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Assessment of 'active investigation' as a potential measure of female sexual incentive motivation in a preclinical non-contact rodent model: Observations with apomorphine

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ABSTRACT

Clinical studies have suggested therapeutic potential for the non-selective dopamine receptor agonist apomorphine, in treating female sexual dysfunction. However, experimental data suggest apomorphine may inhibit sexual behaviour in female rats.

The aims of this study were: Evaluate an alternate behavioural endpoint in a conscious, non-contact model of sexual behaviour; and secondly investigate apomorphine in this model. Proceptive behaviour was determined in sexually naïve ovariectomised female rats as time spent actively investigating an inaccessible sexual incentive (sexually vigorous intact male rat) relative to time investigating a social incentive (castrated male rat) in an open field arena.

Apomorphine (10, 30 and 100 μg/kg SC) induced a dose-related bell-shaped increase in proceptive behaviour, achieving significance ($P<0.05$) at 30 μg/kg, in females given a low (estrogen 1 μg/rat + progesterone 100 μg/rat) hormonal prime. This was equivalent to proceptive activity displayed by females given a high (estrogen 5 μg/rat + progesterone 250 μg/rat) hormonal prime in full behavioural oestrous. In contrast, in females given the high hormonal prime all doses tended to decrease proceptive activity.

This study demonstrates that pro-sexual effects of apomorphine are critically dependent on hormone levels; sexual motivation is enhanced in animals given a low hormonal prime, but attenuated when given to animals in behavioural oestrous.

1. Introduction

The most commonly used method to assess female sexual function in laboratory rodents is the lordosis model. Lordosis is the final element of female rat sexual behaviour, and is a dorsiflexion of the spine evoked by flank stimulation by the male rat to permit intromission (copulation). It is commonly assessed in open field arenas, where the female rat is exposed to a defined number of mount attempts by a vigorous male. Mount attempts resulting in adoption of the lordosis posture are expressed as the 'lordosis quotient' and increasing lordosis quotient is thought to be analogous to an increase in arousal. A criticism of this methodology is that it does not allow the female to control copulation, in that the confines of the arena do not allow her to escape from the male and pace their interactions, which has been shown to be a key element of rat mating in more naturalistic environments (Martines and Paredes, 2001). Additionally it is difficult to dissect out the different elements of sexual behaviour, such as sexual motivation to approach a male, sexual arousal during copulation and orgasm. These different elements are separate clinical diagnoses of sexual dysfunction in women (Diagnostic & Statistical Manual I) and therefore it would be advantageous to model these separately in laboratory studies.

In a recent publication, Ågmo et al. (2004) reviewed several rodent models of sexual behaviour, and identified a non-contact place preference model as having advantages over contact models. This model is considered to assess sexual incentive motivation in the rat, defined as the time spent in an area adjacent to an inaccessible sexual incentive compared with the time spent in an area adjacent to a social, nonsexual incentive. The procedure employs time spent in a particular area rather than quantifying speed or rate of response as a measure of motivation. Locomotor activity can also be assessed and used as a covariate in analysis, an advantage of this procedure. A clear advantage of this model is that female behaviours are not readily overridden or masked by the actions of the male, because there is no physical contact between them. However, a drawback is knowing whether the apparent interest of the female to investigate the male is actually sexual motivation. Whilst it is currently impossible to determine this for certain, it has been established in various forms of choice models (e.g. escape chamber, Erskine, 1985; bi-level chamber, Pfaus et al., 1999; tethered males, Avitsur and Yirmiya, 1999) where the female

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can control contact with the male or in a seminatural environment (McClintock and Adler, 1978), that she will copulate with him after showing approach behaviours towards him.

Experimental data generated with this model, using ovariectomised female rats primed with estradiol benzoate (25 μg/rat) and progesterone (1 mg/rat) to mimic physiological estrus, suggest that apomorphine inhibits sexual incentive motivation in female rats. Saline, in the presence of the hormone prime used in this study induced a sexual preference score significantly different from 'no preference' (i.e. a significant bias towards the vigorous male), whereas apomorphine-treated rats (125 and 500 μg/kg) demonstrated no difference to a neutral preference score, but displayed attenuated locomotor activity (Ellingsen and Ågmo, 2004). A variety of studies in other models also suggests a regulatory role for dopamine in female sexual behaviour. During sexual activity dopamine concentrations have been shown to increase in the nucleus accumbens of female rats (Mermelstein and Becker, 1995; Pfaus et al., 1995) and hamsters (Kohlert et al., 1997), and in the preoptic area of the female rat (Matuszewich et al., 2000). The non-selective dopamine receptor agonist apomorphine induces genital vasocongestive engorgement in the conscious female rat, across the oestrus cycle (Beharry et al., 2003), at a dose of 80 μg/kg SC, equivalent to that which induces penile erection in the conscious male rat (Hsieh et al., 2004). Earlier preclinical studies demonstrated that apomorphine and dopamine when infused into the preoptic area or ventromedial hypothalamus in the estrone-primed rat stimulated lordosis, and dopamine receptor antagonists inhibited lordosis (Foreman and Moss, 1979). Additionally, Mani et al. (1994) demonstrated in the estrogen-primed rat that apomorphine and D_1- but not D_2-selective agonists enhanced lordosis behaviour following infusion into the third ventricle. In contrast, other reports suggest that pharmacological manipulations leading to a reduction in dopaminergic activity stimulate lordosis behaviour in rats (see Ahlenius, 1993), and facilitation of dopaminergic activity inhibits lordosis (Eliasson and Meyerson, 1976; Everitt, 1974; Michanek and Meyerson, 1982).

