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Short communication

# Centrally-administered oxytocin promotes preference for familiar objects at a short delay in ovariectomized female rats

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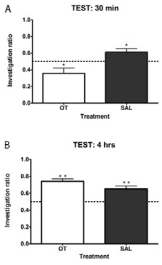
## HIGHLIGHTS

- We tested object preference in female rats in response to oxytocin and saline.
- Oxytocin treatment reversed preference from novelty to familiarity.
- Oxytocin might elicit its effects by increasing the saliency of a familiar stimulus.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Oxytocin has been previously associated with social attachment behaviors in various species, however, most studies focused on partner preference in the socially-monogamous prairie vole. In these, oxytocin treatment was shown to promote partner preference, such that females receiving either central or pulsatile peripheral administration would spend more time with a familiar male. This behavioral outcome was blocked by oxytocin receptor antagonist treatment. The aim of the current study was to further explore the preference-inducing properties of oxytocin by examining its effects on object preference on ovariectomized female rats. In other words, we assessed whether these effects would apply to objects and if they would be persistent across species. Eight rats were infused with oxytocin into the left ventricle and object preference was assessed at two delays: 30 min and 4 h. At the 30 min delay, oxytocin-treated animals showed preference for the familiar object, whereas saline-treated controls exhibited preference for the novel object. At the 4 h delay, both groups showed novel-object preference. Our findings show that oxytocin modulates object preference in the female rat at a shorter delay, similar to the findings from partner-preference studies in the prairie vole, suggesting that the mechanisms driving object preference might be in part similar to those responsible for partner preference.



Oxytocin (OT) is a neurohypophyseal peptide hormone that has been associated with social attachment behaviors in many species, including rodents, sheep, and humans [1–7]. In addition, OT and vasopressin have been shown to mediate social memory in mice [8,9]. The behavioral implications of this neuropeptide have been extensively studied in the socially monogamous prairie vole (*Microtus ochrogaster*), in which OT has been linked to the formation of selective social attachments or partner preferences [10].

Partner preference is quantified by measuring the relative time spent in side-by-side contact (or huddling) between a focal animal and either a familiar or novel conspecific [10–14]. The task starts with a cohabitation phase, during which an experimental vole is

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familiarized to a stimulus vole (generally 6–24 h), and is followed by a test phase, during which the experimental vole is exposed to the familiarized partner and a novel conspecific. An individual is said to display a partner preference if time spent with the familiar conspecific significantly outweighs time spent with the novel conspecific. Partner preference has been shown to be independent of the mode of administration, such that females receiving OT centrally [15,16] or peripherally in a pulsatile manner developed partner preference [17], although the effects of OT on partner preference are not sex-specific [16]. The effect was blocked by co-infusion of an OT receptor antagonist, suggesting that OT is necessary for facilitation of partner preference in the female prairie vole [15,16]. Finally, Witt et al. [7] demonstrated that these effects are not vole-specific, nor are they sex-specific by showing that centrally-infused OT male rats spent more time in close contact with a paired female compared to saline controls.

Although it is clear that OT is a key component in partner preference in the female prairie vole, as well as the rat, the mechanisms by which its effects occur are unclear. One possibility is that the presence of OT increases the reinforcing properties of social interaction. However, Williams and colleagues [14] have shown that female prairie voles prefer anesthetized familiar partners over unfamiliar conspecifics, suggesting that OT is capable of reinforcing preference for familiar social stimuli in the absence of dynamic social interaction. To address this possibility, we assessed object preference in response to OT treatment in adult ovariectomized female rats, hypothesizing that centrally-administered OT would result in preference for familiar objects, whereas saline-infused controls would show preference for novel objects.

Eight Long-Evans rats (Charles River Laboratories, Montreal, QC, Canada) weighing 220–250 g were pair-housed in cages located in a 21 °C room with a 12 h reverse light/dark cycle (lights off at 9 a.m.). All rats had ad libitum access to food and water. Bedding consisted of a 50/50 mixture of corncob and beta-chip. All testing and surgical procedures were performed during the dark phase of the diurnal cycle. All animal protocols were previously approved by Concordia University's animal research ethics committee and were in accordance with the guidelines put forth by the Canadian Council on Animal Care.

Oxytocin (OT: 0.5 μg/μl; Sigma–Aldrich, UK) was dissolved in saline and infused at a rate of 0.5 μl/min for 2 min. Behavioral testing commenced 15 min after each infusion, and consisted of a familiarization session and a testing session, separated by delays.

