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Central oxytocin receptors mediate mating-induced partner preferences and enhance correlated activation across forebrain nuclei in male prairie voles

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ABSTRACT

Oxytocin (OT) is a deeply conserved nonapeptide that acts both peripherally and centrally to modulate reproductive physiology and sociosexual behavior across divergent taxa, including humans. In vertebrates, the distribution of the oxytocin receptor (OTR) in the brain is variable within and across species, and OTR signaling is critical for a variety of species-typical social and reproductive behaviors, including affiliative and pair bonding behaviors in multiple socially monogamous lineages of fishes, birds, and mammals. Early work in prairie voles suggested that the endogenous OT system modulates mating-induced partner preference formation in females but not males; however, there is significant evidence that central OTRs may modulate pair bonding behavior in both sexes. In addition, it remains unclear how transient windows of central OTR signaling during sociosexual interaction modulate neural activity to produce enduring shifts in sociobehavioral phenotypes, including the formation of selective social bonds. Here we re-examine the role of the central OT system in partner preference formation in male prairie voles using a selective OTR antagonist delivered intracranially. We then use the same antagonist to examine how central OTRs modulate behavior and immediate early gene (Fos) expression, a metric of neuronal activation, in males during brief sociosexual interaction with a female. Our results suggest that, as in females, OTR signaling is critical for partner preference formation in males and enhances correlated activation across sensory and reward processing brain areas during sociosexual interaction. These results are consistent with the hypothesis that central OTR signaling facilitates social bond formation by coordinating activity across a pair bonding neural network.

Introduction

The oxytocin (OT) system is an evolutionarily conserved neuroendocrine mechanism that regulates reproductive and social behaviors across divergent taxa, spanning nematodes and humans (Donaldson and Young, 2008; Garrison et al., 2012; Grinevich et al., 2015). In mammals, OT modulates social recognition (Ferguson et al., 2001; Skuse et al., 2014), maternal responsiveness and mother-infant bonding (Numan and Young, 2015; Rilling and Young, 2014), pair bonding behaviors in monogamous species (Hurlemann and Scheele, 2015; Johnson and Young, 2015; Ross and Young, 2009), and even human-dog bonding (Nagasawa et al., 2015; Romero et al., 2014). It has been hypothesized that OT facilitates social bonding behaviors by modulating neural transmission and encoding of social information across sensory and reward processing brain areas (Johnson and Young, 2015; Numan and Young, 2015).

In mammals, OT is predominantly synthesized in the paraventricular, accessory, and supraoptic nuclei of the hypothalamus (Knobloch and Grinevich, 2014). OT is released within the brain in response to stimuli associated with parturition and nursing in females (Lee et al., 2009) and mating in both sexes (Ross et al., 2009a; Waldherr and Neumann, 2007), and OT neurons are activated following tactile stimulation in rats and voles (Barrett et al., 2015; Okabe et al., 2015). In humans, peripheral OT is increased in response to social vocalizations (Seltzer et al., 2010) and eye contact (Nagasawa et al., 2009; Nagasawa et al., 2015), although the relationship between peripheral and central OT remains unclear (Leng and Ludwig, 2015).

OT mediates its central effects by binding with high affinity to OT receptors (OTRs) and/or with lower affinity to vasopressin 1a (V1aR)

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receptors (Schorscher-Petcu et al., 2010; Song et al., 2014). The expression patterns of these receptors throughout the forebrain are extraordinarily diverse both within and across species, and are thought to have contributed to the evolution of diverse patterns of neural and sociobehavioral plasticity during species-typical social contexts (Goodson, 2008).

Microtine rodents, or voles, exhibit rich variation in social behaviors, and the socially monogamous prairie vole, *Microtus ochrogaster*, has been the subject of intense investigation of the neural mechanisms underlying diversity in social behaviors and mating strategies (McGraw and Young, 2010). Voles exhibit both intra- and interspecific variation in forebrain OTR and V1aR distribution (Phelps and Young, 2003; Young, 1999), and these patterns have been associated with variation in social investigation (Ophir et al., 2009), mating strategies and reproductive fitness within mating strategies (Ophir et al., 2012), alloparental care (Olazabal and Young, 2006a,b), sexual fidelity and space use (Ophir et al., 2008), and pair bonding (Insel and Shapiro, 1992; Insel et al., 1994; Lim et al., 2004a; Ross et al., 2009b). Recent investigations have implicated these systems in social affiliation and bonding in additional vertebrate lineages spanning fishes (Oldfield and Hofmann, 2011), birds (Klatt and Goodson, 2013), and mammals (Cavanaugh et al., 2014; Romero et al., 2014; Smith et al., 2010), including humans (Hurlemann and Scheele, 2015; Walum et al., 2012; Walum et al., 2009).

In male and female prairie voles, mating facilitates pair bonding. In the laboratory, pair bond formation is reflected through formation of a robust and enduring preference for the mating partner relative to a novel opposite sex individual, referred to as a "partner preference" (Williams et al., 1992a). Early pharmacological studies revealed that OTR signaling in the brain is critical for partner preference formation in female prairie voles (Insel and Hulihan, 1995; Williams et al., 1994). More specifically, blocking OTR in the prefrontal cortex (PFC) or nucleus accumbens (NA) prevents mating-induced partner preferences in females (Young et al., 2001). Although exogenous central OT infusions facilitate partner preference formation in both sexes (Cho et al., 1999), a single seminal paper failed to show that OTR blockade inhibits mating induced partner preferences in males (Winslow et al., 1993). It appeared that endogenous vasopressin, and not OT, regulated pair bonding in male prairie voles by acting at V1aR receptors in the ventral pallidum and lateral septum (Lim et al., 2004b; Lim and Young, 2004; Liu and Wang, 2003). These sexually dimorphic roles for OT and AVP have pervaded the literature on pair bonding for the past 20 years.

