Neuroscience 303 (2015) 261–269

REGION-SPECIFIC ASSOCIATIONS BETWEEN SEX, SOCIAL STATUS, AND OXYTOCIN RECEPTOR DENSITY IN THE BRAINS OF EUSOCIAL RODENTS

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Abstract—Naturally occurring variations in neuropeptide receptor distributions in the brain contribute to numerous mammalian social behaviors. In naked mole-rats, which live in large social groups and exhibit remarkable reproductive skew, colony-related social behaviors vary with reproductive status. Here we examined whether variation in social status is associated with variations in the location and/or density of oxytocin binding in this species. Autoradiography was performed to assess forebrain oxytocin receptor (OTR) densities in breeding and non-breeding naked mole-rats of both sexes. Overall, males exhibited higher OTR binding in the medial amygdala in comparison to females. While there were no main effects of reproductive status in any region, a sex difference in OTR binding in the nucleus accumbens was mediated by status. Specifically, breeding males tended to have more OTR binding than breeding females in the nucleus accumbens, while no sex difference was observed in subordinates. These effects suggest that oxytocin may act in a sex- and region-specific way that corresponds to reproductive status and associated social behaviors.

Key words: amygdala, naked mole-rat, nucleus accumbens, oxytocin, receptor autoradiography, social behavior.

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Abbreviations: BNST, bed nucleus of the stria terminalis; MPOA, medial preoptic area; OT-neurophysin-ir, oxytocin-neurophysin immunoreactive; OTR, oxytocin receptor; VMH, ventromedial nucleus of the hypothalamus.

http://dx.doi.org/10.1016/j.neuroscience.2015.06.043
0306-4522/

INTRODUCTION

Social interactions are an important aspect of an organism's life-history. Whether a species is gregarious or solitary, the appropriate response to social stimuli has consequences for an animal's reproductive success and survival. The recent rise in papers in the field of social neuroscience highlights a concerted effort toward understanding the underlying mechanisms that regulate specific responses to social stimuli (Insel, 2010). A great deal of investigation on the neurochemistry of social behavior has focused on the role of hypothalamic nonapeptides (Goodson, 2013). Oxytocin, in particular, plays diverse roles in mammalian social behaviors, ranging from social recognition and preferences to anxiolysis. For example, oxytocin is crucial for opposite-sex partner preference formation in prairie voles and social recognition in mice (Insel and Hulihan, 1995; Ferguson et al., 2000, 2001). Central oxytocin administration increases nonsexual same-sex partner preferences in meadow voles (Beery and Zucker, 2010), and reduces aggression in Syrian hamsters (Harmon et al., 2002). These effects may be, in part, mediated by oxytocin's anxiolytic properties (reviewed in Neumann, 2002). Even peripheral administration of the peptide can have diverse prosocial effects in cooperatively breeding mammals (Madden and Clutton-Brock, 2011; Mooney et al., 2014). Determining where and how oxytocin acts in particular species may shed light on how this diversity of behavioral outcomes arises.

One means of investigating species- or state-dependent differences in oxytocinergic action is by examining central oxytocin binding sites in rodents. Receptor distributions are often studied in a comparative context; while oxytocin cell locations appear to be fairly well conserved, binding sites show pronounced differences with species, population, and life-history (Francis et al., 2000; Goodson, 2008; Beery and Zucker, 2010; Ophir et al., 2012; Anacker and Beery, 2013; Beery et al., 2014; Veenema, 2012; Wang et al., 1996). Of particular interest are species from close evolutionary lineages that demonstrate different sociosexual organization. Various species of voles and Peromyscus mice, which demonstrate different mating patterns (monogamy or polygamy), also differ in their oxytocin receptor (OTR) distributions (Insel et al., 1991; Insel and Shapiro, 1992). In the case of prairie voles, oxytocin binding in certain regions, particularly the nucleus accumbens, is crucial to the maintenance of monogamous

attachments (Young et al., 2001; Liu and Wang, 2003), suggesting a potentially broad functional significance to species differences in the anatomical distribution of this receptor. More recently, it has become apparent that other animals such as tuco-tucos, mole-rats, singing mice and even estrildid finches demonstrate interspecific differences in OTR distribution together with differences in social and/or reproductive organization (Beery et al., 2008; Campbell et al., 2009; Goodson et al., 2009; Kalamatianos et al., 2010; Anacker and Beery, 2013).

