that in partially-primed females, the stimulation of D2 receptors, thereby increasing the ratio of D1/D2 activation in favour of D2 receptors, increases precopulatory behaviours, specifically solicitations. Tilting the ratio in the other direction, by stimulating D1 receptors, results in a decrease in hops and/or darts. A general increase in precopulatory behaviour, including hops and/or darts and solicitations, is seen when the activity of both receptors are equally stimulated, following infusion of a non-specific agonist. This may signify the dominance of D2 activity in EB-alone females.

Compared to their roles in EB-alone females, D1 and D2 receptor activity is the opposite in EB+P females. When D1 receptor activity is greater than D2 receptor activity, solicitations increase. When D2 activity is greater, solicitations decrease. This suggests that in fully-primed females, D1 receptor activity is responsible for precopulatory behaviours, while D2 receptor activity inhibits them. When receptor activity is equally diminished following infusion of a nonspecific dopamine receptor blocker, no effects on behaviour are observed. A behavioural effect caused by stimulation of one DA receptor population relative to another is not a new concept. Dopamine receptor densities can change over a lifespan, for example going from a 1:1 ratio in D1 to D2 receptor binding sites at approximately 20 years of age in normal human neostriatum, to twice as many D1 receptors than D2 at age 75 (Morgan et al., 1987).

Shifts in densities can cause increased activation in one receptor subtype over another in response to endogenous DA release. These density shifts can occur from either the formation of

new receptors, or the degradation of old ones. These changes have the potential to result in transformed reactions to DA and dopaminergic drugs, in terms of responsiveness and sensitivity. Steroid gonadal hormones have been found to affect the number of DA receptors, particularly in the striatum. Receptor density is increased both in terms of D1 receptors (Hruska & Nowak, 1988; Levesque & Di Paolo, 1989) and D2 receptors (Hruska, Ludman, & Silbergeld, 1980; Hruska, 1986) after chronic estradiol treatment. Progesterone was found to decrease striatal DA D2 receptors when administered to females independently, but increased the density of DA D2 receptors when estradiol was given previously (Fernandez-Ruiz, Amor, & Ramos, 1989).

A dopaminergic ratio effect has previously been found by Hull and colleagues in the mPOA in male sexual behaviour (Hull et al., 1989). Essentially, it is theorized that stimulation of D1 receptors (or both receptor subtypes, as with a nonspecific agonist such as APO) promote erectile responses, a parasympathetic response. Shifting the stimulation ratio to D2 receptors would then result in a switch to control of the ejaculatory mechanism, a sympathetic response (Hull et al., 1989; 1999). These receptor ratios are believed to control the timing of copulation (Hull et al., 1999).

Thus, the current results suggest a similar control in the timing of female sexual behaviour, only with different roles under different hormonal conditions. Since lordosis postures and precopulatory behaviours are mutually exclusive, they cannot occur at the same time. It could be that shifts in dopaminergic activity in the mPOA affect which of these behaviours is predominant within the copulatory session. In fully-primed rats, if D1 receptor activity is increased over that of D2, an increase in precopulatory behaviour occurs. A shift towards D2 receptor activity then decreases those precopulatory behaviours. In rats that are partially-primed, the ratio is reversed, but still important. Increasing D2 receptor activity over D1 results in increased precopulatory behaviours; conversely shifting the ratio towards D1 decreases them.

The idea that the mPOA, in conjunction with the VMH, is important in the timing of female sexual behaviour (including pacing, lordosis and solicitations) has been previously put

forward (Pfaus, Giuliano, & Gelez, 2007). The present results suggest that the ratio of DA activity within the mPOA on its own could be responsible for precopulatory behaviours. These solicitations then help control the rate of copulation and thereby contribute to reproductive success (Erskine, 1989). Evidence for this stems from findings that precopulatory behaviours determine what kind of stimulation females receive from the male, and when this stimulation will occur (McClintock & Adler, 1978; Erskine, 1989). These precopulatory behaviours then decrease as the male stimuli increases in intensity (and presumably success; Bermant, 1961; Erskine, 1985). Thus, precopulatory behaviours, such as solicitations and hops and/or darts, serve to manipulate the timing and type of stimuli received from the male rat (Erskine, 1989), and this control could be dependent on DA activity, and the dominance of one receptor subtype over the other, within the mPOA of the female. The switch in DA receptor activity, and the subsequent decrease in precopulatory behaviours, would help the female receive ejaculations more efficiently, which in turn would promote neuroendocrine changes necessary for pregnancy (Adler, 1983; Erskine, 1989; Pfaus et al., 2007).