Although preclinical data in rats are conflicting, limited clinical studies have suggested therapeutic potential for apomorphine in treating both erectile dysfunction (Heaton et al., 1995) and enhancing the sexual arousal phase of women with orgasmic dysfunction (Bechara et al., 2004).

In the models described above the use of a hormone priming regime sufficient to induce physiological estrus may preclude studies investigating agents which enhance sexual behaviour if hormonally-induced sexual interest is maximal. However the use of such a prime may identify agents which attenuate sexual behaviour. Allers et al. (in press) recently described a rodent model of vaginal blood flow (vaginal spectral analysis, VSA) thought to be representative of arousal in the clinic, and have demonstrated in this model that apomorphine decreases the VSA signal during oestrus in naturally cycling rats. Also, the doses of apomorphine used in the Ellingsen and Ågmo study have been demonstrated to increase stereotyped behaviour (Fletcher and Starr, 1985), which could act as a confound on the behavioural preference score.

The aim of our study was to evaluate 'active investigation' in a non-contact place preference model in rats. Active investigation was defined as an obvious sniffing, licking or chewing of the grill separating the animals. This alternate measure of sexual motivation is potentially superior to the passive place preference model described by Ågmo et al. (2004) as it assesses continuing active interest by the test animal in the sexual and social incentives. In addition, evaluate the potential preclinical utility of the model by using 'active investigation' to investigate the reported pro-sexual activity of apomorphine, in the female rat.

2. Methods

2.1. Animals

All experiments were conducted in strict accordance with the UK Animals (Scientific Procedures) Act (1986) and Home Office guidelines. Male and ovariectomised (ovx) female rats (Long Evans, approx 200 g on arrival) were purchased from Harlan, UK. On arrival they were single sex housed in fours and given free access to food (RM1 from SDS, UK) and water. The animal stock room was maintained under standard laboratory conditions (21 ± 2 °C, 50–60% relative humidity) and reverse light/dark cycle under sodium lighting (lights off between 09:30 and 21:30 h). All rats were given at least 10 days to acclimatise before any habituation and/or experiencing commenced.

2.2. Apparatus

The test arena was based on that described by Ågmo (2003), but circular, approx 94 cm in diameter with 50 cm high walls, and two attached, opposing satellite boxes (25 × 25 × 25 cm). The satellite boxes were separated from the main arena by a perforated Perspex screen which allowed the test and incentive animals to hear, see and smell each other, but prevented contact. The arena had black walls, with a white base to enable a video track system (Ethovision, Noldus) to detect the black and white rats effectively against the background. A virtual area (30 × 21 cm) adjacent to each incentive animal satellite box was defined using Ethovision, as a passive investigation zone. Using the Ethovision program, time spent in each incentive zone, distance moved and movement velocity during the test period was captured.

Sexual experiencing of male rats was carried out in circular Perspex arenas (40 cm diameter, 60 cm high), observed and recorded manually.

All behavioural studies were carried out during the middle phase of the animal's dark cycle, under sodium lighting.

2.3. Design and procedure

2.3.1. Males

Prior to commencing studies all 16 males (including males due for castration) were sexually experienced with ovariectomised females primed with estradiol benzoate (Sigma; 10 μg/rat) followed 48 h later by progesterone (Fisher; 1 mg/rat). Both steroids were dissolved in corn oil and injected subcutaneously in a volume of 0.2 ml/rat. This hormone prime induces behavioural oestrus in the ovariectomised female rat, ensuring they are receptive to the male during the experiencing period 3 to 6 h post progesterone. Twice weekly for four weeks, all males were allowed to intromit until ejaculation. Eight of the males were then castrated (anaesthesia induced with Isoflurane (~4%) in an anaesthetic chamber and maintained by inhalation of oxygen and isoflurane (~2%)) and after recovery the experiencing continued twice a week to determine when they no longer displayed copulatory behaviour. At this point studies commenced. During the studies, all males received sexual experience twice weekly to ensure consistent behaviour in both intact and castrate males.

2.3.2. Females

A colony of 120 sexually naïve ovariectomised females were maintained and used for studies on a three week rotation.

2.3.3. Habituation

All test animals were habituated to the arena before their first study. Habituation involved placing the test female in the arena for 10 min on two occasions prior to inclusion in studies. At the same time, the males were also placed in the satellite boxes to ensure they too were familiar with the surroundings.

2.3.4. Study procedure

The methodology is based on that described previously by Ågmo (2003). In experiment 1 (optimising social stimulus), a vigorous intact male was used as the sexual stimulus; with either a sexually experienced ovariectomised female hormone primed (estradiol benzoate 10 μg plus 48 h later progesterone 1000 μg) to induce behavioural

oestrus, or a castrate male as social stimulus. The ovariectomised test female received either an identical hormone prime (as above) or corn oil vehicle. Studies commenced 4 h post progesterone prime during the dark phase of the light/dark cycle. Prior to commencing the study, and between study runs intact males were placed in the experiencing arena and allowed to intromit three times with a female primed to induce behavioural oestrus, which was then removed. This ensured that the males were in a state of sexual vigour. Similarly primed females were also placed in with castrate males to ensure the absence of copulatory behaviour, and provide identical treatment of all stimulus males.

