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) density 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.

1. 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 towards 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; Ferguson et al., 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 in 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

(Anacker and Beery, 2013; Beery and Zucker 2010; Beery et al. 2014; Francis et al., 2000; Goodson, 2008; Ophir et al., 2013; 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, specifically the nucleus accumbens, is crucial to the maintenance of monogamous attachments (Liu and Wang, 2003; Young et al., 2001), 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 (Anacker and Beery, 2013; Beery et al., 2008; Campbell et al., 2009; Goodson et al., 2009; Kalamatianos et al., 2010).

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, where 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 et al. 2014; Beery and Zucker, 2010). These within-species relationships between OTR and behavior raise interesting questions as to whether the distribution and intensity of binding might 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 3 males (Jarvis, 1981; Brett, 1991a; Lacey and Sherman, 1991). 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 towards 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; Holmes et al., 2008; Holmes et al., 2009; Holmes et al., 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 research on OTRs in naked 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.

2. Methods

2.1 Animals

Adult naked mole-rats were housed in colonies in polycarbonate cages connected by lengths of tubing and kept on a 12:12 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.). 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 4 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.

2.2 Tissue Collection

Animals were overdosed with avertin (350mg/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 separate experiment. The remaining halves were frozen at -80°C and were used for OTR ligand binding autoradiography.

2.3 OTR Autoradiography

Brain tissue was sectioned into four coronal series at 20 μm using a cryostat. Sections were thaw-mounted onto Superfrost Plus slides (Sigma-Aldrich, Poole, United Kingdom) and stored at -80°C. One series was used for oxytocin receptor autoradiography using the

selective ^{125}I -ornithine vasotocin analog vasotocin, $\text{d}(\text{CH}_2)_5[\text{Tyr}(\text{Me})_2, \text{Thr}^4, \text{Orn}^8, (^{125}\text{I})\text{Tyr}^9\text{-NH}_2]$ (^{125}I -OVTA, PerkinElmer, Inc.), which has previously been used successfully on naked mole-rat tissue (Kalamatianos et al., 2010). Briefly, slides were thawed to room temperature and dried in racks. They were then fixed for 2 min in 0.1% paraformaldehyde (0.1% paraformaldehyde in 0.1 M PBS). Sections were rinsed for 20 minutes (2x10 minutes) in 50 mM Tris (pH 7.4) and then incubated for 60 min at room temperature in a solution containing the radioactively labeled ^{125}I -ornithine vasotocin analog (50 mM Tris, 10 mM MgCl_2 , 0.1% BSA, 50 pM radioligand). Non-specific binding was determined by incubating a parallel series of tissue with 50 μM non-radioactive ligand [Thr^4Gly^7]-oxytocin (Bachem). Tissue was then rinsed for 3x5 minutes in cold Tris- MgCl_2 (50 mM Tris, 10 mM MgCl_2 , pH 7.4), dipped in cold distilled water, and air dried. Sections were apposed to Kodak BioMax MR film (Carestream Health, Rochester, NY, USA) for 5 days. Film was subsequently developed and scanned at 1200dpi (Mustek ScanExpress A3 USB 2400 Pro) at uniform settings.

2.4 Anatomical Localization

An acetylcholinesterase stain was performed on post-autoradiography tissue as in Lim et al. (2004) to delineate anatomical boundaries of scored regions (see below). Slides were incubated in a solution of 0.0072% ethopropazine, 0.075% glycine, 0.05% cupric sulfate, 0.12% acetylthiocholine iodide, and 0.68% sodium acetate (pH 5.0) for 18 hours. Slides were rinsed 3x5 min in dH_2O , developed in 0.77% sodium sulfide (pH 7.8) for 30 min, and rinsed 3x5 min in dH_2O . Staining was intensified by exposure to a 1% silver nitrate solution in the dark for 10 min, followed by 3x5 min rinses in dH_2O . Slides were air dried and scanned. Anatomical

classifications were performed using the *Atlas of the Brain of the Naked Mole Rat* (Xiao et al., 2006).

