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The role of oxytocin and vasopressin in 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.
- Female rats that display mate guarding show enhanced activation of OT and AVP neurons.
- Injection of OT or AVP facilitates different aspects of mate guarding behavior.

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ABSTRACT

We have shown previously that female rats given their first copulatory experiences with the same male rat display mate guarding behavior in the presence of that male provided a female competitor is also present. Females given access to the familiar male show more Fos induction within regions of the brain that contain oxytocin (OT) and vasopressin (AVP) cell bodies, notably the supraoptic (SON) and paraventricular nuclei (PVN) relative to females given sexual experience with different males. The present experiments examined whether the Fos induction we previously observed within the SON and PVN occurred within OT and/or AVP neurons, and whether exogenous administration of OT or AVP prior to female rats first sexual experience could potentiate the acquisition of mate guarding behavior. Female rats that display conditioned mate guarding had significantly more double-labeled Fos/OT neurons in both SON and PVN, and significantly more Fos/AVP neurons in the PVN. Peripheral administration of OT or AVP prior to their first sexual experience with the familiar male facilitated different aspects of mate guarding: OT augmented affiliative behaviors and presenting responses whereas AVP augmented interference behavior. These results indicate that female rats' first experiences with sexual reward when paired with the same male induce changes to bonding networks in the brain. Moreover peripheral administration of OT or AVP during their first sexual experience can augment different aspects of mate guarding behavior.

1. Introduction

Socially monogamous animals form enduring associations with one another that are characterized by selective affiliation, contact, and preferential copulation with their partners relative to strangers. These partnerships are called pair bonds [1]. In rodents, the hallmark example of social monogamy is displayed by the prairie vole (*Microtus ochrogaster*), and this rich literature has provided the scientific community with ground breaking and in depth analyses that have laid the groundwork for other studies looking into monogamy, bonding, and mate guarding. Prairie voles inhabit a very harsh environment, with limited food, water, and mate access. It is thought that they adapted a socially monogamous mating strategy in order to increase the likelihood of their survival and reproductive success [2,3]. For example, if a prairie vole happens to come across a potential mate, it would make sense to bond with this mate, since there is not a large likelihood of encountering another one. Bonding would allow for access to a mate, reproduction, would allow for biparental care of the young, and ultimately enable the couple to defend territory and gather resources more efficiently. However, the influence of the environment on bonding behavior in prairie voles can is most clearly observed during the winter months, when voles cluster together to form communal groups [4]. When living in communal groups, more extra pair copulations occur among pair bonded voles than in spring and summer months when the vole population is more widely dispersed, demonstrating that population density and mate access can create shifts toward promiscuity within the mating strategy of the prairie vole [4]. In the laboratory, pair bonding is determined when male prairie voles display a preference to spend more time with the first female they copulated with relative to a novel female [5], or

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when male/female prairie voles mate guard the first mate they copulated with [5].

The neuroanatomical and neurochemical bases of pair bonding have been elaborately examined in monogamous vs. promiscuous voles. Oxytocin (OT) and vasopressin (AVP) were early targets of this research, as these neuropeptides were known to modulate species-specific social behaviors such as sexual behavior, aggression, maternal care, and olfaction related to conspecific identification [6–12]. Neuroanatomical studies have revealed that, although there are minor species differences in cell bodies and fibers, there are dramatic differences in regional receptor distribution between monogamous and promiscuous vole species [13–15]. Specifically, monogamous prairie voles have higher densities of the vasopressin A1 receptor (V1aR), in the BNST, ventral pallidum (VP), central amygdala (CeA), basolateral amygdala (BLA), and accessory olfactory bulbs (AOB) than promiscuous montane voles [16–18]. Monogamous voles also display higher densities of oxytocin receptor (OTR) in the BNST, medial prefrontal cortex (mPFC), and nucleus accumbens (NaC) than promiscuous vole species [18–20]. These regional differences in V1a and OTR expression have been shown to be stable across lifespan and do not exist in other systems such as the opiate receptor system [20].