In experiment 2 (investigating the effect of apomorphine), test females were primed with either (a) estradiol benzoate (1 μg/rat) followed 48 h later by progesterone (100 μg/rat) or (b) estradiol benzoate (5 μg/rat) followed 48 h later by progesterone (250 μg/rat). Studies commenced 4 h post progesterone prime during the dark phase of the light/dark cycle. Prior to commencing the study, intact males were treated as described in experiment 1. The test females were dosed subcutaneously with apomorphine (Sigma) or vehicle (saline containing 0.1% w/v ascorbic acid) 10 min prior to testing. The males were placed into the satellite boxes immediately prior to commencing the test. The test female is placed into the centre of the arena and time spent investigating each male is recorded for 10 min. Behaviour was observed and recorded by operators blind to the treatment groups. The following parameters were recorded:

Active investigation. This was recorded manually by stopwatch. Active investigation consisted of an obvious sniffing, licking or chewing of the grill separating the animals or attempting to reach the male via the grill.

Passive interest. This was defined as the time in which the female occupied a 30×21 cm passive investigation zone adjacent to the grill, not necessarily touching the grill nor displaying any proceptive behaviour. This was recorded by the Ethovision tracking system, which also captured frequency and duration of visits, the distance moved by the rat and the velocity. The distance and velocity data gave a measure of whether a compound affected locomotor activity, which could influence investigation times and act as a confound.

2.4. Cytological examination of vaginal lavages

Microscopic examination of vaginal cytology, following vaginal lavage, was carried out to determine the equivalent stage of the estrus cycle induced by the hormone primes used in this study. The vaginal lavage technique involves the insertion of a plastic pipette containing 0.2 ml saline (room temperature) into the opening of the vagina, dispensing of the saline into the vagina, collection of the saline and smearing of the liquid onto a microscope slide. The stage of estrus was determined based on the following criteria (Montes and Luque, 1988; Hubscher et al., 2005):

Diestrus A predominance of leukocytes. Some medium-sized nucleated and few cornified cells.
Proestrus Moderate numbers of medium-sized, non-cornified, nucleated epithelial cells, often found in clumps ('bunches of grapes').
Oestrus Large cornified cells predominate.
Metestrus Similar proportion of leukocytes, cornified, and nucleated epithelial cells.

2.5. Data analysis

The time spent actively investigating the intact male was expressed as a partner preference score, defined as time spent actively investigating the intact male (sexual stimulus) expressed as a ratio of total active investigation time of sexual and social (castrated male) stimuli. In this study, the term 'partner' is used to identify the preferred stimulus animal and not a preferred choice for copulation.

Passive interest was also expressed as partner preference score but in addition frequency and duration of visits were captured.

Data were expressed as means ± S.E.M. The statistical significance of differences between treatments were analysed either by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test post hoc, or two-tailed Student's *t*-test where appropriate. Significance was determined as $P<0.05$.

3. Results

3.1. Optimising the social stimulus

With the female social stimulus design, the vehicle-treated test female demonstrated a preference for the social over the sexual stimulus ($t(10)=4.05$, $P=0.0023$). Hormone treatment inducing behavioural estrus evoked a significant increase over vehicle in time spent investigating the sexual stimulus ($t(10)=5.14$, $P=0.0004$), however there was no significant difference between time spent investigating either stimulus animal ($t(10)=0.51$) (Fig. 1A: Student's *t*-test).

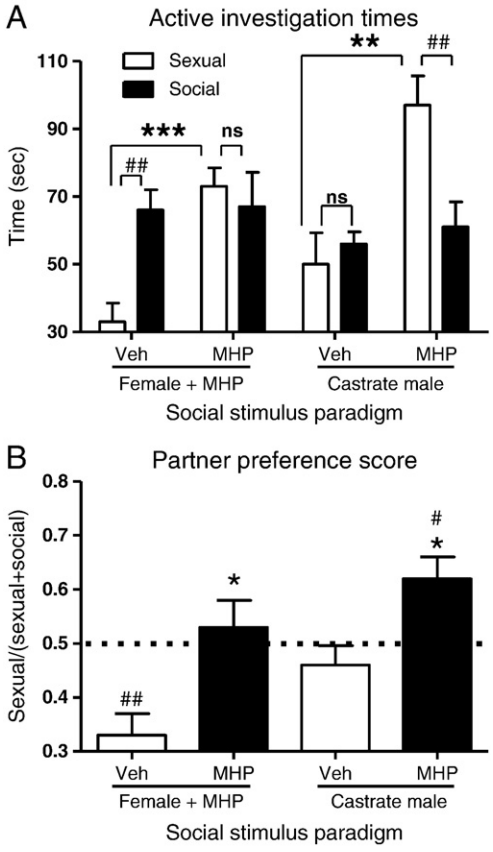

Fig. 1. (A) Time spent actively investigating a sexual stimulus (intact, vigorous male) or a social stimulus (either a castrate male or optimally hormone-primed ovariectomised female). Each column represents mean ± S.E.M. of 6–8 animals. The symbol represents a significant difference ($\#\#P<0.01$) between sexual and social stimulus times, and a significant difference ($^{**}P<0.01$, $^{***}P<0.001$) between vehicle (Veh) and maximal hormone prime (MHP) treated animals (Student's *t*-test). (B) The same data expressed as a partner preference score (sexual time/total time). The symbol represents a significant difference ($^{*}P<0.05$) between vehicle (Veh) and maximal hormone prime (MHP) treated animals (ANOVA followed by Tukey's post hoc test). Dotted line represents 'no preference,' values above represent a preference for the sexual stimulus, and values below the social stimulus. The symbol represents a significant difference ($\#P<0.05$, $\#\#P<0.01$) from 'no preference.'

In the castrate male social stimulus design, the vehicle-treated test females spent comparable times investigating each stimulus animal ($t(14)=0.60$). Hormone treatment inducing behavioural estrus evoked a significant increase over vehicle in time spent investigating the sexual stimulus ($t(14)=3.69$, $P=0.0024$), and also resulted in significantly ($t(14)=3.15$, $P=0.0071$) more time spent investigating the intact male over castrate male (Fig. 1A: Student's *t*-test).

