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Oxytocin promotes functional coupling between paraventricular nucleus and both sympathetic and parasympathetic cardioregulatory nuclei

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ABSTRACT

The neuropeptide oxytocin (OXT) facilitates prosocial behavior and selective sociality. In the context of stress, OXT also can down-regulate hypothalamic–pituitary–adrenal (HPA) axis activity, leading to consideration of OXT as a potential treatment for many socioaffective disorders. However, the mechanisms through which administration of exogenous OXT modulates social behavior in stressful environmental contexts are not fully understood. Here, we investigate the hypothesis that autonomic pathways are components of the mechanisms through which OXT aids the recruitment of social resources in stressful contexts that may elicit mobilized behavioral responses. Female prairie voles (*Microtus ochrogaster*) underwent a stressor (walking in shallow water) following pretreatment with intraperitoneal OXT (0.25 mg/kg) or OXT antagonist (OXT-A, 20 mg/kg), and were allowed to recover with or without their sibling cagemate. Administration of OXT resulted in elevated OXT concentrations in plasma, but did not dampen the HPA axis response to a stressor. However, OXT, but not OXT-A, pretreatment prevented the functional coupling, usually seen in the absence of OXT, between paraventricular nucleus (PVN) activity as measured by c-Fos immunoreactivity and HPA output (i.e. corticosterone release). Furthermore, OXT pretreatment resulted in functional coupling between PVN activity and brain regions regulating both sympathetic (i.e. rostral ventrolateral medulla) and parasympathetic (i.e. dorsal vagal complex and nucleus ambiguous) branches of the autonomic nervous system. These findings suggest that OXT increases central neural control of autonomic activity, rather than strictly dampening HPA axis activity, and provides a potential mechanism through which OXT may facilitate adaptive and context-dependent behavioral and physiological responses to stressors.

Introduction

The neuropeptide oxytocin (OXT), along with the closely related arginine vasopressin (AVP), has critical roles in facilitating selective social preferences (Williams et al., 1994; Winslow et al., 1993; Insel et al., 1998; Baribeau and Anagnostou, 2015; Kelly and Goodson, 2014; Lieberwirth and Wang, 2014; Stoesz et al., 2013). In the context of stress, activation of the OXT system may promote social cohesion by encouraging approach toward, affiliation with, and maintenance of proximity between, familiar preferred conspecifics (Carter, 1998; Engelmann et al., 1996; Williams et al., 1992; Witt et al., 1990). Behaviors that bring preferred individuals closer would promote coordinated responses to stressful stimuli, such as sharing vigilance during stressful events that threaten the safety of the social group (Carter, 1998; Carter et al., 2008; Grabowska-Zhang et al., 2012; Griffiths et al., 2004; Seyfarth and Cheney, 2012; Silk et al., 2003; Silk and House, 2011). For example, previous work has demonstrated that OXT regulates social contact and pair bonding in prairie voles (Cho et al., 1999), defensive aggregation in rats (Bowen et al., 2013), and reduces interpersonal distance in humans (Perry et al., 2014).

Previous research has implicated both OXT and social behavior in the regulation of the mammalian autonomic nervous system (Grippo et al., 2007a,b; Grippo et al., 2011; Gutkowska and Jankowski, 2011; Kenkel et al., 2014; Norman et al., 2011). An organism's autonomic state and

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social functioning dynamically co-regulate each other to adapt to changing environmental conditions (Porges, 2003). Like humans, prairie voles exhibit high levels of sociality and the capacity for selective social bonds (Carter et al., 1995). Prairie voles also have a relatively low resting heart rate, and high levels of parasympathetic activity compared to common laboratory rodents like rats and mice (Grippo et al., 2007a,b).

Here, we examine the hypothesis that exogenous OXT influences the behavioral responses of female prairie voles to a stressor (walking in shallow water) by dampening neural activity in brain regions associated with the HPA axis and autonomic nervous system. Voles were given systemic treatments of OXT or a selective OXT receptor antagonist (L-368,899). We also tested the hypothesis that the behavioral and neural effects of OXT would vary as a function of social housing: voles recovered from the stressor alone or in the presence of a familiar same-sexed social partner (cagemate). Measurements of c-Fos were used as an index of neural activity following the stressor in the paraventricular nucleus of the hypothalamus (PVN), a major site of production of OXT and other stress-related peptides (e.g. AVP, corticotropin releasing hormone) that is critical to the stress buffering and anxiolytic properties of OXT (Smith et al., 2015; Smith and Wang, 2014). The PVN functions in coordination with specific brainstem nuclei known to regulate the autonomic nervous system, including the dorsal vagal complex (DVC), nucleus ambiguous (AMB), and rostral ventrolateral medulla (RVLM) (Herman et al., 1996; Piñol et al., 2012). Plasma corticosterone concentrations provided an index of the functional output of the HPA axis. To examine possible functional coupling among neural activities in these regions correlations also were run between c-Fos activity in the PVN and in the DVC, AMB and RVLM, and between plasma levels of corticosterone and c-Fos activity in each of these brain areas.

