

CHARACTERIZATION OF THE OXYTOCIN SYSTEM REGULATING AFFILIATIVE BEHAVIOR IN FEMALE PRAIRIE VOLES

H. E. ROSS,^{a,b} C. D. COLE,^{a,b} Y. SMITH,^{a,b,c}
I. D. NEUMANN,^d R. LANDGRAF,^e A. Z. MURPHY^f AND
L. J. YOUNG^{a,b,g*}

^aCenter for Behavioral Neuroscience, Emory University, Atlanta, GA, USA

^bYerkes National Primate Research Center, Emory University, Atlanta, GA, USA

^cDepartment of Neurology, Emory University, Atlanta, GA, USA

^dDepartment of Behavioral Neuroendocrinology, Institute of Zoology, University of Regensburg, Regensburg, Germany

^eDepartment of Behavioral Neuroendocrinology, Max Planck Institute of Psychiatry, Munich, Germany

^fNeuroscience Institute, Georgia State University, Atlanta, GA, USA

^gDepartment of Psychiatry, Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA

Abstract—Oxytocin regulates partner preference formation and alloparental behavior in the socially monogamous prairie vole (*Microtus ochrogaster*) by activating oxytocin receptors in the nucleus accumbens of females. Mating facilitates partner preference formation, and oxytocin-immunoreactive fibers in the nucleus accumbens have been described in prairie voles. However, there has been no direct evidence of oxytocin release in the nucleus accumbens during sociosexual interactions, and the origin of the oxytocin fibers is unknown. Here we show for the first time that extracellular concentrations of oxytocin are increased in the nucleus accumbens of female prairie vole during unrestricted interactions with a male. We further show that the distribution of oxytocin-immunoreactive fibers in the nucleus accumbens is conserved in voles, mice and rats, despite remarkable species differences in oxytocin receptor binding in the region. Using a combination of site-specific and peripheral infusions of the retrograde tracer Fluorogold, we demonstrate that the nucleus accumbens oxytocin-immunoreactive fibers likely originate from paraventricular and supraoptic hypothalamic neurons. This distribution of retrogradely labeled neurons is consistent with the hypothesis that striatal oxytocin fibers arise from collaterals of magnocellular neurons of the neurohypophyseal system. If correct, this may serve to coordinate peripheral and central release of oxytocin with appropriate behavioral responses associated with reproduction, including pair bonding after mating, and maternal respon-

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Key words: pair bonding, nucleus accumbens, paraventricular nucleus, supraoptic nucleus, neurohypophyseal peptides, alloparental behavior.

Oxytocin (OT) released from the neurohypophyseal system has been implicated in the regulation of reproductive physiology in mammals, including uterine contractions during parturition and milk ejection during lactation (Burbach et al., 2006). In addition, OT released within the brain coordinates the onset of maternal responsiveness and maternal bonding at the time of parturition (Pedersen and Prange, 1979; Kendrick et al., 1987). Recent studies in humans have also suggested that central OT modulates social cognition, including increasing interpersonal trust, eye gaze, face recognition, and the ability to infer the emotions of others based on facial cues (Kosfeld et al., 2005; Domes et al., 2007; Donaldson and Young, 2008; Guastella et al., 2008; Savaskan et al., 2008).

Prairie voles (*Microtus ochrogaster*) have become an important animal model for elucidating the behavioral roles of OT and the neurobiology of affiliative behavior (Carter et al., 1995; Young and Wang, 2004). Prairie voles are a highly affiliative rodent species characterized by a socially monogamous mating strategy and high levels of alloparental care. In the laboratory, the formation of selective pair bonds between mates can be assessed using a partner preference test in which the time spent with the partner versus a novel stimulus animal is quantified. Extended cohabitation with a male or mating facilitates the formation of partner preferences in female prairie voles (Williams et al., 1992). Pharmacological and genetic manipulation studies have demonstrated that oxytocin receptors (OTR) in the nucleus accumbens (NAcc) play a significant role in the regulation of behaviors associated with social monogamy and alloparental care. Infusion of an OTR antagonist into the NAcc prevents mating-induced partner preferences in female prairie voles (Young et al., 2001). Conversely, increasing OTR density in the NAcc using viral vector gene transfer can accelerate the formation of a partner preference (Ross et al., 2009). Furthermore, OTR binding density in the NAcc is positively correlated with alloparental behavior, and infusion of an OTR antagonist into the NAcc inhibits spontaneous alloparental behavior in sexually naïve female prairie voles (Olazabal and Young, 2006a). Interestingly, non-monogamous rodent species, including meadow voles, mice and rats have very low levels of OTR binding in the NAcc, which may contribute to the species

*Correspondence to: L. J. Young, Yerkes National Primate Research Center, 954 Gatewood Road, Emory University, Atlanta, GA 30322, USA. Tel: +1-404-727-8272; fax: +1-404-727-8070. E-mail address: lyoun03@emory.edu (L. J. Young).

Abbreviations: AP, anterior-posterior; BT, biotin–tyramine; DAB, 3,3-diaminobenzidine; DV, dorsal-ventral; EM, electron microscopy; FG, Fluorogold; FM, fluorescent microscopy; i.p., intraperitoneal; KPBS, potassium phosphate-buffered saline; LM, light microscopy; ML, medial-lateral; NAcc, nucleus accumbens; OT, oxytocin; OTR, oxytocin receptors; PB, potassium buffer; PVN, paraventricular nucleus; SON, supraoptic nucleus.

differences in social behavior (Insel and Young, 2001; Burbach et al., 2006).