The partner preference score is defined as the time spent actively investigating the sexual stimulus, expressed as a factor of total time spent actively investigating the sexual and social stimuli (sexual/(sexual + social) times).

Expressing these data as a partner preference score demonstrates a similar increase in sexual investigation evoked by hormone priming inducing behavioural estrus over vehicle in both the female ($P=0.011$) and castrate male ($P=0.014$) designs (Fig. 1B: ANOVA; $F(3,26)=8.837$, $P=0.0005$). However, the female social paradigm had a preference score of ~0.5 indicating no preference, whereas the male social paradigm had a preference score of >0.5 indicating a preference for the sexual stimulus.

A pilot study in which we investigated whether high frequency vocalisation played any role in this behaviour was also carried out in the model (data not shown). Using an ultrasound detector we were unable to detect any vocalisation during the ten minute run time, and observed that the intact males only vocalised on transfer from the satellite cage to the experiencing arena (see method 2.3.4). The castrate males did not vocalise. This avenue was not pursued further in this study.

3.2. Optimising hormone primes

Hormone primes of estrogen 5 μg plus progesterone 250 μg, estrogen 1 μg plus progesterone 100 μg, and vehicle (no prime) were identified that, based on cytological examination would place the OVX females in an oestrus state equivalent to oestrus, metestrus and diestrus in naturally cycling rats (Fig. 2).

When animals primed with these doses of hormones were examined in the castrate male social stimulus design, a significant increase over vehicle in partner preference score was observed for the higher, but not lower prime (Fig. 3A: ANOVA; $F(3,18)=6.265$, $P=0.011$). Total investigation times were not affected by either hormone prime (Fig. 3B: ANOVA; $F(3,18)=1.49$, $P=0.257$).

3.3. Effect of apomorphine on partner preference score in hormonally primed OVX rats

Apomorphine demonstrated a 'bell-shaped' dose–response curve in sub-optimally primed (estrogen 1 μg plus progesterone 100 μg) OVX rats. Doses of 10 and 30 μg/kg apomorphine dose-dependently increased time spent actively investigating the intact male, but only achieved significance at 30 μg/kg ($P<0.05$) (ANOVA; $F(7,48)=2.86$, $P=0.016$). A dose of 100 μg/kg evoked a score similar to vehicle (Fig. 4A). Total investigation time was reduced in a dose-related manner, but this did not achieve significance (Fig. 4B: ANOVA; $F(7,48)=0.469$, $P=0.851$). When examined in optimally primed rats (estrogen 5 μg + progesterone 250 μg), which demonstrated increased partner preference scores when compared to sub-optimally primed rats ($P<0.01$), apomorphine evoked reductions in preference scores, although this effect did not achieve significance (Fig. 4A). Total investigation time was not affected by apomorphine under this prime (Fig. 4B).

3.4. Comparison of the effect of apomorphine on 'active investigation' and 'passive interest'

In sub-optimally primed rats apomorphine 30 μg/kg demonstrated a significant effect over vehicle on partner preference score, so this dose was investigated further. Subsequent studies confirmed the positive effect of apomorphine 30 μg/kg ($P<0.05$) on active investigation of the sexual stimulus, but no effect on passive interest was observed (Fig. 5A: ANOVA; $F(3,32)=4.341$, $P=0.0124$). Time spent by a test animal in the 'passive interest zone' is greater than time spent in active investigation, however apomorphine did not affect total times spent on either parameter (Fig. 5B: Student's *t*-test; $t(14)=0.43$ and $t(14)=0.79$ respectively). Vehicle-treated animals demonstrated