2.5 OTR Binding

Relative optical density was quantified in regions with visibly extensive binding. The optical density of three consecutive sections per brain region was scored in MCID™ Analysis 7.0 (MCID, InterFocus Imaging Ltd., Cambridge, England) for the nucleus accumbens, the bed nucleus of the stria terminalis (BNST), the medial amygdala, and the central amygdala; these analyses were performed at Smith College. The optical density across the three sections was averaged for statistical analysis. The same procedure was conducted in ImageJ (<http://rsb.info.nih.gov/ij/>) at the University of Toronto for the Islands of Calleja (major), the hippocampus, and the ventromedial nucleus of the hypothalamus (VMH). I^{125} standards (2.8 - 1500nCi, ARI 0133, American Radiolabeled Chemicals Inc, St. Louis, MO) were used to convert optical density measures into a measure of nCi/mg equivalent. Nonspecific binding was subtracted from total binding to yield values for specific binding. Cross-sectional areas of measurement were consistent within an area of interest, but varied between regions. The sampling area was selected to remain in the regional boundaries, while still providing substantial coverage of the region. 2 X 2 Univariate analyses of variance (sex-by-reproductive status) were conducted on relative binding in each region of interest. One male was excluded from analyses because we were not able to definitively confirm breeding status. Due to localized tissue damage, an additional breeding male was excluded from analyses for the

medial amygdala and a subordinate female was excluded from the analyses done on the nucleus accumbens.

3. Results

Regions with binding above background included the Islands of Calleja, nucleus accumbens, BNST, medial amygdala, central amygdala, CA1 region of the hippocampus and VMH (Figure 1).

Males showed greater binding than females in the medial amygdala ($F(1,25)=4.363$, $p=0.047$, partial $\eta^2=0.149$; Figure 2a and c). Though no significant status effect ($F(1,25)=1.211$, $p=0.282$, partial $\eta^2=0.046$) or sex-by-status interaction ($F(1,25)=0.420$, $p=0.523$, partial $\eta^2=0.017$) was detected, the sex difference was likely driven by low binding in female breeders (Figure 2a). For example, a comparison of breeding females with all males indicates significantly lower binding in queens ($t(18)=2.412$, $p=0.027$) while a similar comparison of subordinate females with all other males is not significant ($t(20)=1.002$, $p=0.328$). Further, queens had lower levels of binding in this region when compared to all other animals ($t(27)=2.218$, $p=0.037$).

The 2 X 2 Univariate ANOVA revealed a sex-by-status interaction in the nucleus accumbens ($F(1,25)=4.681$, $p=0.040$, partial $\eta^2=0.158$; Figure 2b and d). Post hoc comparisons reveal a trend for a sex difference in breeders ($t(9)=1.873$, $p=0.099$) but not subordinates ($t(16)=-1.222$, $p=0.239$). No main effect of sex ($F(1,25)=0.393$, $p=0.536$, partial $\eta^2=0.015$) or status ($F(1,25)=0.233$, $p=0.634$, partial $\eta^2=0.009$) was found in this region. No significant effects of sex or status or sex-by-status interactions were seen in any other region examined (all $p>0.05$). Estimated means ($\pm$ SEM) of all regions measured are shown in Table 1.

4. Discussion

We explored a role for OTR density in within-species differences in sociosexual phenotype. OTR binding in the medial amygdala varied significantly with sex, with overall binding higher in males than females. We did not detect any significant qualitative or quantitative differences in OTR binding between breeding and subordinate naked mole-rats. However, we report a significant interaction of sex and status within the nucleus accumbens. Breeding males tended to show higher binding in this region when compared to breeding females, while subordinate males and females did not differ.