Pharmacological studies provided direct evidence for the importance of OT and AVP in the formation and maintenance of pair bonding in monogamous voles [16,21]. By administering a vasopression 1a receptor (V1aR) antagonist intracerebroventricularly (icv), researchers were able to block the onset of pair bonding in male prairie voles. Similarly, direct icv administration of AVP facilitated pair bonding in males even in the absence of mating, and in females after 1 h cohabitation [21, 22]. Administration of OTR antagonist, similarly, blocked pair bonding in both males and females, and concurrent administration of OT was able to rescue pair bond formation [22]. These results clearly demonstrate that both OT and AVP are essential for pair bonding in both sexes of prairie vole.

Rats (*Rattus norvegicus*), on the other hand, are considered promiscuous. They tend to live in large underground burrow systems and mate freely with many different partners throughout several copulatory series [40]. Despite this, a growing body of evidence shows that both male and female rats have the ability to display rudiments of monogamy such as sexual partner preferences. For example, through Pavlovian conditioning paradigms, where neutral odors are paired with sexual reward states, female rats have been shown to display preferences for scented partners over unscented ones, and vise versa if rewarding sex is paired with unscented partners [25]. We have also reported that changing the conditions under which the promiscuous female rat has her first experiences of sexual reward, notably by shifting mate access, can alter her mating strategies, much like the population density and partner access creates shifts in the prairie vole mating strategy [4,23]. Female rats given their first 10 paced copulatory experiences with the same male displayed mate guarding behavior in the presence of their paired male and a competitor female. In these studies, mate guarding was defined as any behavior displayed by the female that prevented the male from attending to or mating with the competitor female [23]. Under this definition several sexual (solicitations, presenting), aggressive (biting, tackling accompanied by vocalizations from the victim), dominant (female–female mounting), social (time spent in proximity, body positioning), and competitive (interceptions) behaviors were included to describe mate guarding [23]. When female rats mate guarded, they displayed all of these behaviors to varying degrees, however, in our previous study, over time, paired females displayed significantly more female–female mounting relative to unpaired female rats. Paired female rats that were placed into an open field with their paired male and a female conspecific, would mostly remain in the vicinity of the male. However, if the competitor female approached the male, the paired female would run over to her, and mount her in succession until she left the vicinity of the male. We also demonstrated that mate guarding was dependent on paced mating, because females not allowed to pace the rate of copulations during their first sexual experiences, which decreases the reward value of copulation [24], did not display mate-guarding behavior. Likewise, females that received paced copulation with different males each time did not show mate guarding behavior.

This shift in mating strategy was also accompanied by different patterns of neuronal activation within regions of the brain known to play a role in bonding behavior [23]. For example, female rats conditioned to show mate guarding had significantly more Fos-positive neurons within the PVN and SON (along with other brain regions such as the nucleus accumbens and hippocampus) following exposure to their familiar male relative to females conditioned to show no partner preference. These data suggest that a similar set of neuroanatomic regions are activated by partner cues in monogamous female prairie voles and promiscuous female rats that have been conditioned to show mate guarding behavior. In turn, this opens up the possibility that OT and AVP may play a role in conditioned monogamous behavior.

This purpose of this study was to examine the potential role of OT and AVP in conditioned mate guarding behavior. First we examined whether the Fos induction in the PVN and SON observed in females conditioned to show mate guarding was within OT and/or AVP neurons. We hypothesized that females that had all of their sexual experience paired with the same male would have more Fos induction within oxytocin and vasopressin neurons than females who had received their sexual experience with a variety of males. Second, we tested whether OT or AVP, injected peripherally prior to female rats first rewarding sexual experience, would facilitate the display of mate guarding behavior on their second sexual experience with the same male, relative to females injected with saline in order to elucidate potential roles that OT and AVP might be playing in the early onset of conditioned mate guarding. We hypothesized that peripheral administration of OT or AVP, but not saline, would facilitate different aspects of mate guarding behavior.

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 pairs in shoebox cages in a colony room on a reversed 12:12 h light/dark cycle at approximately 21 °C. Animals had free access to food and water. Females 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, prior to bilateral ovariectomy (OVX) using lumbar incisions. Females had 1 week to recover before behavioral tests began. 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). Males were housed in group cages (4 animals per cage) 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

All conditioned took place in Plexiglas unilevel pacing chambers (38 cm × 60 cm × 38 cm) with wire-mesh floors covering a layer of bedding [25,26]. Chambers were bisected by a Plexiglas divider with four holes cut into the bottom. Holes were large enough for the female to crawl through but too small for the male to crawl through [25,26].