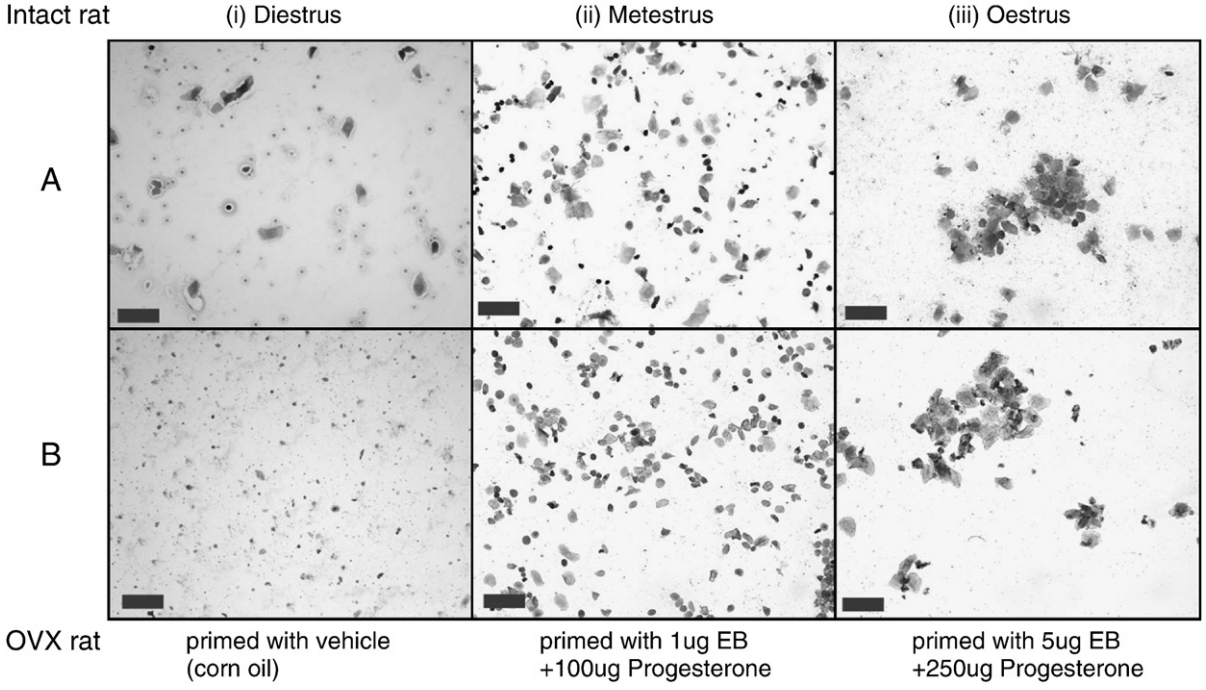

Fig. 2. Microscopic images of vaginal lavage smears from naturally cycling (A) and hormonally primed ovariectomised (B) Long Evans rats comparing stages of the rat natural estrus cycle with artificially induced stages (×10 magnification, scale bar 100 μm, methylene blue stain). (i) Natural diestrus and un-primed ovariectomised female. (ii) Natural metestrus and ovariectomised female primed with estrogen 1 plus progesterone 100 μg/rat. (iii) Natural estrus and ovariectomised female primed with estrogen 5 plus progesterone 250 μg/rat.

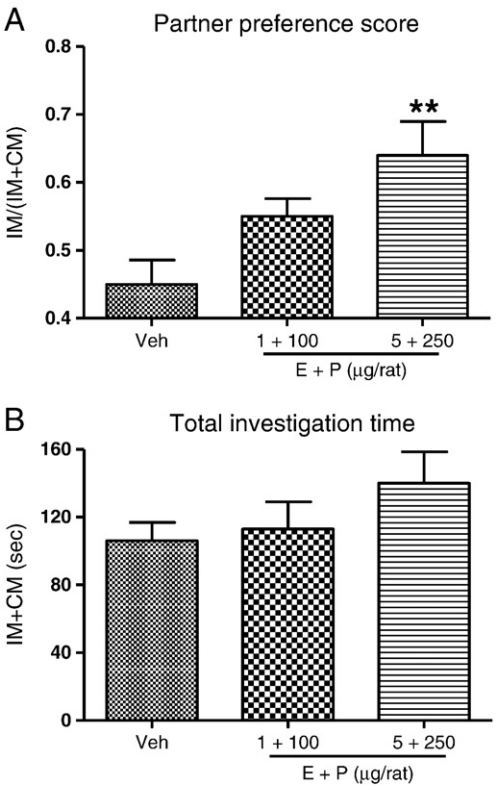

Fig. 3. (A) Time spent actively investigating a sexual stimulus (intact, vigorous male, IM) expressed as a factor of total investigation time (sexual stimulus plus social stimulus (castrate male, CM)). The symbol represents a significant difference (**$P<0.01$) between vehicle (Veh) and estrogen (E) plus progesterone (P) primed animals. (B) Total time spent investigating a sexual and social stimulus animal following vehicle (Veh) or estrogen (E) plus progesterone (P) prime. Each column represents mean ± S.E.M. of 5–8 animals.

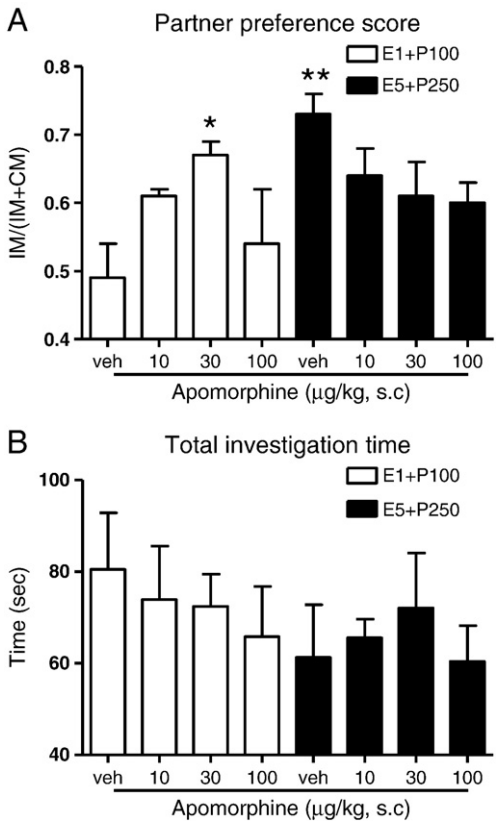

Fig. 4. (A) Time spent actively investigating a sexual stimulus (intact, vigorous male, IM) expressed as a factor of total investigation time (sexual stimulus plus social stimulus (castrate male, CM)) in ovariectomised female rats primed with either estrogen (E) plus progesterone (P) 1 plus 100 or 5 plus 250 µg/rat. The symbol represents a significant difference (*$P<0.05$, **$P<0.01$) between vehicle (Veh, E1 + P100 prime) and apomorphine, or vehicle with optimal hormone prime (Veh, E5 + P250) (ANOVA followed by Tukey's post hoc test). (B) Total time spent investigating a sexual stimulus and a social stimulus in animals primed with estrogen (E) plus progesterone (P) either 1 plus 100 or 5 plus 250 µg/rat, following treatment with vehicle (Veh) or apomorphine 10, 30 or 100 µg/kg. Each column represents mean ± S.E.M. of 6 animals.

a similar frequency of visits to each passive interest zone, as did apomorphine-treated animals (Fig. 5C: ANOVA; $F(3,32)=1.619$, $P=0.207$). Both vehicle- and apomorphine-treated animals spent slightly longer in the sexual rather than social passive interest zone, however this did not achieve significance (Fig. 5D: ANOVA; $F(3,32)=2.879$, $P=0.054$).

