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Behavioural response of sexually naïve and experienced male rats to the smell of 6-methyl-5-hepten-2-one and female rat faeces

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HIGHLIGHTS

- Sexually naïve male rats can detect oestrus based on odour only.
- This ability is unconditioned and may be innate.
- Sexual training augments the significance of oestrous odours.
- Methylheptenone contributes to the olfactory determination of female receptiveness.

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ABSTRACT

Sexually experienced male rats display penile erections when exposed to faeces from mammalian females in oestrus (Rampin et al., Behav Brain Res, 172:169, 2006), suggesting that specific odours indicate female receptiveness across species. However, it is unknown to what extent the sexual response observed results from an odorous conditioning acquired during sexual experience. We tested the behavioural response of male Brown Norway rats both when sexually naïve and experienced to four odours, including oestrous rat faeces and 6-methyl-5-hepten-2-one (methylheptenone; a molecule found in higher concentrations during oestrus in female rats, foxes and horses). Odour had a significant effect on the sexual response of the naïve rats, with oestrus faeces provoking significantly more erections than herb odour, and with methylheptenone and di-oestrus faeces being intermediate. This indicates that sexually naïve male rats have an unconditioned ability to detect oestrous mediated via odour. After gaining sexual experience, the response to methylheptenone, di- and oestrus faeces was significantly higher than that observed with herb odour. These results strongly suggest that methylheptenone is part of the odorous bouquet of oestrus and contributes to the olfactory determination of female receptiveness.

1. Introduction

Sexually experienced male rats display spontaneous (i.e. with no apparent triggering stimulus) penile erections, and the frequency is enhanced when exposed to a non-accessible receptive female [1]. When exposed to faeces from mammalian females in oestrus, male rats have been found to display an increased number of penile erections compared to when exposed to water or to faeces from male or di-oestrous female rats, foxes and horses [2]. Using faeces samples from these three species, we have previously identified eight molecules, which are found in higher or lower concentrations during oestrus compared to males or di-oestrous females of the same species [3]. In the same study, we showed that a mixture of five of these molecules, all found in lower concentrations in oestrous females, elicits penile erections in sexually experienced male rats when presented within a limited range of concentrations (0.1 – 0.3 ppm). In the mixture, the proportions between the five molecules were identical to that found in samples of faeces from female rats in oestrus.

A number of questions, however, arise from our previous work. We used sexually experienced male rats, which mean they had been exposed to the (potentially complex) smells associated with oestrus whilst engaging in sexual behaviour. It is possible that during the subsequent odour tests, the rats recognised and responded to the smell of oestrus simply because they had become conditioned or sensitized to the odour during their previous sexual experience (see [4,5] for reviews on conditioning and sexual behaviour). In the studies published on the sexual response of male rats without copulatory experience, all of them have used the presence of female rats, either inaccessible or hidden from view, as the odour source [6–10]. There is thus a paucity of knowledge regarding the interactive effects of oestrous odours (presented without the presence of a female), sexual experience and conditioning on the occurrence of penile erections in rats.

Of the eight molecules identified as potential oestrus odours, only the five found in lower concentrations were tested in our previous

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work [3]. It is therefore possible that one or more of the three remaining compounds play a role in the elicitation of penile erections found by Rampin et al. [2]. One of these molecules is 6-methyl-5-hepten-2-one (methylheptenone, also known as sulcatone), which gave rise to similar gas chromatography mass spectrometry (GC-MS) profiles in the samples of oestrous faeces from female rats, foxes (vixens) and horses (mares). Methylheptenone is used as a food-flavouring and has a citrus-like smell. Based on the results of a pilot study we decided to investigate if this molecule is implicated in the behavioural response previously obtained with faeces from oestrus females of the three species [2].

The experiment presented here aimed to investigate i) if the odours emitted by oestrous rat faeces have pro-erectile effects in male rats with no previous sexual experience with a female (naïve male rats), and if so ii) whether a single molecule (methylheptenone) found in oestrous rat faeces would have a similar effect. This was compared to the behavioural response of the rats to the same odours after having acquired sexual experience.

2. Material and methods

2.1. Animals

Sixteen male Brown Norway rats (7 weeks of age) were obtained from a commercial breeder (Janvier, France), ensuring that they had had no contact with female rats since weaning. They were housed pairwise, with free access to commercial rat pellets (Diet M25, Special Diet Services, Witham, Essex, United Kingdom) and tap water. Prior to their arrival all female rats were moved to other accommodation in the same building. Care was taken to ensure that the naïve rats did not get exposed to the smell of female rats (e.g. males were always handled prior to the females, gloves were worn and discarded, and lab coats were changed). Over a period of 3 weeks the male rats were accustomed to an inverse light cycle (12 h dark per 24 h starting at 05.15 h). All handling and testing of the rats as well as sexual experience sessions were carried out in red lighting during the dark period in which the rats are naturally active.