Although the ratio of DA receptor subtype activity may modify precopulatory behaviours, the ratio is dependent on the hormonal profile of the female. In EB+P females, a tilt towards D1 receptor stimulation increases solicitational behaviour. In EB-alone females, shifting activity towards the D2 receptor has the same effect. These findings are in line with that of Grierson et al. (1988) who show that D2 receptor activation facilitates lordosis in EB-primed females, but inhibits it in EB+P females. While in the present study there were no effects on lordosis due to D2 receptor manipulation, the results do show that D2 receptor activation increases solicitations in EB-primed females, and decreases solicitations in EB+P females. It would appear then, that when dopaminergic agents are administered systemically, as by Grierson et al. (1988), there are hormonally-dependent lordosis effects, but when D2 agents are infused directly into the mPOA, hormonally-dependent solicitational effects are seen. It seems that lordosis is a behaviour more easily elicited through DA manipulation, possibly due to the number of brain areas that have been

found to affect it. Precopulatory behaviours meanwhile, may be controlled only by DA in the mPOA, and thus systemic injections more easily affect lordosis rather than precopulatory behaviours.

Studies showed that solicitations decrease, and lordosis increases, following ibotenic acid lesions of the mPOA (Hoshina et al., 1994). This suggests that the mPOA is particularly involved in the precopulatory sexual responses that solicit sexual behaviour from males. Dopamine would then be one of the substrates controlling precopulatory behaviours, though the role of DA is not as straightforward as to completely explain the data found by Hoshina et al. (1994). Through lesioning of the mPOA, both DA receptor subtypes would also be eliminated. Based on the present findings, one would expect no change in precopulatory behaviours, as seen following infusion of FLU, and thus some other mechanisms must be at work. The mPOA has also been implicated in pacing, as electrolytic lesions to this area caused an increase in return latencies of the female following intromissions and postejaculatory refractory period (Yang & Clemens, 2000). Finally, Guarraci and colleagues (2004) have further advanced this issue, finding that neurotoxic lesions reduce both precopulatory behaviours and pacing, supporting the two previous studies. These last two studies failed to find any influence of mPOA lesions on lordosis however.

The disparity of the role of the mPOA on lordosis can be explained by the finding by Whitney (1986), who shows that mPOA lesions increase LQs only when the female cannot pace the male. Hoshina et al. (1994) found an increase in LQs when using a no-exit chamber, while Yang and Clemens (2000) and Guarraci et al. (2004) used pacing chambers. In addition, Whitney (1986) reported that following mPOA lesions, when females can exit from the male, LQs do not change but precopulatory behaviours decrease. All of this leads to the conclusion that when females are allowed to pace the copulatory session, either through exits from the male or switching levels in a bilevel chamber, the mPOA is critical in controlling the timing of the female sexual behaviour through control of precopulatory behaviours. The mPOA only plays a role on LQs when the copulation occurs in environments where the female cannot properly escape at her

preferred interval. The inability to time the copulation could then lead the female to take a lordosis posture more frequently.

How DA in the mPOA affects female precopulatory behaviours changes depending on the hormonal profile of the female. D1 receptor activity increases precopulatory behaviours in fully-primed, EB+P females, but it is D2 receptor activity that promotes these behaviours in partially-primed, EB-alone females. Thus, depending on the level of P within the female, the ratio of DA subtype activity has inverse effects, which have been observed previously. As mentioned above, Grierson and colleagues (1988) observed that the D2 receptor has dual effects on lordosis for females tested in chambers where they cannot escape (Grierson et al., 1988). The authors concluded that the differences are based on presynaptic effects stimulating lordosis in non-receptive, EB-primed rats, and large doses acting postsynaptically in EB+P females, inhibiting lordosis. The D2 receptor antagonist had opposite effects, acting presynaptically to inhibit lordosis in EB+P females and acting postsynaptically to stimulate lordosis in EB-primed females (Grierson et al., 1988). Although this hypothesis is one possible explanation of the present results, there are caveats to take into consideration.

Among the issues that limit comparisons between the present results and that of Grierson et al. (1988), the first is the route of administration of drugs. Grierson et al. (1988) injected the dopaminergic agents systemically; in the present set of experiments drugs were microinfused directly into the mPOA. With drug infusions, it is extremely difficult to comment on mechanisms of action since drugs are available in the extracellular space to both presynaptic and postsynaptic receptors. Assuming it was possible to speculate on mechanistic actions, the high doses of QUIN and RAC, the D2 receptor agonist and antagonist, respectively, had opposite effects, though the amount infused was equal. However, it should be noted the binding affinity of each of these drugs may differ. The low dose of the D1 agonist SKF 38393 and the high dose of the D1 antagonist SCH 23390 also had opposite effects, and while this could be perceived as differing via pre- and postsynaptic effects, the differences in amount infused between high and low

dosages are not nearly as high as that of the study done by Grierson et al. (1988). In that study, the systemic dosages that both inhibited LQ in receptive rats and stimulated LQ in nonreceptive rats were 4-80 times higher than doses that solely stimulated LQ in nonreceptive rats. The present data however, used doses that only increased by an order of two; thus the high dose was only four times higher than the low.

