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DISTRIBUTION OF OXYTOCIN IN THE BRAIN OF A EUSOCIAL RODENT

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Abstract—Naked mole-rats are highly social rodents that live in large colonies characterized by a rigid social and reproductive hierarchy. Only one female, the queen, breeds. Most colony members are non-reproductive subordinates that work cooperatively to rear the young and maintain an underground burrow system. Little is known about the neurobiological basis of the complex sociality exhibited by this species. The neuropeptide oxytocin (Oxt) modulates social bonding and other social behaviors in many vertebrates. Here we examined the distribution of Oxt immunoreactivity in the brains of male and female naked mole-rats. As in other species, the majority of Oxt-immunoreactive (Oxt-ir) cells were found in the paraventricular and supraoptic nuclei, with additional labeled cells scattered throughout the preoptic and anterior hypothalamic areas. Oxt-ir fibers were found traveling toward and through the median eminence, as well as in the tenia tecta, septum, and nucleus of the diagonal band of Broca. A moderate network of fibers covered the bed nucleus of the stria terminalis and preoptic area, and a particularly dense fiber innervation of the nucleus accumbens and substantia innominata was observed. In the brainstem, Oxt-ir fibers were found in the periaqueductal gray, locus coeruleus, parabrachial nucleus, nucleus of the solitary tract, and nucleus ambiguus. The high levels of Oxt immunoreactivity in the nucleus accumbens and preoptic area are intriguing, given the link in other rodents between Oxt signaling in these regions and maternal behavior. Although only the queen gives birth or nurses pups in a naked mole-rat colony, most individuals actively participate in pup care.

Key words: sex differences, social hierarchy, naked mole-rat, *Heterocephalus glaber*, sociality, vasopressin.

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Abbreviations: ACB, nucleus accumbens; Amb, nucleus ambiguus; BST, bed nucleus of the stria terminalis; H, habenula; ir, immunoreactive; LC, locus coeruleus; LPO, lateral preoptic area; LSd, dorsal lateral septum; MeA, medial nucleus of the amygdala; MPO, medial preoptic area; NGS, normal goat serum; NTS, nucleus of the solitary tract; NTSm, nucleus of the solitary tract, medial part; Oxt, oxytocin; PAG, periaqueductal gray; PB, parabrachial nucleus; PVN, paraventricular nucleus of the hypothalamus; PVNpm, paraventricular nucleus of the hypothalamus, posterior magnocellular part; PVT, paraventricular nucleus of the thalamus; RN, reticular nucleus; SI, substantia innominata; SNr, substantia nigra; SON, supraoptic nucleus; TBS, Tris-buffered saline; Tris-Triton, Tris-buffered saline containing 0.03% Triton; TTd, tenia tecta, dorsal part; VH, ventral horn; VII, facial nucleus; VP, vasopressin; VTA, ventral tegmental area.

The social structure of the naked mole-rat (*Heterocephalus glaber*) is the closest equivalent to eusociality in a vertebrate. This species lives underground in large colonies of 70–80 individuals (Brett, 1991). Breeding is restricted to the queen and one to three breeding males (Jarvis, 1981; Brett, 1991; Faulkes et al., 1991; Lacey and Sherman, 1991). The remaining colony members are non-breeding subordinates, which participate in pup care, nest building, food carrying, and colony defense (Faulkes et al., 1991; Lacey et al., 1991; Lacey and Sherman, 1991).

