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Distribution of Vasopressin 1a and Oxytocin Receptor Binding in the Basal Forebrain and Midbrain of Male and Female Mongolian Gerbils

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Abstract—The nonapeptide system modulates a diversity of social behaviors, including aggression, parental care, affiliation, sexual behavior, and pair bonding. Such social behaviors are regulated through oxytocin and vasopressin activation of the oxytocin receptor (OXTR) and vasopressin V1a receptor (AVPR1A) in the brain. Nonapeptide receptor distributions have been mapped for several species, however, studies have demonstrated that there is substantial variation across species. Mongolian gerbils (*Meriones unguiculatus*) are an excellent organism for studying family dynamics, social development, pair bonding, and territorial aggression. Although an increasing number of studies are examining the neural mechanisms of social behavior in Mongolian gerbils, nonapeptide receptor distributions have yet to be characterized for this species. Here we conducted receptor autoradiography to map distributions of OXTR and AVPR1A binding throughout the basal forebrain and midbrain of female and male Mongolian gerbils. Further, we assessed whether gonadal sex influenced binding densities in brain regions important for social behavior and reward, however, we observed no effects of sex on OXTR or AVPR1A binding densities. These findings provide mapping distributions of nonapeptide receptors in male and female Mongolian gerbils, laying a foundation for future studies that seek to manipulate the nonapeptide system to examine nonapeptide-mediated social behavior.**© 2023 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: vasopressin, oxytocin, receptor autoradiography, social behavior, mongolian gerbil.

INTRODUCTION

The nonapeptides oxytocin (OXT) and vasopressin (AVP) modulate a wide array of social behaviors including parental care, pair bonding, affiliation, gregariousness, social communication and aggression (Donaldson and Young, 2008; Goodson and Thompson, 2010; Albers, 2012). OXT and AVP are produced in distinct populations throughout the brain. The largest nonapeptide-producing populations are located in the hypothalamus – those of the paraventricular and supraoptic nuclei – and several smaller nonapeptide neuronal populations are found within and outside of the hypothalamus. OXT and AVP act on the OT-receptor (OXTR) and AVP 1a-receptor (AVPR1A) through direct axonal projections as well as paracrine modulation (i.e., peptide can travel to distal sites via dendritic release) (Ludwig and Leng, 2006; Rood and De Vries, 2011; Kelly and Goodson, 2014). While the distribution of nonapeptide neuronal populations exhibits relatively strong conservation across vertebrates, there is a great deal of variation in receptor distributions and densities.

A recent meta-analysis determined that OXTR and AVPR1A binding distributions are more consistent within than across genera in rodents, suggesting that a within-genus comparison of nonapeptide receptor distributions is more appropriate than comparing across distant species (Freeman et al., 2020). However, even between closely related rodent species, OXTR and AVPR1A densities can vary widely. For example, while prairie voles show dense OXTR binding in the nucleus accumbens (NAcc), their close relative the montane vole shows little binding in this region (Insel and Shapiro, 1992); meanwhile the colonial tuco-tuco show little to no OXTR binding in the NAcc (Anacker and Beery, 2013). Similarly, the colonial tuco-tuco exhibits no detectable OXTR binding in the lateral septum (LS) whereas their relative the Patagonian tuco-tuco shows dense binding in this region (Beery et al., 2008). Together, this stresses the importance of mapping nonapeptide receptor distributions for

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Abbreviations: AH, anterior hypothalamus; AVPR1A, vasopressin V1a receptor; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; LS, lateral septum; MPOA, medial preoptic area; NAcc core, nucleus accumbens core; NAcc shell, nucleus accumbens shell; OXTR, oxytocin receptor; PVN, paraventricular nucleus of the hypothalamus; VTA, ventral tegmental area.

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a study organism of interest because results from one species likely will not generalize to others. Although nonapeptide receptor distributions have been mapped for numerous species of rodents, the distributions of OXTR and AVPR1A throughout the basal forebrain and midbrain have yet to be systematically characterized for Mongolian gerbils (*Meriones unguiculatus*).