In view of the fact that the doses do not differ by a very large margin, it is very unlikely that the high dose is high enough to only act postsynaptically, and the low dose is low enough to only act presynaptically. This is especially evident when taking into consideration that drugs infused in the extracellular space are in contact with receptors at both ends of the synapse. Instead of the dopaminergic drugs acting differentially pre- and postsynaptically based on doses, it is possible that the hormonal profile is altering what postsynaptic receptors do. In other words, in the presence of P, activation of postsynaptic D1 receptors may stimulate precopulatory behaviours, and in the absence of P activation of these receptors may be inhibitory. Postsynaptic D2 receptors could then have the opposite profile in the presence and absence of P.

Through the use of in vivo microdialysis it has been determined that both the hormonal state of the female (generated either through endogenous and exogenous hormones) and acts of copulation have influences on DA activity within the mPOA (Luine, 1993; Matuszewich et al., 2000). Although extracellular DA levels were too low to measure in naturally cycling females, 3,4-dihydroxyphenylacetic acid (DOPAC), a DA metabolite, increased from the start of Proestrus to 3-5 hours later; a time when females are receptive (Luine, 1993). Though DOPAC is not a direct measure of DA, the data imply that DA increases at a time when gonadal hormones induce sexual receptivity.

In ovariectomized females primed with a low dose of estrogen (2 µg), P injections increase extracellular DA in addition to sexual receptivity. Higher doses of EB (20 µg) do not change either the extracellular DA or receptivity when followed by a P injection. There is however a decrease in DA metabolites, which is presumed to indicate a decrease in DA turnover

(Matuszewich et al., 2000). This DA synthesis decrease could be a result of P, as part of a mechanism to gradually reduce sexual receptivity. Interestingly, the data show equal LQs for females receiving a P injection following priming with 20 µg of EB compared to when they receive 20 µg of EB alone; and the 20 µg EB+P group show fewer precopulatory behaviours (Matuszewich et al., 2000). This high dose of EB could cause a receptive state in the female mirroring that of rats receiving 2 µg EB+P, and thus an injection of P may not be necessary for increasing DA levels in the mPOA. As such, sexual behaviour would not be increased further (Matuszewich et al., 2000). In the present study, this may explain why DA agonists given to females treated with EB+P did not result in any behavioural differences. Perhaps there was too much circulating EB present for P to have an effect on DA activity in the mPOA (data not shown).

Therefore, it may be that the level of E present in the female directly influences P manipulations on DA activity in the mPOA on female sexual behaviour. This is logical since E upregulates P receptors in the mPOA and other areas that known to influence female sexual behaviour (MacLusky & McEwen, 1978; 1980). Conversely, while it is widely known that E alone can affect sexual behaviour (i.e. Pfaff, 1980; Blaustein, Finkbohner, & Delville, 1987), the mechanism(s) through which this occurs is not known. Consequently, it may be that instead of E having an effect on P's control of DA in the mPOA, it is possible that females primed with a high dose of E use different neural mechanisms than those that are primed with both EB+P.

In the VMH, DA has been shown to promote lordosis by activating progesterin receptors (PR) in a "ligand-independent manner" (Mani et al., 1994). After learning *in vitro* that DA could stimulate PRs through activation of transcription factors, it was later discovered that DA (and the D1 subtype in particular) could mimic the effects of P when infused *icv* (Mani et al., 1994). Two ways DA affects PR are through activation of the chicken ovalbumin upstream promoter (COUP) transcription factor (Power, Lydon, Conneely, & O'Malley, 1991), and when in an *in vitro* cell transfection system, the translocation of the transcription factors PRs and ERs from the cytoplasm

to the nucleus (Power, Mani, Codina, Conneely, & O'Malley, 1991). This mimicking effect could then be blocked by infusion of PR antagonists, as well as antisense oligonucleotides to PR mRNA (Mani et al., 1994), indicating that PRs were required for DA to have an effect. It has since been narrowed down to the D5 receptor subtype (also known as D1B) that has PR-mediated behavioural effects (Mani et al., 2001), and these effects have been found only in the VMH, but not the mPOA or arcuate nucleus (Apostolakis et al., 1996). However, coexpression of D5 receptors and PRs has been found in all three of these areas in female rats (Blaustein et al., 1999).

So whereas the D1 receptor subtype (D5 specifically) in the VMH can facilitate lordosis in the absence of P, the present study indicates the potential for a related mechanism occurring in the mPOA. In the mPOA, and in the absence of P, the D2 receptor subtype facilitated precopulatory behaviours, which could be working synergistically with the D1 subtype activation in the VMH. This would potentiate the full cascade of female sexual behaviour. Specifically, D1 activation in the VMH increases lordosis, and D2 activation in the mPOA increases precopulatory behaviours, both potentially in a ligand-independent manner via PRs. Since lordosis and solicitations are mutually exclusive behaviours, these facilitations help bolster both the precopulatory and consummatory aspects of female sexual behaviour, thereby increasing the likelihood of successful pregnancy. In females primed with both EB+P, the role of the specific DA receptor subtypes change in the mPOA (and possibly in the VMH), as D2 activation now inhibits precopulatory behaviours, perhaps a mechanism for ceasing sexual behaviour as a result of too much stimulation. Meanwhile, the D1 subtype facilitates precopulatory behaviours. This hypothesis could be tested by administering antisense oligonucleotides to PR mRNA before testing the effects of D2 activity in partially (EB-alone) primed females. If there is no effect of increased D2 activity, that would suggest that D2 is operating via a ligand-independent mechanism on PRs as Mani and colleagues describe as occurring in the VMH (Mani et al., 1994).