Cooperative breeding with reproductive suppression is seen in a variety of mammalian taxa, including primates, canids, viverrids, and rodents (Jennions and Macdonald, 1994; Solomon and French, 1997). However, the majority of studies on the biological bases of sociality or social effects on reproduction have focused on only a few rodent species (e.g. voles and mice; reviewed in Carter and Roberts, 1997). Several key traits distinguish naked mole-rats from these traditional models. The majority of subordinates never achieve reproductive status, and it has been suggested that in nature, fewer than 5% of naked mole-rats ever become breeders (Jarvis, 1981; Lacey and Sherman, 1991). In addition, naked mole-rats exhibit a marked reduction in sexual dimorphisms in anatomy and behavior. Males and females do not differ in body size (Jarvis, 1991), have virtually indistinguishable genitalia (Jarvis, 1991; Peroulakis et al., 2002), and perform very similar behaviors (Lacey and Sherman, 1991). The naked mole-rat nervous system also lacks many of the sexual dimorphisms found in other mammals (Peroulakis et al., 2002; Seney et al., 2006; Rosen et al., 2007; Holmes et al., 2007). Instead, differences in some of the classically sexually dimorphic areas appear to depend on social status rather than sex, with reproductive individuals showing differences from non-breeders (Seney et al., 2006; Holmes et al., 2007).

The rich social behaviors of naked mole-rats have been well described (Lacey and Sherman, 1991; Lacey et al., 1991; Jarvis, 1991), but the underlying hormonal and neural mechanisms are largely unknown. As a first step, we previously examined the distribution of vasopressin (VP) in the brains of subordinate and breeding naked mole-rats (Rosen et al., 2007). Some social behaviors exhibited by naked mole-rats are modulated by VP in other species, such as vocal communication, pair-bonding, parental behavior, dominance–subordinance, and social memory (reviewed in de Wied et al., 1993; Goodson and Bass, 2001; Young and Wang, 2004). Unlike the majority of vertebrate species studied to date, subordinate and breeding naked mole-rats lacked VP innervation of the lateral septum and VP-immunoreactive (ir) cells in the bed

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nucleus of the stria terminalis (BST) (Rosen et al., 2007). Instead, the dorsomedial septum contained a particularly dense VP-ir innervation, which did not vary with sex or breeding status (Rosen et al., 2007). For the present study, we examined the immunohistochemical distribution of oxytocin (Oxt) in naked-mole rats.

Oxt is best known for its role as a posterior pituitary hormone involved in milk ejection and parturition. Oxt also modulates social behaviors, such as social memory, pair-bonding, sexual behavior, and parental behavior when released as a neuropeptide in the CNS (McCarthy et al., 1992; Pedersen et al., 1992; Insel et al., 1997; Argiolas, 1999; Bales et al., 2004; Winslow and Insel, 2004; Lim and Young, 2006). Physiological and autonomic functions, food intake, the stress response, and heart rate are also influenced by neural Oxt (reviewed in de Wied et al., 1993; Verbalis et al., 1995; Neumann, 2002; Petersson, 2002). However, unlike vasopressinergic innervation of the forebrain, Oxt pathways generally do not exhibit consistent sex differences (Buijs et al., 1978; Wang et al., 1996; but see Häussler et al., 1990). Here, we mapped the Oxt distribution in the naked mole-rat brain to gain a more complete picture of neuropeptide pathways that might modulate behavior and physiology in this uniquely social species.

EXPERIMENTAL PROCEDURES

Animals

Naked mole-rats in the present study came from colonies maintained at the University of Connecticut, which are descendants of 20 wild-caught animals bred by J. U. M. Jarvis (University of Cape Town, South Africa). Housing conditions have previously been described (Riccio and Goldman, 2000; Seney et al., 2006). A total of four male and four female subordinates were used in the present study, ranging in age from 3 to 8 years. Naked mole-rats reach adult body size at about 1 year of age and it is not uncommon for them to survive over 20 years in the laboratory (Buffenstein, 2005). Due to limited availability, breeders were not included in this study. All procedures were approved by the University of Connecticut Animal Use and Care Committee, and conform to the guidelines of the National Institutes of Health. Every effort was made to minimize the number and suffering of the animals used in the study.

Tissue preparation

Animals were anesthetized (40 mg avertin/100 g b.w.) and rapidly decapitated. Brains were removed, immersion fixed for 4 h in 5% acrolein and 0.5 M phosphate buffer (pH 7.6), and sunk in 30% sucrose. The brains were then cut in the transverse plane at 30 μm on a freezing microtome and the sections stored in cryoprotectant at −20 °C until use.