Mongolian gerbils are native to desert grasslands of China, Mongolia, and Russia (Liu et al., 2009). They are a socially monogamous, biparental rodent that dwells in burrows in small family groups comprised of a male and female pair and a few litters of offspring (Agren, 1976; Deng et al., 2017). Although gerbils are affiliative in reproductive contexts with mates, offspring, and siblings they exhibit aggression during interactions with novel, same-sex individuals (Roper and Polioudakis, 1977; Gromov, 2008; Liu et al., 2009; Pan et al., 2020; Gonzalez Abreu et al., 2022). This behavioral phenotype makes gerbils particularly amenable for research on parental behavior; indeed, studies have examined steroid-mediated paternal care (Martinez et al., 2019) and the role of tactile stimulation in the onset of parental behavior (Gromov, 2008). Given their socially monogamous mating system, gerbils are also excellent for the study of prosocial behavior between pair bonded mates as well as territorial aggression (Hendrie and Starkey, 1998; Pina-Andrade et al., 2020; Kelly et al., 2022).

Although research examining mechanisms of social behavior in gerbils has focused more on steroids (Martinez et al., 2015; Martinez et al., 2019; Pina-Andrade et al., 2020; Romero-Morales et al., 2021; Kelly et al., 2022), a few studies have examined nonapeptide-mediated behavior in Mongolian gerbils. For example, neonatal AVP manipulation results in increased play behavior with littermates in male, but not female, juveniles, demonstrating a role of early life organizational effects of nonapeptides on behavior in gerbils (Taylor et al., 2017). Additionally, peripheral injections of OXT decreases overt aggression in female gerbils (Razzoli et al., 2003). Lastly, hypothalamic OXT neurons preferentially respond more to social than nonsocial stimuli (Gonzalez Abreu et al., 2022), and hypothalamic OXT, but not AVP, neurons exhibit increased neural responses to interactions with a pair bond partner after an injection of testosterone (Kelly et al., 2022).

Although the distributions of OXT and AVP neuronal populations have been characterized in Mongolian gerbils (Wang et al., 2013), there remains a need to map distributions of nonapeptide receptors in this species. Doing so will enable the use of this species for neuromanipulative studies targeting nonapeptide circuits or sites of action, thereby increasing the viability of using Mongolian gerbils in social neuroscience research. Thus, here we use receptor autoradiography to characterize OXTR and AVPR1A binding throughout the basal forebrain and midbrain of male and female Mongolian gerbils. Identifying sites of binding can provide insight into where nonapeptides act upon functional receptors. Additionally, because a prior study identified sex differences in the consequences of neonatal AVP manipulation (Taylor et al., 2017), we also sought to examine effects of gonadal sex on nonapeptide receptor binding densities, focusing specifically on brain regions involved in social behavior for which distributions of OXTR and AVPR1A receptors have been found throughout for many rodents (Albers, 2015; Caldwell, 2017; Smith et al., 2017).

MATERIALS AND METHODS

Animals

All Mongolian gerbils were obtained as young adults (PND 50-65) from Charles River Laboratories. 10 adult male and 8 adult female gerbils (PND 100-150) were used for the present study. Gerbils were group housed with 2–3 same-sex littermates in standard rat polycarbonate cages (40.64 cm × 20.32 cm × 20.32 cm) and had not been used in any prior experiments. Gerbils can easily live to 4 years of age (and often older) in laboratory settings, and thus our age group in the present study is reflective of a younger portion of adulthood. We captured a relatively large age range to obtain a representative sample of adults that may be used for future experiments. Although in a different species, a previous study in prairie voles found that changes to nonapeptide receptor densities in adulthood are more likely to be related to variation in social experience (i.e., housing or pairbond status) than to age, however, OXTR binding densities in two brain regions exhibited modifications in PND375+ male voles. Whether there is variation in nonapeptide receptor densities within PND 100–150 gerbils is beyond the scope of the present study, yet future studies could investigate the influence of age on binding densities throughout the lifespan of gerbils. All rodent cages were lined with Sani-Chips bedding and included nesting material and rodent igloos. Food and water were provided ad libitum, and animals were kept on a 14-hour light: 10-hour dark cycle. Ambient temperatures were maintained at 24 ± 2 °C. All procedures were approved by the Institutional Animal Care and Use Committee of Emory University (Protocol 201900126).