During the test period apomorphine (30 µg/kg SC) reduced both distance travelled (3415 ± 335 cm from 4482 ± 335 cm), and velocity (17.5 ± 0.5 from 20.4 ± 0.7 cm/s) achieving a significant effect on velocity ($t(14)=3.26$, $P=0.0057$), but just failed to reach statistical significance on distance ($t(14)=2.12$, $P=0.052$) when compared with vehicle (Students *t*-test).

4. Discussion

In the studies presented here we first of all investigated the effect played by the gender (male or female) of the stimulus animals and secondly the gonadal state of the male stimulus animals (castrate or intact). This social stimulus study involved two conditions. When the social stimulus was an ovariectomised female primed to induce behavioural oestrus, both stimulus animals were sexually vigorous, the difference being the gender of the stimulus. In the castrate male paradigm, both stimulus animals were male, the difference being that only one, the intact male, was sexually vigorous, so the difference between social and sexual stimulus was better defined, whilst in other respects they were as similar as possible.

From the social stimulus study, total active investigation times for the vehicle-primed animals were similar for each condition, but the bias was different. The same was true for hormone-primed animals. Consequently, following the social stimulus study, a castrate male social stimulus paradigm was chosen for subsequent studies. This was based on the following observations: Using the active investigation end point an un-primed ovariectomised test female demonstrated no preference between the sexual (vigorous, intact male) and social (castrate male) stimulus animals, hence in the un-primed control condition there was no preference, therefore the model was balanced. However if an ovariectomised female primed to induce behavioural oestrus was used as a social stimulus, the un-primed test female demonstrated a significant preference for the social stimulus compared with the sexual stimulus. In addition, when the test female was primed to induce behavioural oestrus a significant preference for the intact over castrate male was observed, but no such preference was observed over an ovariectomised female in behavioural oestrus (Fig. 1A). These observations from the castrate male paradigm are in broad agreement with data generated by Ellingsen and Ågmo (2004), who report that a test female primed to induce behavioural estrus spent more time in the proximity of an intact male over a castrate male. Our observations using the ovariectomised female conspecific are at variance with a report by Frye et al. (1998) who observed that a test female primed to induce behavioural estrus spent more time in the proximity of an intact male over an ovariectomised female. However differences in test conditions such as their use of a Y-maze with un-primed ovariectomised female conspecific could explain this. In both paradigms we investigated, priming the test female to induce behavioural oestrus resulted in a significant increase in the partner preference score for the sexual stimulus when compared with the un-primed control (Fig. 1B). This observation compares with similar

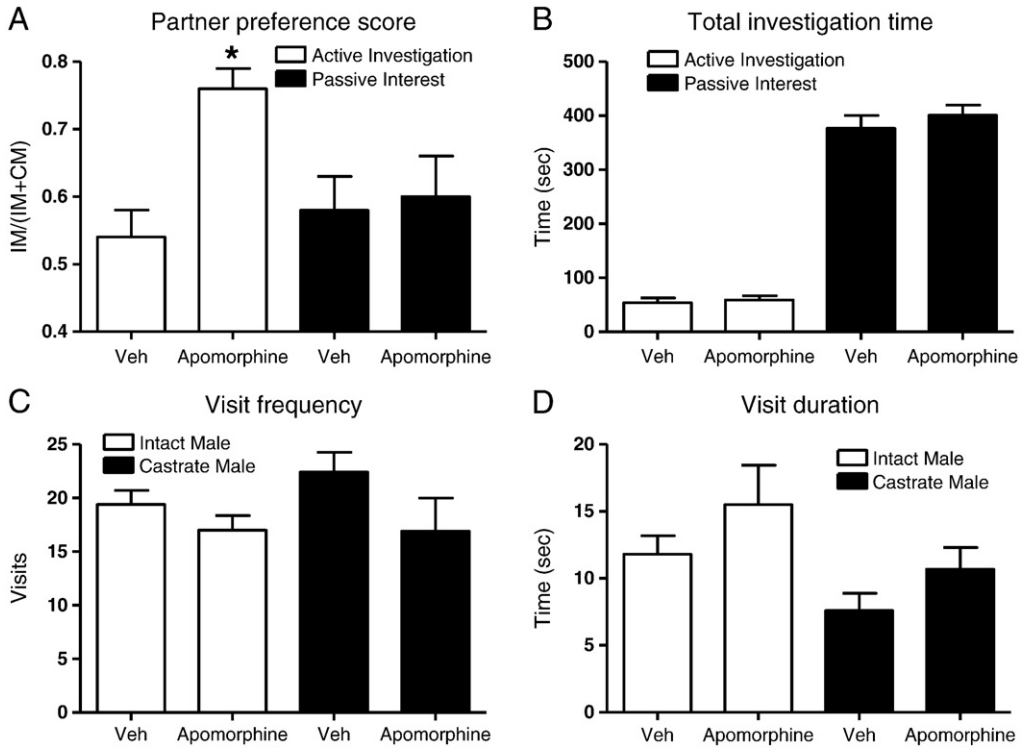

Fig. 5. (A) Time spent in active or passive investigation of a sexual stimulus (intact, vigorous male, IM) expressed as a factor of total investigation time (sexual stimulus plus social stimulus (castrate male, CM)) in ovariectomised female rats primed with estrogen 1 μg/kg plus progesterone 100 μg/kg, following treatment with vehicle (Veh) or apomorphine 30 μg/kg. (B) Total time spent in active or passive investigation of a sexual stimulus and a social stimulus in animals primed and treated as in A. (C) Number of visits made by ovariectomised female rats to either a sexual stimulus (intact, vigorous male) or social stimulus (castrate male), primed and treated as in A. (D) Mean visit duration made by ovariectomised female rats to either a sexual stimulus (intact, vigorous mae) or social stimulus (castrate male), primed and treated as in A. Each column represents mean ± S.E.M. of 8 animals. The symbol represents a significant difference ($^*P<0.05$) between vehicle (Veh) and apomorphine.