An alternative explanation for the interaction between DA and P could be through cellular membrane-mediated actions, such as those found in the VTA for lordosis. In rats primed

with EB+P, either naturally or through hormonal injections following ovariectomy, D1 activation is critical for expressing lordosis (Frye & Vongher, 1999). In concurrence with the present data, D1 facilitation of lordosis in the VTA is not seen in rats primed only with EB (Frye et al., 2004; Petralia & Frye, 2004). Therefore, similar to DA in the mPOA, DA in the VTA may work through different mechanisms depending on the hormonal profile of the female rat to modulate sexual behaviour. It has been proposed that increases in cyclic AMP (cAMP) levels, which amplify by D1 activation, augments the effect of P on lordosis within the VTA.

In the VMH, DA affects PRs in a ligand-independent manner, but in the VTA this mechanism is not present. This has been shown as introducing antisense oligonucleotides for PR mRNA does not reduce P-facilitated lordosis in rats (Frye & Vongher, 1999). Thus, P stimulates lordosis in the VTA via mechanisms independent of PRs. Instead, DA is critical in P having a membrane-mediated effect on lordosis in the VTA, potentially through cAMP stimulation. However, both of these brain areas control lordosis; in the mPOA, where DA facilitates precopulatory behaviours, the mechanism(s) through which DA mediates its effects are not yet known. It may be that this process occurs in the mPOA, especially considering the largest mPOA input goes to the VTA (Conrad & Pfaff, 1976; Swanson, 1976; Brackett & Edwards, 1984). This process could co-exist in both the mPOA and VTA concurrently, thereby allowing P and DA to organize the timing of female sexual behaviour through control of both lordosis and precopulatory behaviours. One potential way of testing whether the DA's effects on precopulatory behaviours in the mPOA is mediated through cAMP stimulation is to block cAMP with an adenylyl cyclase inhibitor 2',5'-Dideoxyadenosine (DDA), which would prevent cAMP release. This should be tested in rats both partially- and fully-primed, and in concurrence with both non-specific and specific DA agonists and antagonists. If the effects of the present studies are altered through manipulation of cAMP levels, this would contribute to a greater understanding of the mechanism of how DA has its effects on precopulatory behaviours within the mPOA.

A third, more recent hypothesis regarding how P and DA interact to modulate precopulatory behaviours comes from a study showing that PR expression can alter the level of activity of POA and DA neurons. Accordingly, it could be that instead of DA having an effect on PRs, it could be that P and/or PRs have an effect on DA neurons resulting in changes downstream. It has been shown that dopaminergic neurons in the POA express PRs (Lonstein & Blaustein, 2004; Leite, Szawka, & Anselmo-Fanci, 2008), and that both gonadal steroids (E and P) have been shown to influence these neurons (Hou, Yang, & Voogt, 2003; Lonstein & Blaustein, 2004; Leite et al., 2008). Although the specific behavioural effects PRs produce on POA DA neurons has yet to be elucidated, Leite and colleagues (2008) theorize that changes in PR expression on DA neurons within the POA could alter DA activity. It should be noted however that while PRs have been found to exist in the mPOA, DA neurons in this area has not yet been shown to express PRs. This is also true of the zona incerta (Sar, 1988; Fox, Harlan, Shivers, & Pfaff, 1990; Auger, Moffatt, & Blaustein, 1996; Lonstein & Blaustein, 2004), the area from which the mPOA receives DA inputs (Wagner et al., 1995). Nevertheless, this is a relatively new theory regarding how DA may interact with the two different hormonal-priming schemes, affecting female sexual behaviour.

Though the microdialysis study noted above has demonstrated that P increases extracellular DA, shown to be critical in the display of precopulatory behaviours by the present study, two other factors were noted to increase the amount of extracellular DA in the mPOA (Matuszewich et al., 2000). The first aspect is male stimulation received, hence perineal stimulation; the second is the environment in which the copulation takes place. These two factors can affect the levels of extracellular DA interactively. In a nonpacing chamber, extracellular DA increases occur independently of male stimulation, depending only on the environment. When pacing can occur, changes in DA levels were not observed, but increases in the metabolites DOPAC and homovanillic acid (HVA) were found following increases in male stimulation (Matuszewich et al., 2000).