Despite the evidence that OT signaling in the NAcc plays a critical role in regulating affiliative behavior in prairie voles, the presynaptic OT system in this region has not been characterized. Specifically, mating-induced OT release has not been directly demonstrated, and the morphology and source of the OT-immunoreactive fibers projecting to the NAcc have not been determined. OT is produced primarily in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. Large diameter magnocellular neurons in these regions form the neurohypophyseal system and are thought to project primarily to the posterior pituitary (Bargmann, 1949). Early tract tracing studies demonstrated that OT-immunoreactive fibers of the brainstem originate from the smaller diameter parvocellular OT neurons in the PVN, leading to the assumption that these neurons also project to the forebrain regions regulating behavior, providing a dissociation between the neurohypophyseal and central OT systems (for review see Landgraf and Neumann, 2004).

In this study we examined the NAcc OT system in detail. Using microdialysis in awake, behaving female prairie voles, we measured OT release within the NAcc as a function of sociosexual interactions with a male. We then compared the distribution of OT-immunoreactive fibers in the NAcc of prairie voles, meadow voles, rats and mice to determine whether the OT innervation of the NAcc is conserved across species with diverse social behavior and OTR distributions in this area. The ultrastructural features of the OT-immunoreactive processes in the NAcc were then examined using electron microscopy (EM). Finally, Fluorogold (FG) tract tracing was used to identify the neuronal origin of the OT projections to the NAcc. The results of these studies add significantly to our understanding of the circuitry involved in regulating affiliative behavior in female prairie voles, and provide a potential mechanism for coordinating central OT release with reproductive physiology in all mammals.

EXPERIMENTAL PROCEDURES

Animals

Prairie and meadow voles were housed in same-sex groups with two to three voles/cage from the time of weaning at 21–23 days of age. Housing consisted of a ventilated 36×18×19 cm Plexiglas cage filled with Bed-o-cobbs Laboratory Animal Bedding (Bed-o-cobbs, Maumee, OH, USA) under a 14:10-h light/dark cycle at 22 °C with access to food (rabbit LabDiet, Richmond, IN, USA) and water *ad libitum*. The prairie voles were obtained from our laboratory breeding colony that originally derived from field-captured voles in Illinois. Meadow voles originated from a colony at Florida State University. For the microdialysis experiment, subjects were sexually naïve female prairie voles 70–90 days of age (30–45 g).

For anatomical studies, subjects were adult (>60 days old) female prairie voles. In addition, five female sexually naïve meadow voles from our breeding colony, five virgin female mice (C57BL/6J), and five virgin Sprague–Dawley (Charles River) female rats were used for a species comparison. All procedures were approved by the Emory University Institutional Animal Care

and Use Committee, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Hormone treatment and microdialysis probe implantation

Subjects were ovariectomized two weeks prior to probe implantation and microdialysis, and administered 1 µg estradiol benzoate in peanut oil, i.p., for four consecutive days prior to testing to induce receptivity. Subjects were anesthetized with isoflurane and U-shaped microdialysis probes were stereotactically implanted unilaterally into the NAcc (nose bar –2.5 mm, AP +1.8 mm, ML –0.9 mm, DV –4.5 mm). After a 1 day recovery, the probe was connected to two lengths of PE20 tubing (Polymicro Tech, Phoenix, AZ, USA). The microdialysis probes were self-made as previously described, and had a molecular cutoff of 18 kDa (for details see Neumann et al., 1993; Bosch et al., 2005). The inlet of the probe was connected to a 10-ml Hamilton syringe controlled by a CMA/100 microinfusion pump (Bioanalytical Systems, West Lafayette, IN, USA). The outlet fed into a refrigerated collector (SciPro, Inc., Sanborn, NY, USA) housing polyethylene tubes (Fisher Scientific, Houston, TX, USA). A single-channel swivel and counterbalanced lever arm allowed the animals to move freely during mating. Ringer's solution was perfused through the probe via the inlet into the NAcc during the experiment at a flow rate of 1.0 µl/min.

OT collection

To determine the effects of mating on OT release, eight consecutive 30-min microdialysates were collected from the NAcc of estrogen-primed female prairie voles ($n=26$). Sample collections were divided into three phases: basal (1–4), restricted exposure (5–8), and free exposure (9–16). Basal dialysates were collected from individually housed females and served as the baseline for extracellular concentration of OT. After the basal phase, a sexually experienced male prairie vole of similar age and weight, housed in a wire mesh cage (restricted exposure phase), was introduced into the test cage. After 2 h the male was removed from the mesh cage and allowed to physically interact with the female (free exposure phase) for 4 h. This portion of the test was videotaped. Female subjects that did not mate during this period because they were not sexually receptive were categorized as non-mated.

Following microdialysis, brains were rapidly removed, frozen on dry ice, and stored at –80 °C until use. Brains were later sectioned on a cryostat into 20-µm slices mounted on Superfrost plus microscope slides (Fisher, Pittsburgh, PA, USA). Slides were stored at –80 °C. Proper placement of the probes was confirmed by Cresyl Violet staining.

Quantification of OT

Dialysates collected during microdialysis were stored at –80 °C until analyzed for content. Samples were lyophilized and the concentration of OT in each dialysate was determined by radioimmunoassay as described previously (Neumann et al., 1993). Cross-reactivity of the polyclonal antiserum with arginine–vasopressin and other related peptides was <0.7%. Intra- and inter-assay coefficients of variation were in the 5%–9% and 8%–12% ranges, respectively; all dialysates to be compared were assayed in the same run. Twenty-five microliters of each dialysate was assayed and the level of detectability of the assay was 0.05 pg/dialysate.

FG infusions

Female prairie voles ($n=18$) were anesthetized with isoflurane and placed in a Kopf stereotaxic apparatus. FG was iontophoretically injected unilaterally into the NAcc (AP +1.7 mm, ML –0.9

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