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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