Due to the fact that these changes occur regardless of the hormonal profile of the female, and only in response to copulation (or the environment in which it occurs), they could be seen as promoting enough DA activity to “turn off” sexual receptivity (See Figure 9). As DA activity in the mPOA builds, the female increases the number of precopulatory behaviours displayed, which in turn increases the intensity of male stimulation received by the female (i.e. clitoral, flank and vaginocervical). The switch in the ratio of D1/D2 activity could come about naturally through the reception of escalating male copulatory stimuli. It is known that a decrease in precopulatory behaviours, and an increase in pacing, is cued by increasing amounts of male stimulation, as the decline is not seen in females with an anaesthetized perineum and vagina (Bermant & Westbrook, 1966). This stimulation received from the male would then feedback on the mPOA, increasing DA activity until the ratio of DA receptor subtypes “switches” to the D2 receptor in fully-primed females, and the D1 receptor in EB-alone females. In doing so, there would be a decrease in precopulatory behaviours, so that by the time male stimulation increases to the point of several ejaculations, the female will no longer be soliciting a sexual response, thereby essentially ceasing sexual receptivity. This effect could be comparable to estrus termination.

A possible future experiment to test this model would consist of measuring DA receptor subtype binding using autoradiography following reception of VCS. VCS could be received naturally, through copulation with a male, and artificially, via the use of a glass rod by the experimenter. This model suggests that following VCS, the ratio of D1/D2 activity would eventually reverse after a certain amount of stimulation is received. This amount could be comparable to the average amount of intromissions females require for successful pregnancy. Thus, the DA ratio switch could coincide with other processes that occur at the time of pregnancy, reducing sexual receptivity.

Whereas the present data suggest that dopamine binding to different DA receptor subtypes in the mPOA can alter the precopulatory behaviours of female rats, no effect on lordosis was observed, except for one notable exception. In rats primed with EB+P and given the D1