Differential distributions of OTR may be important for regulating not only behavioral differences between species, but also within-species individual differences in the display of certain species-specific behaviors. This is exemplified in female prairie voles, in whom alloparental care is positively correlated with OTR density in the nucleus accumbens and the caudate, and negatively correlated with OTR in the lateral septum (Olazabal and Young, 2006a). Site-specific delivery of oxytocin antagonists into the nucleus accumbens decreases alloparental behaviors in this species, demonstrating that OTR binding is important for the display of the behavior and not simply a product of it (Olazabal and Young, 2006b). Increasing the number of receptors in the nucleus accumbens also accelerates the establishment of a sexual partner preference in female prairie voles (Ross et al., 2009). In meadow voles, OTR density in the central amygdala and social behavior vary seasonally with day length, and OTR density in the lateral septum is negatively correlated with same-sex affiliative behavior in females (Beery and Zucker, 2010; Beery et al., 2014). These within-species relationships between OTR and behavior raise interesting questions as to whether the distribution and intensity of binding contribute to individual differences in behavior between members of the same species.

Naked mole-rats (*Heterocephalus glaber*) exhibit the most dramatic reproductive skew documented in mammals. These subterranean rodents live in colonies that can approach 300 animals in number (Brett, 1991a). However, in any given colony, mating opportunity is restricted to a single female, called the queen, and up to three males (Jarvis, 1981; Lacey and Sherman, 1991; Brett, 1991a). Socially-dominant breeders exhibit a number of proceptive and receptive sexual behaviors that are infrequent or absent in the non-reproductive subordinates of the colony (Jarvis, 1991). In addition to sexual activity, breeding animals, particularly the queen, show higher levels of aggression toward familiar conspecifics (Clarke and Faulkes, 2001). While many animals take part in pup care at some level, the breeding female is the only animal that nurses; breeding males also perform more general pup care than subordinates (Lacey and Sherman, 1991). Furthermore, comparisons between breeders and subordinates reveal remarkable neural differences (Holmes et al., 2007, 2008, 2009, 2011; Mooney and Holmes, 2013; Zhou et al., 2013). The interesting relationships between brain, behavior, and reproductive status observed in this cooperatively breeding species offer opportunities for investigating whether OTR binding contributes to within-species phenotypes.

Previous comparative research on OTRs in mole-rats (Kalamatianos et al., 2010) has identified profound differences in the telencephalic distribution of OTR binding and oxytocin-neurophysin immunoreactive (OT-neurophysin-ir) neuronal processes between subordinate eusocial naked mole-rats and solitary Cape mole-rats; animals in the latter species lead essentially solitary lives. For example, OTR binding and OT-neurophysin-ir processes in the nucleus accumbens are present at an intense level in the eusocial naked mole-rats, but nearly absent in the solitary Cape mole-rat, indicating that oxytocinergic activity in the nucleus accumbens may play an important role in eusocial behaviors. Little is known about the behavioral effects of oxytocin in naked mole-rats, though we have previously shown that subordinates have more oxytocinergic neurons in the paraventricular nucleus than do breeders (Mooney and Holmes, 2013) and peripheral administration of oxytocin increases some social behaviors in subordinates (Mooney et al., 2014). Specifically, intraperitoneal injections of oxytocin increase the amount of in-colony huddling, as well as the proximity to and investigation of familiar conspecifics when tested outside of the colony. Interestingly, intraperitoneal injections of the oxytocin antagonist L-368,899®, which has been shown to cross the blood–brain-barrier in rhesus monkeys (Boccia et al., 2007), does not decrease these behaviors by itself but does block the effects of oxytocin administration. Because the social behaviors demonstrated by naked mole-rats vary with both sex and social status, we tested the hypothesis that OTR binding varies between established socially-dominant breeders and their colony-matched non-breeding subordinates of both sexes.

EXPERIMENTAL PROCEDURES

Animals

Adult naked mole-rats were housed in colonies in polycarbonate cages connected by lengths of tubing and kept on a 12:12-h light/dark cycle at 28–30 °C. Animals had *ad libitum* access to a diet of sweet potato and wet 19% protein mash (Harlan Laboratories, Inc., Mississauga, ON, Canada). All animals were older than 1 year and had reached adult body size (O'Riain and Jarvis, 1998; Buffenstein, 2005). All procedures adhered to federal and institutional guidelines and were approved by the University of Toronto Animal Care Committee. The current experiment compared four groups: male and female breeders (n = 6 per sex) were reproductive animals that had previously produced and raised at least one litter and remained in their home colonies until tissue collection; male (n = 10) and female (n = 9) subordinates were non-reproductive animals, from the same colonies as the breeders, which remained in their home colonies until tissue collection.

Tissue collection

Animals were overdosed with avertin (350 mg/kg; i.p.) and rapidly decapitated. Brains were extracted and bisected at midline in the sagittal plane. Half of the brain was immersion fixed in 4% paraformaldehyde for use in a