2.2. Choice and preparation of odours

The oestrous faeces were collected from female Brown Norway rats (mean age 17 weeks) prior to the arrival of the males. The hormonal status of the females was determined from vaginal smears and upon determining the oestrous cycle phase for each female [11], faeces were collected from the cage floors within a few hours and immediately frozen. Only faeces from females where oestrus could be clearly established were used. For the purpose of the behavioural test, frozen faecal pellets from 4 to 5 females were transferred to a small container and allowed to thaw prior to each test.

In a pilot study with sexually experienced rats using the same behavioural test protocol as described below, we found that penile erections were evoked when a 165 ppm solution of methylheptenone (diluted in odourless 1,2-propanediol) was used. This was estimated to be the concentration found in oestrous rat faeces and this concentration was used in all subsequent tests.

Herb (1-hexanol) was chosen as the control odour under the assumption that this odour was unknown to the rats, and did not have nutritional, predatory or sexual connotations; i.e. without biological significance. In addition, the herb compound was found in varying concentrations in the faeces samples from all three species tested by Rampin et al. [2], and is thus a good choice of control odour for comparison with methylheptenone. We wanted the smell of this control odour to be of a similar strength to that of methylheptenone, and this was found to be the case (as experienced by humans) at a dilution of 5000 ppm in 1,2-propanediol. Apart from the oestrus faeces, all compounds used where purchased from Sigma/Aldrich.

2.3. Behavioural tests

The rats were habituated over several days to being moved into a standard test arena (L×W×H: 50×30×30 cm^3) located in an adjacent room. The test arena was made of glass with three opaque and one transparent wall. The test arena was covered with a transparent, perforated lid and the floor covered with clean saw dust. During habituation the test arena contained an empty odour container (see below). Testing began when the rats were sexually mature yet still relatively young at 11 weeks of age.

Prior to a test, a heavy (690 g, thus not movable by the rat) empty container (cylindrical, 8.5 cm high, diameter 6.0 cm, with a lead base) was placed at one end of the arena. The rats were left to acclimatize to the test surroundings for 5 min with the empty container in place. At the end of this period, an open glass vial (D×H: 15 mm×45 mm) containing 4 ml of the odorous solution to be tested was placed into the container and covered with a bolted-on perforated lid (35 holes each with 5 mm diameter). When faecal pellets were used, these were placed on aluminium foil in the bottom of the container. The odour exposure during testing lasted 30 min. During this period the behaviour of the rats was recorded on video, as well as being continuously observed by a trained observer, who registered and time-stamped each behavioural component displayed by the rat (Table 1) together with its position in the arena (in the half of the arena containing the sample or not). At the end of the test, the rat was returned to its home cage. The test arena was thoroughly cleaned and disinfected, and the room ventilated for 20 min prior to each test, including the first of the day.

All rats were exposed to all odours once when they were sexually naïve, and again following sexual experience. In both test series, methylheptenone and herb were tested first. This was done because methylheptenone is a constituent part of oestrus faeces, and we wanted to prevent any prior exposure to this odorant. The two faeces odours were tested last for all rats and both odour pairs were tested in a Latin square design. On average 5.6 rats were tested on a test day (SD = 1.3), and a minimum of 3 days and an average of 6.4 (SD = 3.2) days elapsed between tests of the same rat. A pilot study confirmed that any change in response to the odours upon second exposure was not

Table 1
Ethogram describing the mutually exclusive behavioural components registered during behavioural observations, and subsequently analysed for latency, frequency and duration. In addition the position of the rat in the test arena was noted.

Behavioural category	Description of behavioural components
Investigate odour container	Sniffing the perforated lid of the odour sample container; mounting the container; attempting to bite or gnaw the top of the container.
Penile erection	Registered when hip thrust, hip constriction or tip-toe posture occurred in association with genital grooming (from [9]).
Exploration	All exploration of surroundings not involving sample container, includes rooting and sniffing the litter and sniffing the air.
Freezing	Immobility, often sudden, with ears raised and eyes open
Grooming	Licking paws, washing face, ears, and flanks, not necessarily completing the sequence, as well as genital grooming in the absence of behaviours indicating penile erection (see above).
Resting	Sitting or lying down without discernible alertness or other activity
Position change	Turning around, changing positions between sitting and lying
Other	Any behaviour not included in the above (all infrequent or of short duration)