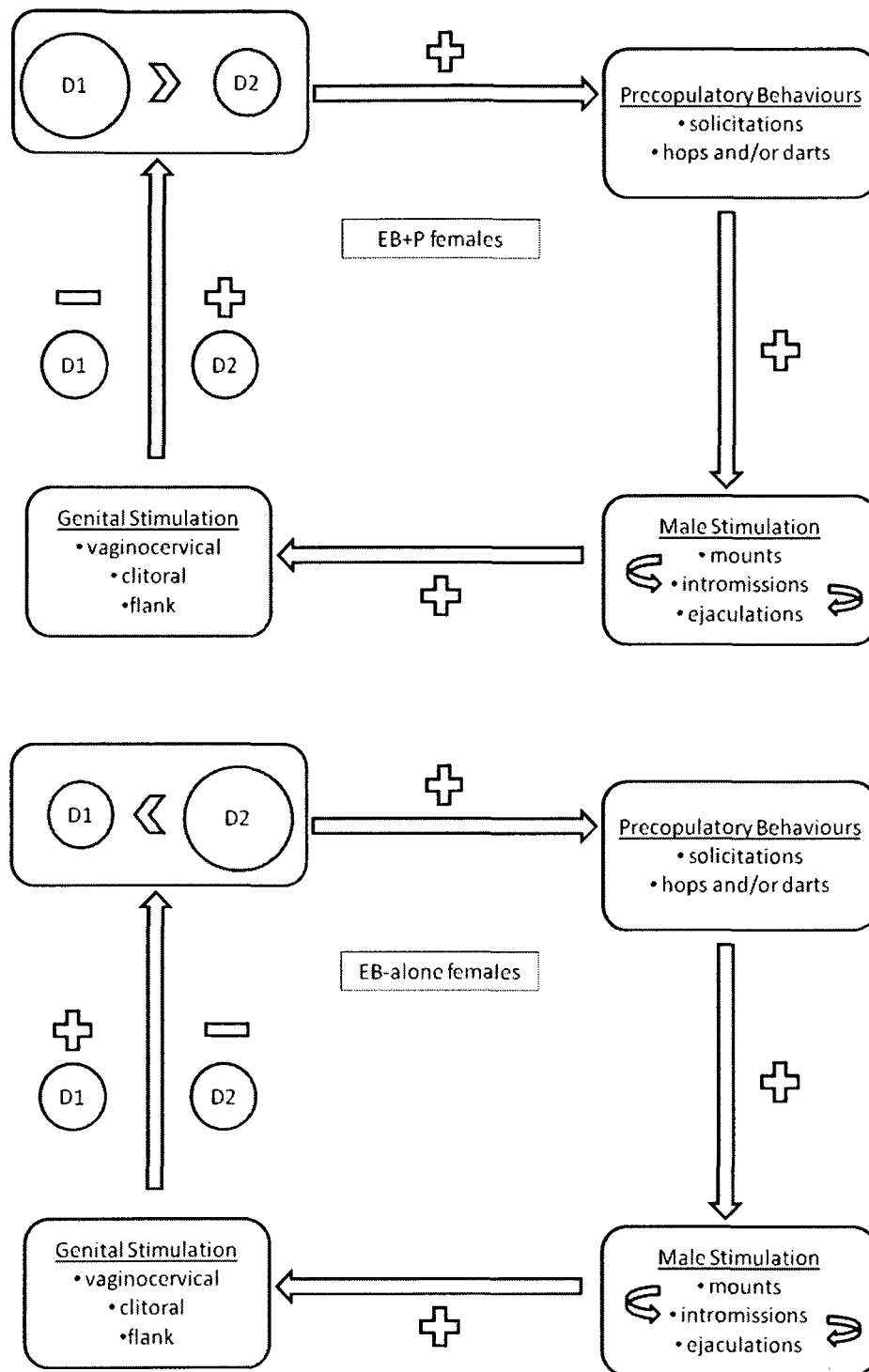


Figure 9. Model for the effect of the ratio of dopamine subtypes in female sexual behaviour, under two hormonal profiles.

receptor antagonist SCH 23390, thus tilting the DA activity in favour of the D2 subtype, an increase towards significance in non-flank stimulated lordosis postures (NFSLP) was found. A NFSLP was defined in the present set of experiments as the display of a lordosis posture in the absence of a mount by the male. Normally, in a receptive female, stimulation of the flanks, followed by pressure on the rump, base of the tail and perineum induce lordosis (Pfaff, Montgomery, & Lewis, 1977). The location and preciseness of the stimulation, as well as the order in which it is received are important (Pfaff et al., 1977).

Flank stimulation activates a spinal circuit, which includes activation of the brainstem, and results in the contraction of axial muscles in the back (Pfaff et al., 1977). This produces a lordosis posture, which in the present experiments would be scored as a lordosis magnitude 1 (LM1) posture, signified as lacking an arching of the back. In normal receptive rats, flank stimulation is the absolute minimum required to elicit any sort of lordosis response; hair deflection has no effect (Pfaff et al., 1977). It was thus concluded that cutaneous mechanoreceptors connected with the rat's hair did not play a role in the lordosis reflex on their own. Instead a different set of mechanoreceptors must respond to pressure of the skin to invoke lordosis (Pfaff et al., 1977).

However, the results of the present study indicate that when blocking D1 receptors in the mPOA of fully-primed rats, thereby increasing the ratio of DA activity in favour of D2, that the sensitivity of the female rat is increased so that a lordosis posture is exhibited more readily and easily, in response to stimulation less than that of flank stimulation. It could be that the females become so sensitive to male stimulation that sniffing of the flanks or light brushes by the male's nose or body, can trigger a lordosis response. This type of lordosis response is found in the hamster, an animal studied for its easily inducible and prolonged lordosis posture (Tiefer, 1970), where simple hair deflections in a sensitive area of the skin can cause a lordosis reflex (Pfaff et al., 1977). An increased area of sensitivity may compensate for a decrease in pressure intensity required to elicit such a response (Kow, Montgomery, & Pfaff, 1979).

It is known that E is necessary to potentiate lordosis in response to flank stimulation, and because it is a reflex it does so in an all-or-none fashion; although the reflex occurs to varying degrees (Kow & Pfaff, 1998). Estrogen has been shown to increase cutaneous receptive fields of the pudendal nerve by 30%, causing the sensory pathways of cutaneous inputs to converge, which results in a decrease in the stimulus threshold for lordosis to occur (Kow & Pfaff, 1973). This stimulus threshold is evident as ovariectomized rats show different LQs when primed with different levels of E (Kow et al., 1979). However it is thought that E only affects the response of the female to the sensory input, by having a permissive influence to neurons responsible for the lordosis response. In the VMH for example, E influences neurons so that they are more likely to be excited, which results in altered motor output and increasing the likelihood lordosis will occur (Kow & Pfaff, 1998). Estrogen does not, however, alter the input itself, as other properties of stimulation necessary for the rat to experience the prerequisite responsible for eliciting the lordosis reflex are unaffected. These include the stimulus threshold, resting firing rate, and receptive field size (Kow, Zemlan, & Pfaff, 1980; Sakuma & Pfaff, 1980; Kow & Pfaff, 1982).

Based on the present finding that EB+P females exhibit more non-flank stimulated lordosis postures following D1 antagonist infusion into the mPOA, it is possible that increased D2 activity (or less D1 activity) can alter these stimulation properties, either increasing the receptive field size or decreasing the stimulus threshold, so that in these females the lordosis reflex occurs much more easily. Perhaps E is acting on DA neurons in the mPOA to facilitate this occurrence.

It is known that neurons in the mPOA are active in response to the male stimulation associated with copulation, as mechanically stimulating the uterine cervix causes firing in the mPOA (Haskins & Moss, 1983). VCS provided naturally by the male rat, or manually by an experimenter with a glass rod, has also been shown to increase *c-fos* immunoreactivity in the mPOA (Pfaus et al., 1993; 1996). Additionally, electrical stimulation of the mPOA increases vaginal blood flow and wall tension (Giuliano et al., 2001; Giuliano, Rampin, & Allard, 2002). It

has been theorized then that the mPOA may be a component necessary in the response and processing to VCS (Yang & Clemens, 2000), and perhaps DA in the mPOA of the female is responsible for this processing. The present results indicate that EB+P females show increased NFSLPs when D2 activity is greater than that of D1, which suggests that this alteration in responding to male stimulation may supersede just VCS, and may encompass that of other types of stimulation provided such as flank, perineum or clitoral.