Rats were anesthetized using Isoflurane (Inhalation Anaesthetic, Richmond Hill, ON, Canada), and were ovariectomized via bilateral lumbar incisions (1 cm). One 4 mm stainless steel cannula (21 Ga, Plastics-One, Ronaoke, VA, USA) was stereotaxically implanted, unilaterally, toward the left lateral ventricle at the following coordinates from bregma: antero-posterior (AP) = +0.8 mm, lateral-medial (LM) = +1.4 mm and dorso-ventral (DV) = −4.0 mm. The cannula was anchored into place with skull-screws using dental cement. An obturator (26 Ga, Plastics-One, Roanoke, VA, USA) was inserted into the cannula. Following surgery, animals were single-housed for the remainder of the experiment and were handled every day for approximately 5 min/day.

Object preference was assessed in an arena constructed of gray PVC, measuring 60 cm × 70 cm × 70 cm [18,19]. The bottom of the arena consisted of a removable stainless-steel tray, covered with wood shavings, while a video camera was placed over the arena to record familiarization and test phases for subsequent analysis. The test stimuli were objects made of glass or porcelain, and varied in height and width between 6 and 10 cm. The objects were glued to the bottoms of small jars (6 cm high), which were screwed into jar lids, attached onto the steel flooring at 27 cm from opposing corners. There were three identical copies of each object, which were used interchangeably, and washed after each use with water.

Investigation ratios (IR = Time spent investigating the novel object/Time spent investigating both objects) during the first minute of the 5-min TEST session were assessed using single-sample, one-tailed *t*-tests, and performance was compared to chance (i.e., IR = 0.5). Total time spent with both objects during the 5-min FAM sessions was also recorded and analyzed using dependent samples *t*-tests. An investigation ratio significantly greater than 0.5 indicates rats spending more time with the novel object. Conversely, a ratio significantly smaller than chance indicates a preference for the familiar object.

NOP testing comprised three phases: habituation (HAB), familiarization (FAM) and testing (TEST). Each rat was given the opportunity to habituate to testing conditions during a 15-min HAB session, one day preceding the FAM session, in which rats were allowed to investigate two identical objects in the same arena where subsequent testing occurred; the two objects encountered during the habituation session were not used for NOP testing. The FAM and TEST sessions lasted 5 min each. Novel object preference was assessed after two different delays (interval between FAM and TEST), 30 min and 4 h. All rats received both treatments (i.e. OT or SAL), but on different testing days. As such, each rat was subjected to a total of four testing sessions: 30 min/OT, 30 min/SAL, 4 h/OT and 4 h/SAL. Each session was video-recorded and object preference was quantified as time investigating the novel object divided by total time investigating both objects for the first minute of the TEST session. A rat was considered to be investigating an object when its head was oriented within 45° and 1 cm from the object. Rearing with the head oriented upward was also considered, if at least one paw was placed on the object, however climbing on the object or sitting on it was not included. Rats that did not investigate an object for at least one second during the first minute of the test, or did not investigate at all during the FAM session were excluded from the analysis. Furthermore, rats with blocked cannula or faulty injectors (i.e. no change in drug/vehicle level during infusion) were also excluded from analysis.

All rats were assigned to both conditions (OT or SAL), counterbalancing for treatment order such that on day 1, four rats received OT infusions 15 min prior to NOP testing at the 30-min delay, while the other four received SAL. Infusions were delivered using 9 mm injectors (26 Ga, Plastics-One, Roanoke, VA, USA), manufactured so that once inserted into the guide cannula, would extend 1 mm from the tip of the cannula. On day 2, rats that received OT treatment on day 1 were assigned to the SAL group and vice-versa. The procedure was identical on days 9 and 10 (NOP at a 4-h delay), except that rats that received OT infusions on day 1 were assigned to the SAL group on day 9. Similarly, these rats received OT infusions on day 10. One rat was excluded from the 30-min NOP analysis due to a possible obstruction in the injector or cannula, and another rat was excluded from the 4-h NOP analysis due to lack of investigation during the FAM session.

There were no significant differences in investigation time between treatments during the FAM sessions at both delays (Fig. 1A and B). When assessed at a 30-min delay, OT-treated rats showed preference for the familiar object (Fig. 2A), indicated by an investigation ratio significantly smaller than chance (IR: 0.35; $t_6 = 2.26$, $P < 0.05$). In contrast, SAL rats tested at a 30-min delay investigated the novel object significantly more than chance (IR: 0.61; $t_7 = 2.64$, $P < 0.05$). At a 4-h delay however (Fig. 2B), both groups yielded investigation ratios significantly greater than chance (OT: $t_6 = 7.94$, $P < 0.01$; SAL: $t_7 = 4.39$, $P < 0.01$).

These data show that centrally-administered OT modulates object preference in the ovariectomized, adult female rat at short delays (i.e. 30 min). Intra-ventricular infusions of OT resulted in preference for familiar objects, whereas SAL treatment yielded preference for novel objects at a shorter delay. Although centrally-administered OT has been previously shown to enhance social