Tissue preparation

Gerbils were euthanized by CO_2 inhalation, and brains were dissected and flash-frozen on powdered dry ice. The brains were then wrapped in tinfoil and stored in a −80 °C freezer until cryosectioning. Brains were thawed to −20 °C and coronally cryosectioned at 20 μm thickness. Multiple series of each brain were collected at 200 μm intervals on to Superfrost Plus slides (Fisher Scientific Co., Pittsburgh, PA, USA). Brain-mounted slides were again stored in the −80 °C freezer until further processing.

Receptor autoradiography and quantification

Tissue slides were processed as described previously (Grieb et al., 2021). Briefly, tissue was lightly fixed for two minutes with 0.1% paraformaldehyde, followed by two ten-minute washes with Trizma buffer (50 mmol L^{-1} Trizma Base [Sigma, St Louis, MO, USA], pH 7.4). To selectively label OXTR, we used the selective radioligand

[^{125}I]ornithine vasotocin analog ([^{125}I]OVTA, NEX254010UC; Perkin Elmer, Waltham, MA, USA), and to selectively label AVPR1A we used the selective radioligand [^{125}I]linear vasopressin antagonist ([^{125}I]LVA, NEX310050UC;; Perkin Elmer, Waltham, MA, USA). Slides were incubated in tracer buffer (50 pM radioligand, 50 mmol L^{-1} Trizma Base, 21 mmol L^{-1} mgCl, 1% w/v bovine serum albumin, 0.5% w/v/ bacitracin, pH 7.4) for one hour, after which excess binding solution was removed, and the slides were washed via two 5-minute washes and one 35-minute wash in buffer (50 mmol L^{-1} Trizma Base, 21 mmol L^{-1} MgCl, pH 7.4). After washing, slides were allowed to dry completely, and then laid on film (BioMax MR Film; Carestream Health, Rochester, NY, USA) with [14C] standards and given 3 days of exposure for [^{125}I]LVA or 7 days of exposure for [^{125}I]OVTA. Developed films were scanned at high resolution on an Epson DS1630 flatbed scanner and analyzed as described previously (Taylor et al., 2020). The brain nuclei that were quantified as regions of interest were the medial preoptic area (MPOA), anterior hypothalamus (AH), lateral septum (LS), bed nucleus of the stria terminalis (BNST), basolateral amygdala (BLA), central amygdala (CeA), nucleus accumbens shell (NAcc shell), nucleus accumbens core (NAcc core), ventral tegmental area (VTA), and the paraventricular nucleus of the hypothalamus (PVN). These nuclei were identified according to the Mongolian gerbil brain atlas (Radtke-Schuller et al., 2016). Some tissue loss occurred during receptor autoradiography; final sample sizes for each brain region are reported in Tables 1 and 2. Non-specific binding of radioligand to tissue was accounted for by subtracting measurements taken from tissue with low background from measurements taken from regions of interest. Radioligand selectivity was confirmed by comparing binding in matching slides composed of adjacent sections. One slide was incubated in the solutions described above, and the matching slide was incubated in the solutions above + 10 uM competitor ligand (OXT or AVP) (Fig. 1). All measurements are expressed in the units of the [14C] standards, disintegrations per minute (DPM).

