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Research report

Oxytocin differentially affects sucrose taking and seeking in male and female rats

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HIGHLIGHTS

- Oxytocin impacts conditioned and unconditioned behaviors in male and female rats.
- Oxytocin suppressed locomotor activity in females to a greater extent than males.
- Oxytocin decreased sucrose intake and seeking greater in females relative to males.
- Overall, females were more sensitive to oxytocin's behavioral effects than males.

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ABSTRACT

Oxytocin has a modulatory role in natural and drug reward processes. While the role of oxytocin in pair bonding and reproduction has been extensively studied, sex differences in conditioned and unconditioned behavioral responses to oxytocin treatment have not been fully characterized. Here, we determined whether male and female rats would show similar dose response curves in response to acute oxytocin on measures of locomotor activity, sucrose seeking, and sucrose intake. Male and freely cycling female rats received vehicle or oxytocin (0.1, 0.3, 1, 3 mg/kg, IP) injections before behavioral tests designed to assess general motor activity, as well as sucrose self-administration and seeking. Lower doses of oxytocin decreased motor activity in a novel environment in females relative to males. Likewise, lower doses of oxytocin in females decreased responding for sucrose during maintenance of sucrose self-administration and reinstatement to sucrose-conditioned cues. However, sucrose seeking in response to a sucrose prime was only decreased by the highest oxytocin dose in both sexes. In general, oxytocin had similar effects in both sexes. However, females were more sensitive to lower doses of oxytocin than males. These findings are consistent with the notion that oxytocin regulates many of the same behaviors in males and females, but that the effects are typically more profound in females. Therapeutic use of oxytocin should include sex as a factor in determining dose regimens.

1. Introduction

Oxytocin is a classical and well-characterized neuroendocrine hormone that is a potent modulator of a variety of brain functions, including emotions, social interactions, and sexual behavior [1]. In females, oxytocin has a critical role in reproduction as it induces uterine contractions during childbirth and facilitates milk ejection during lactation [2]. Oxytocin is primarily synthesized in magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus and is secreted by axon terminals in the posterior pituitary into systemic blood circulation [3]. In addition, oxytocin is produced in parvocellular and magnocellular neurons in the paraventricular nucleus that project to various brain regions and release oxytocin [4]. Centrally acting oxytocin has a number of behavioral and physiological effects, including suppression of food intake [5]. Specifically, peripheral injection of oxytocin in rats inhibits sucrose intake, whereas oxytocin receptor antagonists increase consumption [6,7].

Although oxytocin's mediating role in food consumption has been previously studied, an area that has received little attention is in the regulation of appetitive-related behaviors. It is not surprising that oxytocin should have some impact on natural rewards like sucrose consumption, given its innervation of central appetitive pathways and modulation of drug reward [8,9]. Indeed,

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previous findings have shown that oxytocin knock-out mice displayed enhanced intake of sucrose solution [10] and oxytocin infusion into the ventral tegmental area suppressed sucrose intake [11]. In an earlier study from our laboratory, systemic oxytocin (1 mg/kg) pretreatment attenuated sucrose seeking in a sucrose prime reinstatement test in both male and female rats; however, it did not impact motivation to lever press for a sucrose pellet at this dose [12].

In our previous study, males and females both exhibited oxytocin induced reductions in sucrose seeking. However, females show higher intake/preference of highly concentrated sweetened solutions than males [13]. Additionally, when sweetened concentrations are reduced, ovariectomized females exhibited lower intake than males [14]. Importantly, sex differences have been reported in the oxytocin system. For example, regardless of estrous cycle, females have significantly lower oxytocin receptor binding densities than males in the majority of forebrain regions involved in reward process [15].

Oxytocin also has anxiolytic effects in rats. In an open field test, a relatively low dose of oxytocin increased motor activity, whereas higher doses decreased activity in male rats [16]. To this end, we first evaluated the dose response effects of oxytocin on novelty induced locomotor activity in male and female rats. Second, we studied the impact of oxytocin on operant sucrose self-administration in both sexes. Finally, we determined whether oxytocin would decrease reinstatement of sucrose seeking induced by either sucrose prime or conditioned cues.

2. Methods and procedures

2.1. Subjects

A total of 91 male and female Sprague-Dawley rats (Charles River) were housed on a reversed 12:12 light–dark cycle in a temperature- and humidity-controlled vivarium (lights off at 06:00). Adult male rats weighed 275–300 g and adult females were 205–225 g at the time of delivery. Rats were individually housed and received ad libitum water and standard rat chow (Harlan, Indianapolis, IN, USA) until the locomotor test. After this test, subjects were food-restricted (≈20 g/day) to maintain 85% of ad libitum rats' body weight throughout the study. Procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Rats" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 2011) and approved by the IACUC of the Medical University of South Carolina.

