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# Conditioned mate-guarding behavior in the female rat

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## HIGHLIGHTS

- Female rats can display mate guarding behavior.
- Experience with sexual reward shifts sexual strategies in the female rat.
- Hypothalamic and limbic brain regions are differentially activated.

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## ABSTRACT

Female and male rats are often described as having a promiscuous mating strategy, yet simple Pavlovian conditioning paradigms, in which a neutral odor or strain-related cues are paired with preferred sexual reward states during an animal's first sexual experiences, shift this strategy toward copulatory and mate preferences for partners bearing the familiar odor or strain cue. We examined whether female rats given exclusive rewarding copulation with one particular male would display mate-guarding behavior, a strong index of monogamous mating. Ovariectomized, hormone-primed female Long–Evans rats were given their first 10 paced sexual experiences at 4-day intervals with a particular unscented male of the same strain. A final test was conducted in an open field 4-days later in which the primed, partnered female was given access to the male partner and a fully-primed competitor female. In this situation, the partnered females mounted the competitor female repeatedly if she came near the vicinity of the male. This behavior prevented the male from copulating with the competitor, and was not displayed if partnered females could not pace the rate of copulatory behavior efficiently during the training trials, nor was it displayed by the competitor females. Fos expression was examined in both the partnered and competitor females after the final open field test. Partnered females had significantly higher expression within the supraoptic nucleus and nucleus accumbens shell compared to partnered females that did not develop this behavior or competitor females. These data show that females engaged in paced copulation with the same male display mate-guarding when exposed to that male and a competitor female. Increased activation of the SON and NAc may underlie this behavior.



## 1. Introduction

Mating strategies are specific sets of interactions that determine with whom and how often an individual mates. Several mating strategies can be observed in mammals, and each is accompanied by its own set of defining behaviors. For example, in nature, the rat is described as having a promiscuous mating strategy that is characterized by cooperative and promiscuous group mating in both males (polygamy) and females (polygyny). On the opposite end of the spectrum are prairie voles, one of the few mammals that display social monogamy. Prairie voles are considered unique in that they form preferential associations with a specific sexually mature individual of the opposite sex, known as a pair bond. Pair bonds are characterized by selective contact and affiliation with the partner over a stranger vole, biparental care of the young, nest sharing, and mate-guarding behavior [1]. Thus, although voles and rats are closely related, they have adapted very different mating strategies due presumably to differences in environmental factors such as population density, sex ratio, and predator range, factors that are critical for the development of social monogamy in the prairie vole [2,3].

The flexibility of sexual and reproductive strategies in the rat has been revealed in laboratory studies that employ Pavlovian conditioning paradigms [4–7]. When neutral odors (e.g., wintergreen, lemon, almond) are paired with copulation to ejaculation, male rats form a preference to ejaculate with a familiar partner over an unfamiliar one, a phenomena referred to as a conditioned ejaculatory preference [6,7]. Female rats allowed to control or pace the initiation and rate of

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copulation form conditioned place preferences for distinctive environments paired with the postcopulatory reward state [5]. Paced copulation also supports the conditioning of sexual partner and mate preferences in female rats [4] as measured by selective solicitations and copulations with familiar males relative to novel males in an open field, and a selective preference to receive the familiar male's ejaculations. Thus, rats are capable of displaying rudiments of monogamous sexual partner and mate preference despite being an allegedly promiscuous species. Conversely, both male and female rats learn to avoid partners bearing an odor paired with sexual nonreward. In those studies, rats are paired with a sexually nonreceptive partner bearing an odor, or are administered the opioid receptor antagonist drug naloxone, prior to their first several sexual experiences. Rats are given alternating access to unscented sexually receptive partners (or administration of saline prior to unscented sexually receptive partners). On a final test, rats are given access to two receptive partners, one scented and the other unscented. In both cases, males and females copulate selectively with the unscented partner [8]. Thus, rats can learn to modify their sexual strategy depending on early sexual learning. However, how far the mating strategy of the rat can be shifted by early sexual experience remains elusive.

The present study investigated whether female rats that have exclusive access to a single male during their first paced sexual experiences might display mate-guarding behavior when paired with that male and a competitor female in an open field. We also examined whether the development of such behavior alters the pattern of expression of the immediate-early gene product Fos in the brain of those females following this interaction compared to the competitor females or partnered females that received nonpaced early sexual experience with a male. Comparative studies between monogamous and non-monogamous vole species have lead to the identification of brain regions that are involved in monogamous responding in voles, including the activation of oxytocin and vasopressin systems in the paraventricular and supraoptic hypothalamic nuclei, the mesolimbic dopamine system, and regions such as the ventral pallidum that integrate those systems into behavioral output [9–14]. The activation of these and other regions related to partner preference, sexual reward, and appetitive sexual responding were examined using Fos immunocytochemistry following the open-field test.

