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ROLE OF THE OXYTOCIN SYSTEM IN AMYGDALA SUBREGIONS IN THE REGULATION OF SOCIAL INTEREST IN MALE AND FEMALE RATS

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Abstract—We previously found that oxytocin (OT) receptor (OTR) binding density in the medial amygdala (MeA) correlated positively with social interest (i.e., the motivation to investigate a conspecific) in male rats, while OTR binding density in the central amygdala (CeA) correlated negatively with social interest in female rats. Here, we determined the causal involvement of OTR in the MeA and CeA in the sex-specific regulation of social interest in adult rats by injecting an OTR antagonist (5 ng/0.5 μl/side) or OT (100 pg/0.5 μl/side) before the social interest test (4-min same-sex juvenile exposure). OTR blockade in the CeA decreased social interest in males but not females, while all other treatments had no behavioral effect. To further explore the sex-specific involvement of the OT system in the CeA in social interest, we used *in vivo* microdialysis to determine possible sex differences in endogenous OT release in the CeA during social interest. Interestingly, males and females showed similar levels of extracellular OT release at baseline and during social interest, suggesting that factors other than local OT release mediate the sex-specific role of CeA-OTR in social interest. Moreover, we found a positive correlation between CeA-OT release and social investigation time in females. This was further reflected by reduced CeA-OT release during social interest in females that expressed low compared to high social interest. We discuss the possibility that this reduction in OT release may be a consequence, rather than a cause, of exposure to a social stimulus. Overall, our findings show for the first time that extracellular OT release in the CeA is similar between males and females and that OTR in the CeA plays a causal role in the regulation of social interest toward juvenile conspecifics in males.

Key words: oxytocin, oxytocin receptor, central amygdala, medial amygdala, social interest, sex differences.

INTRODUCTION

Social interest reflects the motivation to investigate a conspecific for the perception and identification of social cues which will, in turn, facilitate context-appropriate social behavior responses. Social interest can therefore be seen as an initial step in mediating the subsequent expression of a wide range of social behaviors, such as aggression, mating, and parental care. Interestingly, there are sex differences in the expression of social interest in both rats and mice. In both species, adult males, compared to adult females, show higher levels of social investigation toward juvenile conspecifics (Thor, 1980; Bluthe and Dantzer, 1990; Johnston and File, 1991; Dumais et al., 2013, 2016). However, the neural mechanisms underlying this sex difference in social interest have not been assessed.

A key candidate for the sex-specific regulation of social interest is the oxytocin (OT) system. OT is primarily synthesized in the paraventricular nucleus and supraoptic nucleus of the hypothalamus. Upon central release, OT modulates the activation of many brain regions via binding to the widely distributed OT receptor (OTR; Gimpl and Fahrenholz, 2001). Importantly, the OT system regulates various social behaviors in humans and rodents (Veenema and Neumann, 2008; Ross and Young, 2009; Guastella and MacLeod, 2012), often in sex-specific ways (for review see Dumais and Veenema, 2015, 2016). The amygdala is of particular interest because it has been shown to be a core region of sex-specific activation by OT. For example, human fMRI studies have shown that exogenous OT modulates amygdala activation in response to social stimuli differently in men compared to women (Domes et al., 2007; Domes et al., 2010; Rilling et al., 2012; Rilling et al., 2014). Furthermore, correlational studies in rodents suggest that the OT system in subregions of the amygdala, namely the medial amygdala (MeA) and central amygdala (CeA), plays a differential role in mediating male versus female social interest. In detail, male mice showing high levels of social investigation have higher OTR mRNA expression in the MeA compared to males showing low levels of social investigation (Murakami et al., 2011). In rats, males have higher OTR binding density in the MeA compared to females, and OTR binding density in the MeA correlates positively with social investigation time in males, but not females (Dumais et al., 2013). In contrast, OTR binding density in the CeA does not show a sex difference, but correlates negatively with social investigation time in females, but not in males (Dumais et al.,

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Abbreviations: CeA, central amygdala; MeA, medial amygdala; OT, oxytocin; OTR, oxytocin receptor.

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2013). Together, this suggests that OTR activation in the MeA facilitates social investigation in males, while OTR activation in the CeA decreases social investigation in females.