Immunohistochemistry

Every fourth section through the forebrain of three females and four males and through the brainstem of two males and three females was immunostained for Oxt according to Rosen et al. (2007). Briefly, floating sections were pre-treated with 3% H_2O_2, followed by 0.01% sodium borohydride in 0.05 M Tris-buffered saline (TBS), and blocked in 20% normal goat serum (NGS) in Tris-Triton (Tris-buffered saline containing 0.03% Triton). Sections were then exposed to 1) anti-Oxt rabbit antiserum (Millipore, Billerica, MA, USA) diluted 1:10,000 in Tris-Triton and 2% NGS overnight; 2) biotinylated goat-anti-rabbit (1.5 μg/ml, Vector Laboratories, Burlingame, CA, USA) in NGS for 45 min; and 3) biotinylated avidin–horseradish peroxidase complex (ABC solution, Vector Laboratories) in TBS for 45 min. The bound antibody complex was visualized with a nickel-intensified 0.05% 3–3′-diaminobenzidine solution.

As a specificity control, sections were treated with antiserum preadsorbed with 50 μM of either purified Oxt or arg^8-VP (both from Calbiochem, La Jolla, CA, USA). Preadsorption with Oxt peptide dramatically diminished immunostaining in the nucleus accumbens (ACB), dorsomedial septum, paraventricular nucleus (PVN), and supraoptic nucleus (SON), and eliminated staining in all other areas. Immunostaining was not reduced in sections exposed to Oxt antiserum preadsorbed with VP, consistent with this antibody's low cross-reactivity (<1.0%) with VP, as reported by the manufacturer. Additional positive and negative controls included sections from an adult male C57Bl/6 mouse that were also exposed to the Oxt antiserum, or to each of the preabsorbed antisera.

Artwork and digital photomicrographs

Camera lucida drawings of the distribution of Oxt cells and fibers were made from a representative male naked mole-rat brain. Designation of neuroanatomical structures are based on the recently published atlas for naked mole-rats (Xiao et al., 2006). Structures not identified in the published atlas were labeled based on the rat brain atlas (Swanson, 1992), which is in general agreement with the nomenclature used for the naked mole-rat (Xiao et al., 2006).

All photomicrographs were taken with a digital camera. Adobe PhotoShop 6.0 was used to adjust brightness/contrast and remove background artifacts as necessary. Final images were sized, assembled and labeled in CorelDRAW 11.0.

RESULTS

The overall distribution of Oxt-ir cells and fibers was similar in males and females with no obvious sex differences, and resembled that of other rodents (Buijs, 1978; Buijs et al., 1978; Sofroniew et al., 1979; Hermes et al., 1988; Dubois-Dauphin et al., 1989), including the mouse (Castel and Morris, 1988 and present study). However, Oxt-ir cell bodies were generally more numerous and Oxt-ir innervation more extensive in the mouse than in naked mole-rat, with several notable exceptions discussed below.

Cells

The PVN and SON contained the majority of Oxt-ir cells (Fig. 1C–E; Fig. 2A–D). Most of these appeared to be magnocellular (cross-sectional area approximately 100–120 μm^2) and were darkly stained (Fig. 2A, B). The lateral portion of the anterior PVN contained many such Oxt-ir cells (Figs. 1E, 2A), with a few lightly staining somata in the medial PVN (Fig. 2A, B) and in the accessory magnocellular nucleus of the PVN (PVNpm) (Fig. 1D). A few lighter-stained Oxt-ir cells in the posterior PVN (Fig. 1F) had the appearance of parvocellular neurons, (i.e. small, round, and with few processes). Within the SON, Oxt-ir cells were distributed throughout the medial–lateral axis (Figs. 1C–E, 2C). This differs from the distribution in the mouse (Fig. 2D), where Oxt-ir cells of the SON are clustered on the lateral edge of the optic tract. A moderate number of darkly-staining accessory magnocellular Oxt-ir cells were also scattered throughout the preoptic area and anterior hypothalamus (Fig. 1B–D), with a small cluster of cells occurring at the base of the preoptic area, dorsal to the