4.1 OTR Binding Distribution and Density

We confirmed oxytocin binding in the regions reported by Kalamatianos et al. (2010), including the Islands of Calleja, nucleus accumbens, BNST, central and medial amygdala nuclei, and the CA1 region of the hippocampus. The BNST and amygdala contribute to the social behavior and social decision making networks, which regulate a number of social behaviors (Newman, 1999; O'Connell and Hofmann, 2011). Specifically, oxytocinergic action in the medial amygdala has been implicated in social investigation and recognition in mice and rats (Ferguson et al., 2001; Choleris et al., 2007; Arakawa et al., 2010; Lukas et al., 2013). These are particularly important behaviors for a species that lives in colonies numbering in the tens or hundreds and is very sensitive to familiar conspecifics versus intruding mole-rats (O'Riain and Jarvis, 1997). In the central amygdaloid nucleus and BNST of rats, oxytocin attenuates maternal aggression (Lubin et al., 2003; Consiglio et al., 2005). In addition, a positive correlation between levels of

oxytocin receptor binding in these regions and responsiveness to pups has been reported for rat dams (Francis et al., 2000; Champagne et al., 2001). Whether oxytocin action in these regions is related to parental care in naked mole-rats is not yet known. However, naked mole-rats exhibit high levels of alloparental behavior (Lacey and Sherman, 1991); this means that tolerance of colony-mates in the nesting chamber by the queen and pup-responsiveness by breeders and subordinates are important for colony success. Future work will highlight what role, if any, oxytocinergic action at these sites has in this species.

In addition to binding in the CA1 region of the dorsal hippocampus, we report low level binding in the CA1 of the ventral hippocampus (Figure 1). While binding in the dorsal hippocampus may contribute to spatial navigation, as proposed by Kalamatianos et al. (2010), the ventral hippocampus has been implicated in emotional processing and defensiveness in rats (Moser and Moser, 1998; Pentakowski et al., 2006; Fanselow and Dong, 2010). Specifically, OTR binding in the ventral hippocampus of rats is enhanced with stress (Liberzon and Young, 1997) and interruption of oxytocinergic action here may lead to increased social avoidance (van Wimersma Greidanus and Maigret, 1996). Thus, oxytocin functioning in this region may be important for group living where animals are in close proximity.

We also identified low level OTR binding in the VMH. OTR in this region appears to be a common feature of hystricognath rodents measured to date, including guinea pigs and tuco-tucos as well as mole-rats, while its presence may be more variable and species-specific in myomorph rodents (Tribollet et al., 1992; Beery et al., 2008). Oxytocin in the VMH decreases feeding and promotes activity in rats (Noble et al., 2014). While it remains to be tested whether oxytocin in the VMH has these effects in naked mole-rats, they might be beneficial to species

that rely on subterranean group foraging for survival (Brett, 1991b). However, the lack of status or sex differences in this region was surprising as oxytocin action in the VMH has also been linked to sexual activity in female rats (McCarthy et al., 1994) and OTR binding density is increased in the VMH by male and female gonadal steroids (Tribollet et al., 1990).

Absolute levels of OTR binding were low compared to similar assays we have conducted in other species: *M. pennsylvanicus*, *R. norvegicus*, *O. degus*, *S. cyanus*, *C. sociabilis*, *C. haigi*, and 4 additional *Ctenomys* species (Beery and Zucker 2010; Starr-Phillips and Beery 2013; Beery et al. 2008; Beery, Kamal, Sobrero and Hayes, *in review*; and unpublished data). Film exposure time was double that typically needed for these other rodent species (including concurrently run vole tissue), and revealed relatively sparse binding. This may reflect low absolute levels of OTR binding in naked mole-rats, or reduced affinity for the OTR ligand in comparison to other rodents, including relatively closely related histricognaths.