results for lordosis studies — only following appropriate hormone priming will ovariectomised female rats display lordosis (Gonzalez et al., 1976). A key requirement of a preference model is that there is no preference between the two stimuli in the control condition, such that the effect of hormones, drugs or other experimental manipulations can be effectively assessed in the model. Therefore the use of a female rat as a social stimulus was not appropriate in this model and the castrate male social stimulus paradigm was therefore used for all subsequent studies.

One disadvantage of a non-contact model of sexual motivation is the difficulty of knowing what exactly underlies the preference of a hormone-primed or drug-treated female rat to display a preference for a sexually vigorous male rat. The use of two males, one castrated one intact, helps to control for some of the potential confounding factors, but plenty of others may exist. One likely factor is auditory cues. In the presence of a devocalised male rat ovariectomised female rats brought into behavioural estrus will vocalise in response to recordings of male calls. In the absence of the male this response is diminished (White et al., 1993). Pilot experiments to determine if auditory cues were influencing investigative preference in our study were inconclusive. This suggests that auditory cues in themselves are insufficient to produce a preference. An alternative factor is olfactory cues. Proximity to the urine from intact but not castrate male mice has been demonstrated to shorten and synchronise the estrus cycle in female mice — the Whitten effect (Jemiolo et al., 1986). However the attraction by ovariectomised mice for male soiled bedding is independent of hormone prime, whereas proceptive behaviour in the presence of the male is hormone dependent (Moncho-Bogani et al., 2004). However a limitation of this method is only olfactants that were absorbed onto the bedding and were sufficiently stable could be investigated. On the other hand, female rats in estrus prefer the odor of sexually vigorous over castrate males, with time spent sniffing increased when physical contact is hampered (Sakuma, 2008). In addition olfactory impairment reduces mounting behaviour displayed by the sexually vigorous female rat (Afonso et al., 2006). These data coupled with the sniffing behaviour integral to active investigation suggest that olfactory cues play a significant role in investigative preference.

Increases in ear wiggling, hop and darting have been reported to reflect an increase in arousal in female rats, occurring in response to proximity to and stimuli provided by the male (see Erskine, 1989). These behaviours were manifest rarely in this model, so consequently were not used as outcome measures. The probable explanation for the complete absence of ear wiggles in this model is that this is a non-contact model. Alternatively it could be due to the strain of rat used. We have noted that the Long Evans strain of rat we use displays significantly less obvious and less frequent ear wiggling compared with literature reports (for example Frye et al., 1998) or Sprague–Dawley rats from the same supplier. This observation comes from lordosis studies as well as an escape chamber model both of which have physical contact between the male and female (personal communication).

It has been known for some time that in naturally cycling rats, a higher sexual preference score is observed in the proestrus/estrus condition (Eliasson and Meyerson, 1975). Allers et al. (in press) have demonstrated that in a rodent vaginal spectral analysis (VSA) model of vaginal blood flow, representative of arousal in the clinic, apomorphine increases the VSA signal during metestrus and decreases the signal during oestrus in naturally cycling rats. We were therefore interested to identify a hormonal prime that would mimic oestrus and metestrus in the ovariectomised rat confirmed by examining vaginal cytology and using these primes investigate the effects of apomorphine in the partner preference model. Distinctive changes in vaginal cytology across the oestrus cycle have been described (Hubscher et al., 2005; Montes and Luque, 1988) and we have observed similar changes over the natural cycle. Whilst ovariectomised rats primed to

be in behavioural estrus display readily identifiable behaviors, other stages of the cycle are less obvious. We are not aware of any reports confirming the experimental hormone prime has induced the desired stage of the cycle at the point of conducting a behavioural study. Our use of vaginal smears confirms we have achieved a state equivalent to the cycle stage we were interested to investigate. Having investigated a range of hormone primes we identified a hormone prime of estrogen 5 μg per rat followed 48 h later by progesterone 250 μg per rat that induced vaginal cytology equivalent to oestrus (Fig. 2iii). A prime of estrogen 1 μg per rat followed 48 h later by progesterone 100 μg per rat induced vaginal cytology equivalent to metestrus (Fig. 2ii). In the absence of a hormone prime, the vaginal cytology resembled diestrus in naturally cycling rats (Fig. 2i). When ovariectomised rats primed with these paradigms were examined in the partner preference model using the active investigation end point, we observed a significant increase in sexual partner preference score for the higher prime but not for the lower prime, relative to vehicle-treated animals (Fig. 3A). These data suggest that at the higher prime rats were in behavioural oestrus, and were therefore actively seeking a sexual partner. Total investigation times were similar for all treatment groups (Fig. 3B), suggesting that the increase in partner preference score was reflecting an increase in sexual motivation and not due to marked changes in locomotor or exploratory activity. Similar observations were made by Clark et al. (2004) who demonstrated an increase in sexual partner preference during proestrus over diestrus in naturally cycling rats and ovariectomised rats primed to induce behavioural oestrus over vehicle prime.