The MeA is part of a network that processes olfactory social cues in rodents (Halpern and Martinez-Marcos, 2003; Guthman and Vera, 2016) and is well-known to regulate various social behaviors (Bergan et al., 2014; Noack et al., 2015). While the CeA is more commonly known for its role in fear and anxiety (Bale et al., 2001; Viviani et al., 2011; Knobloch et al., 2012), it has also been implicated as a region involved in the modulation of social behavior, such that c-Fos expression (an indirect marker for neural activity) in the CeA was higher in adolescent male rats exposed to a social context compared to adolescent rats exposed to a non-social context (Varlinskaya et al., 2013). Importantly, previous studies have implicated the OT system in both the MeA and CeA in mediating social behaviors, further supporting our hypothesis that these amygdala subregions may be involved in modulating social interest. For example, OT in the MeA is known to facilitate social approach and social recognition (Arakawa et al., 2010; Lukas et al., 2013), and OT in the CeA has been implicated in maternal and intermale aggression (Lubin et al., 2003; Bosch et al., 2005; Consiglio et al., 2005; Calcagnoli et al., 2015). However, comparisons between males and females regarding the role of OT in these amygdala subregions in the regulation of social behavior is largely lacking.

In the current study, we aimed to determine the causal role of the OT system in the MeA and CeA in the regulation of social interest in male and female rats. Based on the previously observed correlations of OTR binding and social interest (Dumais et al., 2013), we tested the hypotheses that OT acting on OTR in the MeA facilitates social investigation in males, while OT acting on OTR in the CeA decreases social investigation in females. To this end, we determined the effects of acute pharmacological OTR blockade and OT administration in the MeA and CeA on social investigation time in adult male and female rats. Because these pharmacological manipulations showed a sex-specific effect of OTR blockade in the CeA on social investigation time, we also investigated possible underlying mechanisms for this sex-specific effect by determining potential sex differences in extracellular OT release in the CeA at baseline and during exposure to the social interest test using *in vivo* microdialysis.

EXPERIMENTAL PROCEDURES

Animals

Male and female Wistar rats were obtained from Charles River Laboratories (Raleigh, NC, USA) at 8–9 weeks of age for experimental rats and at 22 days of age for stimulus rats. Rats were maintained on a 12-h light/dark cycle, lights on at 0700 h, and food and water were available *ad libitum*. Experimental rats were housed in same-sex pairs in standard rat cages (26.7 × 48.3 × 20.3 cm) unless otherwise mentioned, and were given at least one week to acclimate to our facilities. Stimulus rats were housed in same-sex groups of four per cage, and were used at 25–30 days of age. All experiments were conducted in accordance with the guidelines of the NIH and approved by the Boston College Institutional Animal Care and Use Committee (IACUC).

Stereotaxic surgery

Cannulation. After daily handling for one week to familiarize them with the injection procedure, experimental rats were anesthetized using isoflurane and mounted on a stereotaxic frame. A heating pad was used to regulate body temperature of rats while anesthetized. Guide cannulae (22 gauge; Plastics One, Roanoke, VA, USA) were implanted bilaterally 2 mm dorsal to the MeA (2.8 mm caudal to bregma, 3.3 and −3.3 mm lateral to midline, and 7.3 mm ventral to the skull surface) or CeA (2.5 mm caudal to bregma, 4.2 and −4.2 mm lateral to midline, and 5.9 mm ventral to the skull surface) according to Paxinos and Watson (1998). Guide cannulae were fixed to the skull with four stainless steel screws and acrylic glue and closed with dummy cannulae (26 gauge; Plastics One, Roanoke, VA, USA). After surgery, rats were individually housed in standard rat cages, and behavioral testing was performed 3–4 days after surgery.

Microdialysis probe placement. A separate cohort of rats was used for *in vivo* measurement of extracellular OT release in the CeA. Handling and surgical procedures were similar to the procedures described above except for the placement of microdialysis probes instead of cannulae. Microdialysis probes (BrainLink, the Netherlands) were implanted unilaterally into the CeA (2.5 mm caudal to bregma, −4.2 mm lateral to midline, and 8.9 mm ventral to the skull surface). Two-inch pieces of polyethylene tubing were fixed to the ends of the microdialysis probes to allow for attachment to the microinfusion pumps and eppendorf tubes for sample collection. After surgery, rats were individually housed in standard rat cages. Microdialysis and behavioral testing were performed 2 days after surgery. This short postoperative recovery period is necessary for optimal detection of neuropeptides in microdialysates of chronically implanted probes (Horn and Engelmann, 2001). We have demonstrated that prior surgery and ongoing microdialysis had no effect on social investigation time in rats (Dumais et al., 2016).

Behavioral testing

Social interest test. To test for social interest, the time rats spent investigating an unfamiliar same-sex juvenile rat was measured according to Dumais et al. (2013). A juvenile rat was used in order to assess general social approach of the experimental rat toward a conspecific that does not elicit aggressive or sexual behaviors. A juvenile rat was placed into the experimental rat's home cage for 4 min, and the time spent investigating the juvenile was