Statistical analysis

All statistical analyses were performed in SPSS 28 (IBM Analytics, USA). We were interested in characterizing sex differences in functional protein in brain regions important for the modulation of social behavior, and therefore analyzed ten brain regions of interest. Normality was assessed using the Shapiro-Wilks test. For normally distributed data, we used independent samples *t* tests. For non-normally distributed data, we used Mann Whitney U tests. The false discovery rate (FDR) procedure was used to correct for multiple comparisons, which were calculated separately for OXTR and AVPR1A binding. Corrected and uncorrected p-values are reported in Tables 1 and 2. Figures were made using PRISM 9 (GraphPad, USA).

RESULTS

Mapping of OXTR and AVPR1A binding in the basal forebrain and midbrain

To map expression of OXTR and AVPR1A binding in the brains of male and female Mongolian gerbils, we conducted receptor autoradiography on brain sections extending from the olfactory bulbs to the periaqueductal gray. We observed I-125 OVTA and I-125 LVA binding across several brain regions. We observed especially dense OXTR binding in the BLA, CeA, MeA, BNST, and hippocampus. For AVPR1A binding, the densest binding was observed in the BLA, CeA, LS, and VTA, as well as throughout the cortex. Representative autoradiograms demonstrating receptor distributions are provided for OXTR in Fig. 2 and AVPR1A in Fig. 3.

Sex effects in brain regions associated with social behavior and reward

Previous studies have found that gonadal sex influences nonapeptide receptor densities in brain regions important for social behavior and reward, including the BNST, LS, MPOA, and NAcc (Dumais et al., 2013; Powell et al., 2022). However, here we found no associations with gonadal sex on any OXTR (all $p > 0.475$; Fig. 4 (A); Table 1) or AVPR1A (all $p > 0.665$; Fig. 4(B); Table 2) binding sites in the AH, BLA, BNST, CeA, LS, MPOA, NAcc core, NAcc shell, PVN, or VTA.

Table 1. Statistical details of associations with gonadal sex for OXTR binding densities in brain regions important for social behavior and reward of Mongolian gerbils. T(df) values are provided for independent-samples *t* tests on normally distributed data and U values are provided for Mann Whitney U tests on non-normally distributed data

Brain region	T(df) or U	*p*-value uncorrected	*p*-value FDR corrected	Effect size Cohen's d	Female sample size	Male sample size
AH	T(13) = 0.634	0.537	0.767	0.347	5	10
BLA	U = 23	0.859	0.953	0.286	5	10
BNST	T(13) = 2.043	0.062	0.475	1.119	5	10
CeA	T(13) = −0.946	0.361	0.722	−0.518	5	10
LS	U = 24	0.953	0.953	0.070	5	10
MPOA	T(13) = 1.801	0.095	0.475	0.986	5	10
NAcc core	T(13) = −1.257	0.231	0.578	−0.662	6	9
NAcc shell	U = 15	0.181	0.578	−0.881	6	9
PVN	T(13) = −0.656	0.523	0.767	−0.346	6	9
VTA	T(11) = −0.391	0.703	0.879	−0.223	5	8

Table 2. Statistical details of associations with gonadal sex for AVPR1A binding densities in brain regions important for social behavior and reward of Mongolian gerbils. T(df) values are provided for independent-samples *t* tests on normally distributed data and U values are provided for Mann Whitney U tests on non-normally distributed data

Brain region	T(df) or U	*p*-value uncorrected	*p*-value FDR corrected	Effect size Cohen's d	Female sample size	Male sample size
AH	T(14) = 1.158	0.266	0.665	0.579	8	8
BLA	T(14) = −1.162	0.265	0.665	−0.581	8	8
BNST	T(14) = 0.243	0.812	0.958	0.121	8	8
CeA	T(14) = −0.320	0.754	0.958	−0.160	8	8
LS	T(14) = −0.086	0.933	0.958	−0.043	8	8
MPOA	T(14) = −0.053	0.958	0.958	−0.027	8	8
NAcc core	T(14) = −0.963	0.352	0.704	−0.048	8	8
NAcc shell	T(14) = −0.053	0.602	0.958	−0.267	8	8
PVN	T(13) = −1.255	0.232	0.665	−0.649	8	7
VTA	T(13) = −1.282	0.222	0.665	−0.664	8	7