2.2. Locomotor activity

To explore the effect of oxytocin on unconditioned locomotor activity in both males and females, rats underwent a single locomotor test. Locomotor activity was assessed in clear acrylic chambers (approximately 40 cm × 40 cm × 30 cm) equipped with Digiscan monitors (AccuScan Instruments Inc., Columbus, OH, USA). Each chamber contained a 16 × 16 photobeam array for the *x* and *y* axes and 16 photobeams for the *z* axis. Photobeam breaks were detected by a Digiscan analyser and recorded by DigiPro software (Version 1.4).

2.3. Sucrose taking, extinction, and reinstatement

Responding for sucrose was conducted in standard operant chambers (30 cm × 20 cm × 20 cm, Med Associates) housed inside sound-attenuating cubicles fitted with a fan for airflow and masking noise. Each chamber also contained two retractable levers, two stimulus lights, a speaker, and a house light. Rats were given daily 2 h sessions to lever press for sucrose on a fixed ratio (FR) 1 schedule of reinforcement. During the sessions, a response on the active lever resulted in delivery of a sucrose pellet (45 mg, BioServe) combined with a 5 s presentation of a light + tone stimulus complex, followed by an un-signaled 15 s time out. Responses occurring during the time out and on the inactive lever were recorded without scheduled consequences.

Following sucrose self-administration, lever responding was extinguished in daily sessions, whereby operant responding no longer resulted in delivery of the sucrose reinforcement or cues. Extinction consisted of daily 2-h sessions for at least 7 days and responding on either lever had no scheduled consequences. Extinction criterion was ≤20 presses for two consecutive days. When extinction criterion was met, behavior was reinstated by presentation of conditioned reinforcers (cued reinstatement) or by non-contingent sucrose delivery (primed reinstatement). During the cued reinstatement tests, active lever presses resulted in presentation of the light + tone stimulus in the same manner as during sucrose taking. During the sucrose prime tests, rats received one non-contingent pellet every 2 min for the first 10 min of the session and one pellet every 30 min thereafter, but responding on either lever had no scheduled consequences [12]. Daily extinction sessions occurred for at least 2 days between reinstatement tests.

2.4. Estrous cycle monitoring

Females underwent daily post-session vaginal cytology procedures starting at least 2 days before the first test until the end of experiment for habituation purpose. Samples were collected with a sterile saline-dipped pipette tip and smeared onto glass slides, stained with Quik-Dip Hematology Stain (Mercedes Medical, FL), examined using a light microscope set at 10× magnification, and classified according to previously published criteria [17].

2.4.1. The effect of oxytocin on locomotor activity

Males and females (n = 8–11 per group) were injected IP with vehicle or one dose of oxytocin (0.1, 0.3, 1, or 3 mg/kg) at the volume of 1 ml/kg. Oxytocin was purchased from Cell Sciences (Canton, MA) and dissolved in ddH_2O. Following injection, rats were placed into their home cage for 30 min, and then placed in the locomotor chamber for an additional 90 min.

2.4.2. The effect of oxytocin on sucrose intake

In this experiment, we tested the effects of oxytocin administration on established sucrose maintained responding using an FR1 schedule of reinforcement. Males and females rats (n = 9–10) first learned to lever press for sucrose for at least 7 days (with ≥10 pellets/session). Once sucrose intake stabilized (within 20% difference in pellets received between the last 2 days), each rat was tested with a unique order of vehicle, 0.1, 0.3, 1 and 3 mg/kg oxytocin (IP). Each solution was administered 30 min before daily sucrose sessions. To reach criteria between tests, rats were required to have two consecutive days in which the numbers of pellets earned were within 20% of each other.

2.4.3. The effect of oxytocin on sucrose conditioned cue and primed reinstatement

Males (n = 21) and females (n = 18) were first trained to lever press for sucrose on an FR1 schedule of reinforcement for 10 days, followed by at least seven sessions of extinction. When extinction criterion was met, all rats were divided into two groups and underwent either cue-induced or sucrose-primed reinstatement. Before each discrete reinstatement trial, with a minimum of two extinction sessions between reinstatement tests, rats received an injection of oxytocin (0.1, 0.3, or 1 mg/kg) or vehicle in a counterbalanced order for a total of four reinstatement tests. Our laboratory