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Administration of an oxytocin receptor antagonist attenuates sexual motivation in male rats

D.S. Blitzer [a], T.E. Wells [a], W.R. Hawley [a,b,*]

[a] Franklin and Marshall College, Department of Psychology, United States
[b] Edinboro University of Pennsylvania, Department of Psychology, United States

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ABSTRACT

In male rats, oxytocin impacts both sexual arousal and certain types of consummatory sexual behaviors. However, the role of oxytocin in the motivational aspects of sexual behavior has received limited attention. Given the role that oxytocin signaling plays in consummatory sexual behaviors, it was hypothesized that pharmacological attenuation of oxytocin signaling would reduce sexual motivation in male rats. Sexually experienced Long-Evans male rats were administered either an oxytocin receptor antagonist (L368,899 hydrochloride; 1 mg/kg) or vehicle control into the intraperitoneal cavity 40 min prior to placement into the center chamber of a three-chambered arena designed to assess sexual motivation. During the 20-minute test, a sexually experienced stimulus male rat and a sexually receptive stimulus female rat were separately confined to smaller chambers that were attached to the larger end chambers of the arena. However, physical contact between test and stimulus rats was prevented by perforated dividers. Immediately following the sexual motivation test, test male rats were placed with a sexually receptive female to examine consummatory sexual behaviors. Although both drug and vehicle treated rats exhibited a preference for the female, treatment with an oxytocin receptor antagonist decreased the amount of time spent with the female. There were no differences between drug and vehicle treated rats in either general activity, exploratory behaviors, the amount of time spent near the stimulus male rat, or consummatory sexual behaviors. Extending previous findings, these results indicate that oxytocin receptors are involved in sexual motivation in male rats.

1. Introduction

Oxytocin (OT) is well known for regulating a variety of social behaviors. For example, in male rats, oxytonergic signaling has been shown to impact affiliative behaviors and aggression (Calcagnoli et al., 2015a, 2015b), social memory (Lukas et al., 2013), the rewarding aspects of social interactions (Ramos et al., 2015), and social defeat-induced avoidance of a conspecific (Lukas et al., 2011). Interestingly, OT also influences different types of sexual behavior in male rats (Argiolas, 1999). Male rats exhibited higher levels of sexual arousal, as indicated by the occurrence of penile erections, following direct administration of OT into either the ventral tegmental area (Succu et al., 2008), the paraventricular nucleus (PVN) of the hypothalamus (Melis et al., 1986), the posteromedial cortical nucleus of the amygdala (Melis et al., 2009), area CA1 of the hippocampus (Melis et al., 1986), or the subiculum (Succu et al., 2011). In accordance with these findings, penile erections were reduced following either intraventricular administration of an OT receptor antagonist (Argiolas et al., 1987; Melis et al., 1999, 1992) or lesions of the PVN (Liu et al., 1997), which is partially comprised of oxytonergic neurons (George et al., 1976).

In addition to its role in sexual arousal, OT is involved in the consummatory aspects of male rat sexual behavior. Levels of OT in blood plasma increased following sexual experience in both sexually naive male rats and male rats with previous sexual experience (Hillegaart et al., 1998). Furthermore, levels of OT in the cerebrospinal fluid have been shown to increase two to three-fold after ejaculation (Hughes et al., 1987; Waldherr and Neumann, 2007), an effect abrogated by lesions of the PVN (Hughes et al., 1987). Sexual experience increased OT receptor mRNA and protein expression in the medial preoptic area (MPOA), the area of the brain that is critical for the execution of consummatory sexual behaviors (Gil et al., 2013). As might be expected, OT administered either centrally or peripherally decreased the number of mounts (Gil et al., 2011) and intromissions (Stoneham et al., 1985) prior to ejaculation, reduced the latency to first mount and first intromission (Arletti et al., 1990), as well as the ejaculation latency and the post ejaculatory interval (Arletti et al., 1992, 1990, 1985; Gil et al., 2013, 2011). Conversely, central administration of an OT receptor antagonist, either into the medial preoptic area or ventricles, resulted in a longer latency to first intromission (Gil et al., 2011), a reduction in both mounts and

* Corresponding author at: Edinboro University of Pennsylvania, Department of Psychology, Compton Hall, Room 106, 210 East Normal St., Edinboro, PA 16444, United States.
E-mail address: whawley@tulane.edu (W.R. Hawley).