4.2 Sex Difference in OTR Binding in the Medial Amygdala

We found that OTR binding density in the medial amygdala was higher in males than females, particularly when comparing reproductively active members of the colony. Kalamatianos et al., (2010) did not find a similar sex difference, however, that report analyzed binding in subordinate animals only. While there was no significant effect of status, visual examination of the data suggested that the inclusion of the breeding females, with their relatively low OTR binding density, may have driven the sex difference (Figure 2a and c). A male bias in OTR binding is found in a wide number of species. For example, male Wistar rats have higher binding than females in many regions including the posterior BNST, the medial

amygdala, the posterior nucleus accumbens, the hippocampal CA1 region, and the VMH (Dumais et al., 2013). *Peromyscus* mice species also show sex differences in many regions, including the BNST, with males generally showing higher OTR binding across regions (the exception is the CA1 region of the hippocampus, where binding is higher in females; Insel et al., 1991). Species that show few sites with sex differences in the OTR system, such as singing mice (*Scotinomys xerampelinus*) and colonial tuco-tucos (*Ctenomys sociabilis*), also show a male bias. Male *S. xerampelinus* show higher binding in both the medial amygdala and the CA1 in comparison to females (Campbell et al., 2009) while male *C. sociabilis* show higher OTR binding in the medial preoptic area (MPOA) and VMH than females (Beery et al., 2008). Mandarin voles (*Lasiopodomys mandarinus*) show high levels of male parental care and live in a subterranean environment (Smorkatcheva, 2003), similar to naked mole-rats. Interestingly, mandarin voles might closely approximate the region-specific pattern of sex differences in OTR system that we report for naked mole-rats. Male mandarin voles show higher OTR mRNA expression than females in the medial amygdala but not nucleus accumbens (Cao et al., 2014).

The functional significance of the sex difference in the medial amygdala of naked mole-rats has yet to be determined. However, evidence from a study on OT-knockout mice suggests that OT in the medial amygdala can attenuate the hypothalamic-pituitary-adrenal axis response to psychogenic stress (Mantella et al., 2004). While the common pattern for cooperatively breeding primates, birds and carnivores is for basal levels of circulating stress hormones in the dominant animals to be greater than or equal to those in subordinates (Creel, 2001), there is some evidence suggesting that subordinate mole-rats have higher basal levels of glucocorticoids in comparison to queens (Faulkes and Abbott, 1997). More extensive

investigation into presumed sex and status differences in stress reactivity is needed to better understand the relationship between stress and oxytocin in naked mole-rats.

4.3 Effects of Reproductive Status on OTR Binding?

Naked mole-rats exhibit status differences in a variety of behaviors, including sexual behavior, parental care, and aggression (Lacey and Sherman, 1991; Clarke and Faulkes, 2001). In the present study, OTR was found in regions that control expression of these behaviors in other species. However, we only detected one statistical effect of status: a significant sex by status interaction in the nucleus accumbens suggests that status is mediating sex differences in OTR. Specifically, breeding males tended to have more OTR binding than breeding females, with no difference between male and female subordinates. Oxytocin receptors in the nucleus accumbens are necessary for the expression of alloparental care in prairie voles (Olazabal and Young, 2006b), and the density of OTR binding at this site is positively correlated with the duration of hovering over pups (Olazabal and Young, 2006a). Given that we report the unexpected discovery of less OTR binding in breeding queens—the only animals that provide milk for the pups—relative to breeding males, it may be relevant that the previously reported positive relationship between OTR and pup care in voles was found in sexually naïve juvenile females. In rats, caring for direct offspring is associated with increased OTR binding in other regions, such as the lateral septum, medial preoptic area, BNST, and the central nucleus of the amygdala (Francis et al., 2000; Champagne et al., 2001). In the present study, we did not collect

behavioral data on the animals investigated and were thus unable to test for correlations between OTR binding and individual differences in parental or alloparental behavior.