Methods

Animals

Seventy-two pairs of adult, reproductively naïve female prairie voles (*Microtus ochrogaster*) were used in this study. Prairie voles were F3 or F4 descendants of prairie voles originally captured near Champaign, Illinois, and bred in-house. Following weaning at 20 days of age voles were pair-housed with a same-sex littermate, and maintained on a 14 h:10 h light:dark cycle (lights on at 06:00) in a temperature and humidity controlled vivarium. Experiments occurred during the light period. Food (Purina rabbit chow) and water were available ad libitum, and cage changes were performed on a weekly basis. 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 University of Illinois at Chicago Institutional Animal Care and Use Committee.

Experimental paradigm

One prairie vole per pair was randomly designated as the focal vole. Focal voles were momentarily restrained to receive an intraperitoneal (i.p.) injection of oxytocin (OXT), oxytocin antagonist (OXT-A), or sterile isotonic saline vehicle (VEH), and were placed back into their home cage with their same-sex littermate for 30 min. OXT (Bachem, Bubendorf, Switzerland) was dissolved in sterile isotonic saline at a concentration of 100 μg/mL and injected at a dose of 0.25 mg/kg, a dose that has been previously shown to ameliorate autonomic stress responses in voles (Grippo et al., 2009). Oxytocin antagonist (L-368,899, Merck Pharmaceuticals, Kenilworth, NJ) was dissolved in sterile isotonic saline at a concentration of 8 mg/mL and injected at a dose of 20 mg/kg, a dose that has been previously shown to block OXT activity when delivered peripherally (Smith et al., 2010). Treatment with sterile isotonic saline vehicle was delivered at the same injection volume (100 μL). Following injection, focal voles experienced a wet cage stressor (WCS), by being transferred in a plastic cup to a mouse cage containing ~2 cm of water at room temperature. The WCS was designed to model a flooded burrow, mimicking a stressor voles might encounter in their natural habitat. After 5 min of exposure to the WCS, voles were transferred by a plastic cup back into their dry home cage to recover in the presence or absence of their familiar cagemate. After a 60 min recovery period in the home cage voles were euthanized by isoflurane anesthesia (<2 min) followed by decapitation and collection of blood and tissue samples. The reason voles were killed 60 min after termination of the stressor is twofold: 1) plasma corticosterone concentrations are typically elevated by 60 min following an acute stressor, and 2) Fos expression in activated neurons can be detected between 30 and 90 min following neural activation. A schematic depicting the timeline of the experiment is presented in Fig. 1.

Hormone validation of the wet cage stressor

A separate cohort of female voles ($n = 12$) were used to assess the degree to which a 5 min exposure to a wet cage represents a stressor. Voles were injected with sterile saline, and after 30 min exposed to the WCS. Small volumes (<100 μL) of whole blood were collected to assess the time course of corticosterone release in response to the WCS. Repeated sublingual phlebotomy was performed under light (<1.5%) isoflurane anesthesia immediately following the vehicle injection (Baseline), immediately following the WCS (WCS), 1 h following the WCS (WCS + 1 h), and 24 h following the WCS (WCS + 24 h). Whole blood was collected into heparinized microcentrifuge tubes and kept on ice until centrifugation for plasma collection. Plasma was assayed for corticosterone as reported below (Hormone measurement).

Behavior

Behavior was recorded and scored by two independent observers (>85% concordance) for the first 5 min following reintroduction into the home cage with the familiar cagemate following removal from the wet cage stressor. The frequency and duration of both initiations and receptions of affiliative contact were quantified with Behavior Tracker (v. 1.0). All contact observed during the allotted time period was categorized as affiliative, and never resulted in agonistic interactions. Total time in sedentary social contact was defined as the amount of time that a vole either initiated or received a bout of social affiliation in which both voles were immobile in side-by-side contact. In addition, the frequency and duration of stress-related behaviors were quantified in the first 5 min following removal from the wet cage stressor. An initial survey of behaviors revealed that the non-social behaviors displayed during this early post-stressor period were largely confined to rearing, digging, and autogrooming.

Immunohistochemistry

After behavioral testing and euthanasia, brain tissue was carefully extracted from the skull and placed in a scintillation vial containing a small stir bar and 10 mL of 4% buffered paraformaldehyde (Fisher Scientific, pH 7.5). The vial was placed on a stir plate and allowed to spin for 2 h. Fresh paraformaldehyde was exchanged and the brain was allowed to spin for an additional 2 h. Finally, brains were placed into fresh paraformaldehyde for overnight post-fixation. Brains then were immersed in a 25% sucrose solution and stored at 4 °C until sectioned. Brains were cut in 40 μm coronal plane sections using a freezing sliding microtome (Leica Microsystems, Bannockburn, IL). Sections were stored in cryoprotectant in 24-well plates at −20 °C until processed.

Brains were stained for oxytocin and c-Fos using standard avidin-biotinylated enzyme complex (ABC) immunohistochemistry (Vector Laboratories, Burlingame, CA, USA). Serial sets (every third section) of free-floating tissue sections were rinsed in 0.05 M potassium phosphate-buffered saline (KPBS) for 1 h to remove excess