The dose-related increase in partner preference score induced by apomorphine in the sub-optimally primed (estrogen 1 μg plus progesterone 100 μg per rat) ovariectomised female rat is in line with the observations made by Allers et al. in the VSA model, and also the lordosis study by Foreman and Moss (1979) where apomorphine increased lordosis behaviour after a low but not high hormone prime. However, changes in partner preference score only achieved significance at 30 μg/kg, whereas at 100 μg/kg the score was similar to vehicle (Fig. 4A). The peak effect at 30 μg/kg is in agreement with the apomorphine dose-related increase in penile erection in the rat reported by Hsieh et al. (2004). The optimal prime itself induced a significant increase in partner preference score when compared with the sub-optimal prime. The same doses of apomorphine caused a downward trend in partner preference scores in optimally primed (estrogen 5 μg plus progesterone 250 μg per rat) ovariectomised rats (Fig. 4A). The lack of pro-sexual effect in partner preference score in the optimally primed ovariectomised rat is also in line with observations from rats in natural oestrus reported in the VSA model. Alternatively, in the presence of a high hormone prime, the test females will be displaying a high degree of sexual partner preference (see Fig. 1B), so it would perhaps be difficult to induce a further increase above an already elevated baseline. In this study, the partner preference index rarely achieved values greater than 0.75, reflecting similar data described by Ellingsen and Ågmo (2004) and Frye et al. (1998). This variation in response to apomorphine is possibly explained by the affinity state of dopamine receptors changing in response to fluctuating hormone levels across the oestrus cycle (Di Paolo et al., 1988) and hence possibly in response to the different hormone primes employed in the ovariectomised rats. In addition, Di Paolo et al. (1986) demonstrated increased striatal dopaminergic activity in ovariectomised rats following a 50 μg progesterone prime. Further evidence in a recent study in the cynomolgus monkey demonstrating changing dopamine D2 receptor availability across the oestrus cycle (Czoty et al., 2008) lends additional support to this concept.

Previously, Ellingsen and Ågmo (2004), using a similar behavioural model reported that apomorphine inhibits sexual incentive motivation. However there are several differences in methodology between the two studies. Firstly, Ellingsen and Ågmo used a greater hormonal prime than employed here, and secondly used doses of 125 and 500 μg/kg apomorphine, again greater than employed here. Data we have generated with our optimal prime, and highest dose of apomorphine are broadly in agreement with the earlier study, suggesting that with high hormonal priming high doses of apomorphine will attenuate sexual incentive behaviours. The earlier studies by Eliasson and Meyerson (1976) and Michanek and Meyerson (1981) also employed a high hormonal prime (10 μg estrogen plus 400 μg progesterone) with high dose of apomorphine (200 and 500 μg/kg), demonstrating an attenuation of lordosis. Additional support for this concept was generated by Allers et al. (in press) who demonstrated that apomorphine decreases arousal in oestrus animals.

In a further study comparing the effect of apomorphine 30 μg/kg on both active and passive interest in the sub-optimally primed ovariectomised rat, we confirmed initial observations of a significant increase in partner preference score in active investigation paradigm. Interestingly, there was no increase in partner preference score when using the passive interest paradigm (Fig. 5A). Whilst the time animals spent in the passive interest zones was greater than the time spent actively investigating stimulus animals, there was no difference in total times spent between vehicle- and apomorphine-treated groups in either investigation paradigm (Fig. 5B). The passive interest paradigm described here is in fact analogous to the 'incentive zone' used by Ellingsen and Ågmo (2004) originally described by Ågmo (2003). Passive interest data described here is in general agreement with observations made by Ellingsen and Ågmo, in that apomorphine does not increase the partner preference score (Fig. 5A). In addition, we have also demonstrated that apomorphine neither modulates visit frequency to either stimulus animal (Fig. 5C), nor visit duration (Fig. 5D). However, there was an upward trend in visit duration in the sexual passive zone compared with social passive zone following both vehicle and apomorphine treatments (Fig. 5D).

The apparent loss of effect at higher doses of apomorphine in the conscious female behavioural but not anaesthetised VSA model is possibly due to competing stereotypic behaviours interfering with the active investigation. Indeed, stereotyped behaviour has been reported at doses similar to the maximum used in this study (Beck et al., 1986; Cameron et al., 1988), and Davis et al. (1986) demonstrated that a dose of 120 μg/kg apomorphine increases climbing behaviour in female rats. Analysis of total investigation times revealed a modest, but non-significant decrease induced by 100 μg/kg apomorphine in the sub-optimally primed rat which could also be associated with emergence of competing stereotypic behaviours. Apomorphine 30 μg/kg attenuated locomotor activity, significantly reducing velocity. This suggests that the 'bell-shaped' dose–response to apomorphine is possibly a result of increasing locomotor effects with the higher dose. The Ellingsen and Ågmo study reported significant, apomorphine-induced attenuation of both distance moved and velocity. In addition, they report significant increases in sniffing behaviour at the doses of apomorphine used. Apomorphine-induced sniffing and stereotyped behaviour have been reported at doses equivalent to those used by Ellingsen and Ågmo (Cameron et al., 1988; Lee et al., 1995) further suggesting that the attenuation of sexual behaviour is a result of increasing stereotyped behaviours.

In conclusion, the results of the current study suggest that observing active investigation in sub-optimally primed ovariectomised rats could provide a sensitive measure of sexual incentive motivation, and is superior to previously described place preference methodology. Data generated with apomorphine reflect clinical data in man where pro-sexual effects are dose-limited by adverse events including nausea, visual and cardiovascular effects (Heaton et al., 1995). It remains to be seen whether this model is predictive of clinical efficacy in women.

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