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intromissions, as well as a reduction in the likelihood of ejaculation (Argiolas et al., 1988). Collectively, these results indicate that OT influences the consummatory aspects of male rat sexual behavior, and that alterations in oxytonergic signaling are not merely a byproduct of ejaculation (Murphy et al., 1987).

Although considerable attention has been given to the role of OT in sexual arousal and consummatory sexual behaviors in males, the role of OT in sexual motivation has received limited attention (Arletti et al., 1992, 1990, 1985; Gil et al., 2013). The notion that OT influences sexual motivation is supported by evidence which indicates that oxytonergic signaling is modulated by testosterone and estrogen, hormones that play a significant role in male rat sexual motivation (Attila et al., 2010). Gonadectomized male rats exhibited reduced OT receptor binding and reduced expression of OT receptor mRNA in the ventromedial hypothalamus (VMH) (Bale and Dorsa, 1995; Johnson et al., 1991, 1989; Tribollet et al., 1990). Administration of testosterone (Bale and Dorsa, 1995; Johnson et al., 1991, 1989; Tribollet et al., 1990), or a combination of estradiol and 5-α dihydrotestosterone (DHT) (Johnson et al., 1991), reversed the effects of gonadectomy on OT receptor binding and levels of OT receptor mRNA. Notably, in vitro, estradiol has been shown to modulate the release of OT from hypothalamic neurons (Wang et al., 1995). Additionally, the aromatase enzyme (El-Emam Dief et al., 2013), G-protein coupled receptor 30 (GPR30) (Hazell et al., 2009; Sakamoto et al., 2007) and estrogen receptor β mRNA (Hrabovszky et al., 1998; Laflamme et al., 1998; Simonian and Herbison, 1997), colocalize with OT, which may explain why levels of OT receptor mRNA and OT receptor binding in male rats are influenced by alterations in estrogen signaling alone (Bale and Dorsa, 1995; Johnson et al., 1991; Tribollet et al., 1990).

Given the relationship between OT and gonadal hormones, as well as the role that OT plays in consummatory sexual behaviors in male rats, it is reasonable to hypothesize that administration of an OT receptor antagonist would reduce sexual motivation, as well as consummatory sexual behaviors. However, characterizing the role of oxytocin in sexual motivation as either the latencies to achieve first mount or intromission, or the post-ejaculatory interval, does not fully capture the motivational aspects of sexual behavior because these outcomes do not occur independent of changes in consummatory behaviors (Everitt, 1990). With this in mind, it is advantageous to examine the influence of OT on the motivational aspects of male rat sexual behavior independent of consummatory behaviors. Therefore, it was predicted that administration of an oxytocin receptor antagonist would reduce the amount of time male rats would spend adjacent to a sexually receptive female rat that could not be physically contacted.

2. Methods

2.1. Animals

All procedures were approved by the Franklin and Marshall College Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals (2011). Thirty behaviorally naive test ($N = 25$) and stimulus ($N = 5$) male Long-Evans rats were obtained from a breeding colony in the animal care facility at Franklin and Marshall College. No more than three test male rats were used from each litter. Each litter was comprised of both drug and vehicle treated rats. Male rats were between 121 and 126 days of age on the day of sexual motivation testing. Ovariectomized stimulus female Long-Evans rats ($N = 24$), which were between 118 and 120 days of age on the day of sexual motivation testing, were purchased from Harlan Laboratories (Indianapolis, IN).