In these experiments, we revisit the role of OTR in partner preference formation in male prairie voles, and demonstrate conclusively using a highly selective OTR antagonist (OTA) that, as in females, central OTR signaling is critical for pair bonding in males. We then introduce an abbreviated cohabitation paradigm to investigate the role of central OTR signaling in modulating sociosexual behavior and neural activity across a hypothesized pair bonding network (PBN), with the aim of identifying potential neural mechanisms by which central OTR signaling may modulate pair bond formation. To achieve this goal, we infuse OTA or aCSF intracerebroventricularly (ICV) into male prairie voles and measure sociosexual behaviors as well as induction of the immediate-early gene transcription factor, Fos, following cohabitation with a sexually receptive female. We first demonstrate that this paradigm is a useful tool for investigating sexual behavior and neural activity in male prairie voles by replicating findings from investigations in other rodents. Next, we restrict our analysis to nuclei within the PBN and find no evidence that central OTR signaling during sociosexual interaction modulates Fos expression within any of the analyzed brain nuclei. However, our data suggest that central OTR signaling during sociosexual interaction plays a critical role in modulating a robust pattern of correlated Fos expression *across* PBN nuclei. These results provide novel insights into how OTR modulates neural activity across the PBN, and are consistent with previously outlined hypotheses that OTR modulates functional connectivity (Goodson and Kabelik, 2009) and transmission of social information across conserved brain networks (Johnson and Young, 2015; Numan and Young, 2015) during species-typical social contexts.

Materials and methods

Subjects

Male prairie voles were housed in groups of two or three until stereotaxic surgery during adulthood (60–200 days), after which they remained singly housed until behavioral testing. Housing consisted of a ventilated 26x18x19 cm Plexiglas cage filled with Bed-o-cobbs Laboratory Animal Bedding under a 14:10 h light/dark cycle at 22 °C with ad libitum access to food (rabbit LabDiet) and water. Subjects were drawn from our laboratory breeding colony originally derived from field captured voles in Illinois. Stimulus animals were sexually experienced, ovariectomized, estrogen-primed (see below), adult female prairie voles. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Emory University Institutional Animal Care and Use Committee.

Experiment 1: partner preference

Intracranial cannulation. Adult male prairie voles were anesthetized with 2.5% isofluorane inhalation and stereotaxically implanted unilaterally into the left hemisphere with a 22 gauge guide cannula (catalog no. C313GS-5/SPC; Plastics One; Roanoke, VA) aimed ICV using stereotaxic coordinates (A/P + 0.6 mm; M/L ± 1.0 mm; D/V − 3.0 mm). The guide cannula was fixed to the skull with a combination of Jet Acrylic Liquid and Jet Denture Repair Powder (Lang Dental Manufacturing Co., Inc.; Wheeling, IL). All subjects recovered for 4 days following surgery.

OTA administration. 4 days following surgery, subjects received microinjections of either 2 μL of control artificial cerebral spinal fluid (aCSF, n = 18) or 2.5 ng/μL of a selective oxytocin receptor antagonist (OTA), des Gly-NH_2d$(CH_2)_5$-[D-Tyr^2,Thr^4]OVT (Manning, Miteva et al., 1995) dissolved in 2 μL of aCSF (n = 21) using a 28 gauge internal cannula (Plastics One) extending 0.5 mm beyond the end of the guide cannula into the lateral cerebral ventricle. Injections were performed with a 10 μL Hamilton syringe (Hamilton; Reno, Nevada) connected to polyethylene-20 tubing (Plastics One), which was in turn secured to the internal cannula. Infusions were administered over the course of 60 s, and the internal cannula was left in place for 3 min following infusion to prevent backflow. Two infusions were administered to each subject; one immediately prior to the 48-h cohabitation, and the second 24 h into the cohabitation.

Cohabitation and behavioral analysis. Immediately following OTA infusion, subjects were placed in a clean cage (26 × 18 × 19 cm) with a sexually experienced, ovariectomized, estrogen-primed, adult stimulus female for 48 h. In the three days preceding cohabitation, stimulus females were brought into estrus with daily injections of 4.0 μg estradiol benzoate dissolved in sesame oil (Sigma; St. Louis, MO; S3547) injected subcutaneously. The first 3 h of cohabitation were video recorded and analyzed for mating; only one pair did not mate during this period and was excluded from final analysis. After 24 h of cohabitation, males were briefly removed from the home cage, anesthetized, infused with a second dose of OTA as described above, and immediately returned to cohabitation. Following 48 h total of cohabitation male subjects and stimulus females were separated overnight. The following day, all subjects underwent a 3-h partner preference test. In this paradigm, the male subject is placed in a central "neutral" zone of a 3-chambered apparatus in which the familiar female "partner" is tethered in one far chamber and the novel female "stranger" is tethered in the opposite chamber (Williams et al., 1992b). The experimental animal is free to