## 2. Materials and methods

### 2.1. Animals and surgery

Sexually naïve Long–Evans female rats (200–250 g) were obtained from Charles River Canada (St-Constant, QC, Canada). Animals were housed in shoebox cages in groups of two in a colony room on a reversed 12:12 h light/dark cycle at approximately 21 °C and given free access to food and water. Female rats were ovariectomized (OVX) bilaterally via lumbar incision. Prior to surgery, female rats were anesthetized using a 1 ml/kg intraperitoneal injection of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 ml/kg), mixed in a ratio of 4:3 respectively. Female rats were given 1 week to recover from the procedure prior to the conditioning trials. Throughout the duration of the experiment, female rats were maintained on hormone replacement by subcutaneous injections of estradiol benzoate (EB; 10 μg in 0.1 ml of sesame oil) 48 h prior to testing and progesterone (P; 500 μg in 0.1 ml of sesame oil) 4 h prior to testing.

Sexually naïve male rats (300–350 g) were also obtained from Charles River Canada (St-Constant, QC, Canada). They were housed in group cages (4 animals per cage) and housed under conditions identical to those of the female rats.

All animal procedures complied with the guidelines of the Canadian Council on Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

### 2.2. Conditioning apparatus

Conditioning occurred in Plexiglas unilevel pacing chambers (38 cm H × 60 cm W × 38 cm deep) with wire-mesh floors covering a layer of bedding [4]. Chambers were bisected by a Plexiglas divider with four holes cut into the bottom which were large enough for the female to crawl through but too small for the male to crawl through [4,15,16].

### 2.3. Conditioning procedure

Conditioning sessions occurred at 4-day intervals, 4 h after P injections, during the middle third of the rats' circadian cycle (lights off at 08:00). Females were assigned randomly to one of 2 cohorts. Cohort 1 consisted of self-paced paired and self-paced unpaired females. Cohort 2 contained non-self-paced paired and non-self-paced unpaired (N = 12/group) females. All paired females copulated with the same male across all trials, whereas unpaired females copulated with a variety of males across all trials. Males were placed onto one side of the conditioning chambers and allowed a 5-minute habituation period before each trial. Females were then placed into the opposite side of the conditioning chamber and allowed to have paced sex with the male for 20 min. Each group received 10 trials, which were all recorded on video and scored for mate-guarding behavior.

A third cohort of animals was run following the same protocol, however, self-paced (N = 15 paired + 15 unpaired) and non-self-paced (N = 16 paired + 16 unpaired) groups were run within the same test session so that a direct comparison could be made between the two conditions.

### 2.4. Mate-guarding test

Four days after the final conditioning trial each mate-guarding was assessed using an open-field (123 cm × 123 cm × 46 cm) with a thin layer of bedding [4]. Each open-field contained a paired female with her corresponding male and an unpaired competitor female. Before the test, males were placed into the open field for a 5-minute habituation period, after which both the paired and unpaired females were placed into the open-field at 2 diagonal corners. Rats were allowed to copulate freely for a 1-h period. After the open-field test half of female rats (N = 6/group) were perfused and their brains were collected to examine Fos induction as a function of the behavior both of these groups displayed. The remaining animals underwent 2 reconditioning trials, which followed the same protocol as the conditioning trials. After the second reconditioning, the female rats were exposed to the pacing chamber alone for 1 h and then perfused so their brains could be examined for Fos expression in response to the contextual cues alone.

All open-field tests were recorded on video and scored afterward using a computerized event recorder customized for rat sexual behavior in an open-field [17]. The frequency of solicitations, hops and darts, and defensive responses were recorded, as were the incidents and reflex magnitudes of lordosis when males mounted. Mate-guarding behavior appeared as female–female mounting, typically initiated by the paired female toward the unpaired female competitor. Interceptions and time spent near the male were also recorded for both females.

### 2.5. Perfusions

Animals were euthanized with an overdose of sodium pentobarbital (120 mg/kg) administered via intraperitoneal injection. They were then perfused intracardially with 250 ml of phosphate-buffered saline (PBS) followed by 250 ml of 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 4 h. After which brains were placed into a 30% sucrose solution for 48 h then flash frozen and stored at −80 °C until slicing.