All of the test and stimulus rats were group housed by sex and provided unrestricted access to food and water in polycarbonate cages that were lined with wood chip bedding. Rats were maintained in a temperature-controlled environment on a reverse 12:12 light-dark schedule (lights on at 10:00 pm). All behavioral testing was conducted at least 2 h after the beginning of the dark cycle.

2.2. Drug and hormone treatments

Sexual receptivity in stimulus female rats was induced by intramuscular injections of estrodial benzoate (10 μg; Custom Prescriptions of Lancaster, Lancaster, PA) and progesterone (500 μg; Custom Prescriptions of Lancaster) that were delivered 48 h and 4–6 h, respectively, prior to either sexual experience or the sexual motivation test. Both hormones were suspended in a 0.1 mL vehicle solution that consisted of sesame oil and benzyl alcohol (3%; Custom Prescriptions of Lancaster).

A timeline depicting the behavioral and pharmacological procedures conducted with test male rats is shown in Fig. 1. Test male rats received a single injection into the intraperitoneal cavity of either an OT receptor antagonist, L368.899 hydrochloride (1 mg/kg; Santa Cruz Biotechnology, Dallas, TX), or vehicle (sterile water; 1 mL/kg) 40 min prior to the beginning of sexual motivation testing (Boccia et al., 2007). The non-peptide OT receptor antagonist L368,899 was found in both the cerebrospinal fluid and hypothalamus of female rhesus monkeys at this dosage and time point following peripheral administration, an effect that corresponded with a reduction in sexual behavior (Boccia et al., 2007). In addition, administration of L368,899 directly into the amygdala has been shown to counteract the anti-aggressive effects of OT in male rats (Calcagnoli et al., 2015b) and intraperitoneal administration of L368.899 attenuates social reward in wild-type male mice (Wei et al., 2015).

2.3. Sexual behavior prescreen

Test male rats were individually acclimated to temporary home cages on three separate 10-min sessions in a behavioral testing room that was illuminated by red light with a standard house fan on in a corner of the room to minimize background noise (Hawley et al., 2011). Prior to the beginning of sexual motivation testing, all test male rats were given three separate 45-minute sexual experiences with a novel sexually receptive stimulus female rat in the temporary home cage. Each experience was separated by one week. The frequencies of mounts, intromissions, and ejaculations were scored by a trained observer and recorded by a video camera. Test male rats that did not exhibit at least one mount on any of the three prescreen tests were removed from the study ($N = 1$). In addition to confirming sexual activity in test male rats, stimulus male rats were given two sexual experiences in temporary home cages prior to the beginning of sexual motivation testing to confirm sexual activity. All five stimulus male rats exhibited at least one ejaculation on the first experience (40 min) and at least two ejaculations on the final experience (1 h).

2.4. Sexual motivation and consummatory sexual behavior testing

As indicated in Fig. 2, the sexual incentive motivation arena consisted of a primary arena (100 cm × 50 cm × 40 cm) with outer walls that were constructed of clear Plexiglas. Two internal walls of black Plexiglas created three equal sized chambers. Each of the internal walls had an opening in one of the lower corners (10 cm × 10 cm) that was placed opposite of the opening on the other internal wall and opposite to the smaller stimulus rat chamber to which it provided access. Stimulus rat chambers (25 cm × 15 cm × 40 cm) were comprised of three sides constructed of clear Plexiglas and a fourth side constructed of perforated metal (0.1 cm wide). The side of the stimulus rat chambers

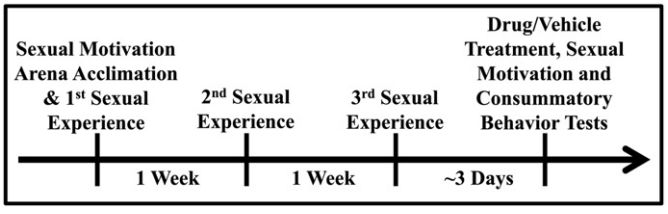

Fig. 1. Experimental timeline of all behavioral and pharmacological treatments involving test male rats.