Evidence supporting the role of the mPOA on the sensitivity of the female to genital stimulation comes from differences found in return latencies following different types (and thus amounts) of male stimulation received. Lesions of the mPOA result in a lengthening of the time it takes a female to return to the male following intromissions and ejaculations, and not mounts, as they do not provide VCS (Yang & Clemens, 2000). This is potentially due to a reduction in the analgesic effect of VCS (Komisaruk & Whipple, 1986; Gomora, Beyer, Gonzalez-Mariscal, & Komisaruk, 1994), which could cause an aversive response to copulation. This may be why the female rats displayed more precopulatory behaviours, since while they were receptive to copulation, they also may have found it aversive. It is possible that the mPOA inhibits sensitivity of not only the cervix but also of the other areas incorporated in producing the lordosis response, including the flanks and perineum.

A final hypothesis that attempts to explain the increase in NFSLPs observed is that they are not lordosis postures at all, but rather a different type of precopulatory behaviour. Although Pfaff (1980) defined this behaviour as a pre-lordosis crouch, it has also been denoted in the literature as posing. Characterized by ear wiggling and abrupt immobility (Whitney, 1986), it occurs when a female presents a posture that orients her hindquarters towards the male (Emery & Moss, 1984; Erskine, 1989). In the present study neither ear wiggling nor orientation of the female in the NFSLP was noted, therefore it remains possible that the NFSLPs exhibited could be a different type of precopulatory behaviour, displayed in the fully-primed female when D2 activity in the mPOA is greater than that of D1.

The possibility thus remains that the NFSLPs, which were the only difference in lordosis postures observed, could instead be a type of output fulfilling the goals of precopulatory behaviours. Regardless of what classification it fits under, its role is hypothesized to induce male mounting and stimulation, thereby increasing DA. When this DA reaches a certain maximum, it is expected that the ratio of activated receptor subtype will switch, a process that will decrease precopulatory behaviours, and thus male stimulation, increasing the likelihood of the female becoming pregnant.

Previously it has been shown that glutamate and GABA compete in the VMH to control lordosis, with activation of glutamate receptors inhibiting these consummatory behaviours (Georgescu & Pfaus, 2006a,b). Connections between the VMH and mPOA are known to exist, but have yet to be fully examined. The potential exists for the mPOA and VMH to interact and thus control the timing of female sexual behaviour, with a relationship between precopulatory and consummatory behaviours occurring in concert (see Figure 10). As this relationship contributes to the goal of receiving increasing male stimuli, genital stimulation experienced would feed back on the two areas. As proposed in the model above, this genital stimulation would eventually switch the ratio of neurotransmitter activity responsible for each aspect of the sexual cascade, specifically the DA D1/D2 ratio in the mPOA and glutamate/GABA ratio in the VMH. When the ratios switch, copulation would decrease, so that following ejaculation there is a post-ejaculatory interval, which serves the purpose of increasing the possibility of impregnation. This is essentially what is seen following estrus termination.

The connections between the mPOA and VMH on the control of the timing of female sexual behaviour should be tested in order to determine the accuracy of these proposed models. Notably, how the different processes are controlled between the two areas, so that precopulatory behaviours and lordosis postures are not occurring at the same time. This would include determining the neurons that make up this connection, and what type of neurotransmitters are