We found no group differences in OTR density in other regions examined. As mentioned, oxytocinergic activity in the central amygdala and the BNST seems to contribute to the inhibition of aggressive behaviors in rats (Lubin, 2003; Consiglio et al., 2005). However, these studies only looked at maternal aggression, which might not translate to aggression for the purpose of maintaining a hierarchy. Within naked mole-rat colonies, breeders – particularly queens – are more aggressive than subordinates (Clarke and Faulkes, 2001). The MPOA, which is sensitive to oxytocin in the display of dominance-related aggression in hamsters (Harmon et al., 2002), showed little OTR binding in the naked mole-rat. Therefore, it remains to be seen whether oxytocin is related to the status difference in intra-colony aggression in naked mole-rats.

Finally, it is possible that oxytocin contributes to status-specific behavioral phenotypes through differences in its local release rather than solely through differences in receptor density. We have previously demonstrated that subordinates have more oxytocin-producing neurons in the paraventricular nucleus of the hypothalamus (Mooney and Holmes, 2013). Further research is needed to establish whether this increase in detectable immunoreactivity for oxytocin reflects altered synthesis and/or release of the peptide.

4.4 Conclusion

The naked mole-rat provides a remarkable model for examining sociosexual phenotypes. Although animals within a colony inhabit the same physical and social

environment, their interactions with and responses to stimuli in that environment may differ considerably depending on the status of the animal. Here, we tested the hypothesis that some of these differences may be associated with OTR distribution and/or density and found that binding does not directly relate to the breeding status of an animal. Rather, social status appears to mediate sex differences in OTR density in the medial amygdala and nucleus accumbens. Future investigations will help elucidate whether oxytocin binding is important for the behaviors that individual mole-rats exhibit within their colony.

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539 Figure captions

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541 Figure 1. Coronal hemi-sections showing regions of oxytocin receptor binding in the naked
542 mole-rat from rostral (A) to caudal (F). For comparison, the corresponding sections depicting
543 acetylcholinesterase are shown to the right of panels depicting oxytocin binding.

544 Figure 2. Group comparison of oxytocin receptor binding. (A) Higher binding in males than in
545 females in the medial nucleus of the amygdala ($*p<0.05$). (B) A sex-by-status interaction in the
546 nucleus accumbens ($p<0.05$), in which breeding males tended to show higher binding than
547 breeding females ($p=0.099$). Representative images of OTR binding in the medial amygdala (C)
548 and nucleus accumbens (D) are shown from an established breeding pair.

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551 Table 1. Oxytocin binding by region

Region	Status	Sex	Mean (nCi/mg 125I-OVTA)	Std. Error
Major Islands of Calleja	Breeder	F	0.548	0.071
		M	0.585	0.078
	Subordinate	F	0.487	0.061
		M	0.432	0.055
Nucleus Accumbens	Breeder	F	0.193	0.018
		M	0.239	0.019
	Subordinate	F	0.221	0.015
		M	0.195	0.014
Bed Nucleus of the Stria Terminalis	Breeder	F	0.102	0.011
		M	0.117	0.013
	Subordinate	F	0.109	0.009
		M	0.090	0.008
Amygdala (Medial Nucleus)	Breeder	F	0.066	0.007
		M	0.084	0.008
	Subordinate	F	0.077	0.005
		M	0.087	0.005
Amygdala (Central Nucleus)	Breeder	F	0.166	0.015
		M	0.162	0.019
	Subordinate	F	0.165	0.013
		M	0.149	0.012
Hippocampus (Ventral)	Breeder	F	0.111	0.109
		M	0.282	0.122
	Subordinate	F	0.301	0.092
		M	0.135	0.092
Hippocampus (Dorsal)	Breeder	F	0.224	0.046
		M	0.323	0.050
	Subordinate	F	0.246	0.038
		M	0.285	0.036
Ventromedial Nucleus of the Hypothalamus	Breeder	F	0.107	0.017
		M	0.108	0.019
	Subordinate	F	0.110	0.014
		M	0.099	0.013

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Figure 1

Figure 2