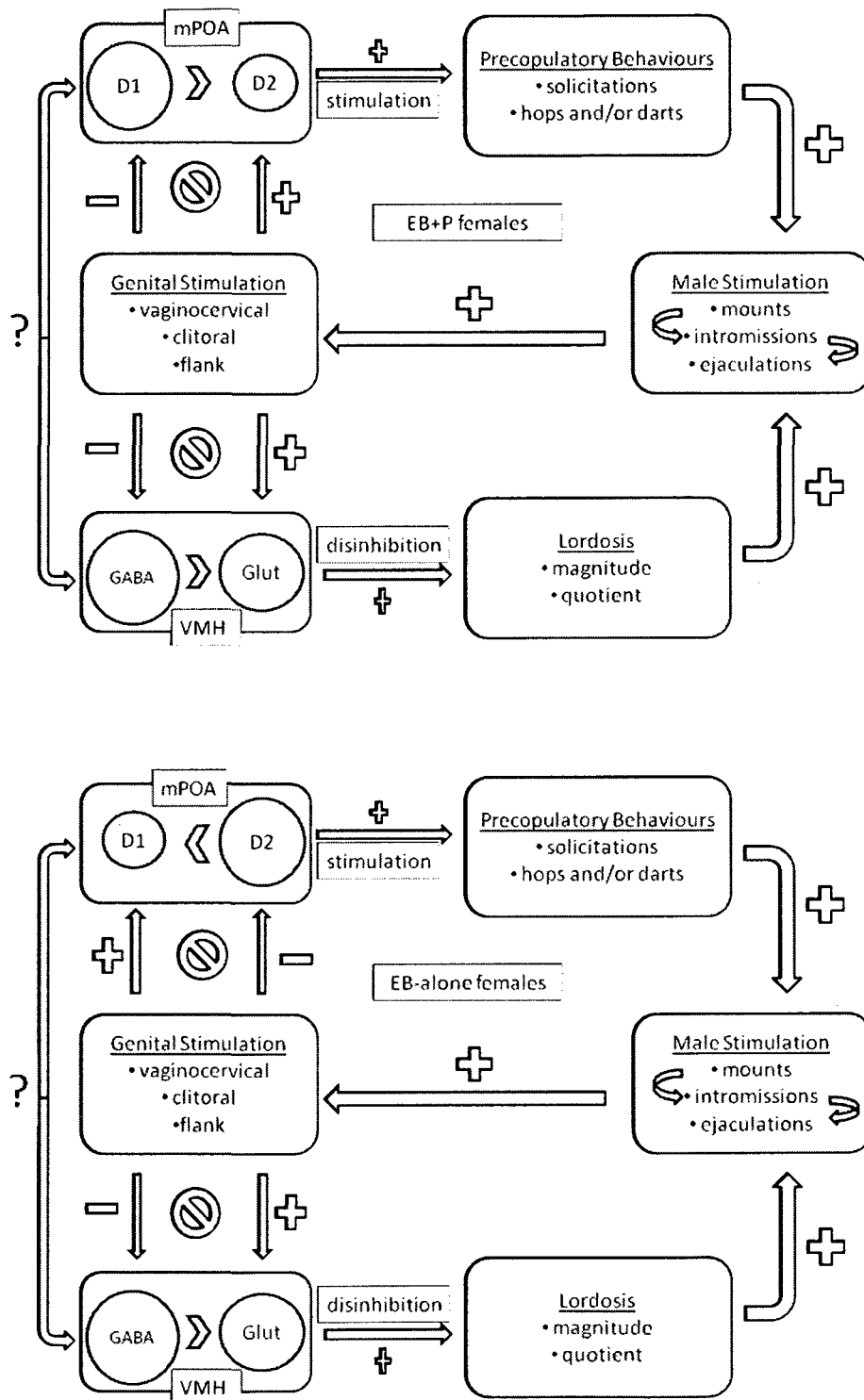


Figure 10. Model for the interaction between mPOA and VMH on the control of the timing of female sexual behaviour, under two hormonal profiles.

acting on them. Also of interest would be what effect activations of one area, either the VMH or mPOA, have on the other, and whether this changes under different hormonal profiles.

It is likely that multiple pathways exist for different aspects of female sexual behaviour. For example, it has already been noted that multiple areas have shown to influence lordosis, including the VMH and VTA. Therefore, multiple pathways influencing precopulatory behaviours may also exist. This is especially important when considering that the mPOA is interconnected with many other areas, receiving inputs from areas such as the main olfactory cortex, and sending outputs to the nucleus accumbens and VTA (Pfaus et al., 2007). Examination of these connections should be considered, in order to complete the brain circuitry responsible for female sexual behaviour.

In addition to testing the proposed models, future experiments should attempt to replicate the role of the dopamine subtypes, and their effects on precopulatory behaviour. Of particular importance is further examination of the ratio of D1/D2 activation. This could be accomplished by infusing nonselective DA, and selective D1 and D2 receptor antagonists into females that are EB-primed only. Note that there may be a floor effect observed following D2 receptor antagonist injections, as this would tilt the ratio in favour of D1 receptor activity; possible ineffective doses were observed testing the agonists on fully-primed females (data not shown). It would also be interesting to examine a change in the ratio of D1/D2 activation within a copulatory session, potentially through the use of reverse microdialysis. This would allow for the testing of the proposed models mentioned above, and whether a switch in DA subtype activity would result in a cessation of sexual behaviour. Finally, as Yang and Clemens (2000) have noted, a closer look at mPOA placement may be necessary, as the anterior portion of the mPOA seems particularly important in the control of the timing of the copulatory session.

In summary, DA in the mPOA plays a vital and hormonally-dependent role on precopulatory behaviours in female sexual behaviour. In female rats primed with EB+P, increasing D1 receptor activity promotes precopulatory behaviours, while increasing D2 receptor

activity decreases these behaviours. In EB-alone females, the opposite role is seen, as increasing D2 receptor activity facilitates precopulatory behaviours, and increasing D1 receptor activity decreases these behaviours. These altered ratios of D1/D2 activity, through stimulation of one subtype over another, could be critical in the timing of female sexual behaviour. This would be possible through controlling precopulatory behaviours in order to control the amount of male stimulation, and thus genital stimulation, they receive.

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