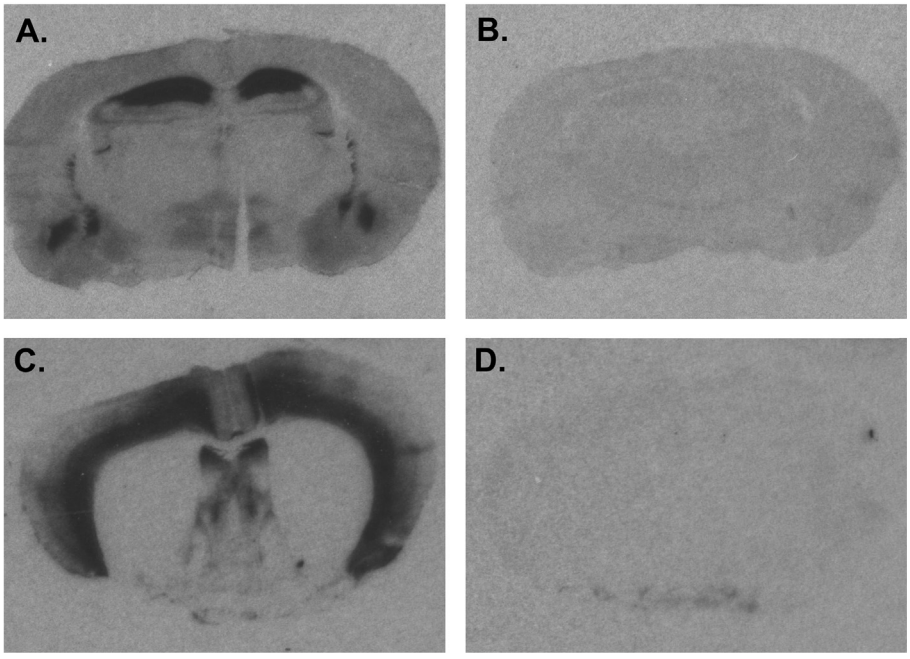

Fig. 1. Radioligand selectivity. **(A)** Total OXTR binding. **(B)** OXTR binding buffer + 10 uM OT. **(C)** Total AVPR1A binding. **(D)** AVPR1A binding buffer + 10 uM AVP. Representative images are from female Mongolian gerbil tissue.

DISCUSSION

Distributions of AVPR1A binding have previously been described in male Mongolian gerbils in relation to transient cerebral ischemia (Vallet et al., 1995), however, to our knowledge, here we provide the first characterization of distributions of OXTR and AVPR1A binding throughout the basal forebrain and midbrain of male and female Mongolian gerbils. We examined the effect of sex on functional protein throughout brain regions important for social behavior and reward yet observed no influence of gonadal sex on nonapeptide receptor densities. Below we briefly discuss similarities and differences in nonapeptide receptor distributions in gerbils compared to other rodents, as well as discuss potential functions of nonapeptide receptors in the BLA – the densest receptor binding site that we identified in gerbils.

OXTR and AVPR1A distributions in gerbils and other rodents

There is considerable variation in nonapeptide receptor densities across species. Such variation likely arises from distinct evolutionary trajectories and contributes to species differences in behavior (Freeman et al., 2020; Froemke and Young, 2021). Although we characterized nonapeptide receptor binding throughout the basal forebrain and midbrain, we focused analyses on brain regions that are important for modulating social behavior and reward (O'Connell and Hofmann, 2011). Numerous studies have characterized the presence and/or absence of nonapeptide receptors in the brain regions examined here, with more extensive characterizations in prairie voles, Wistar rats, tuco tucos, ground squirrels, and spiny mice (Young et al., 2000; Beery et al., 2008; Smith et al., 2017; Freeman et al., 2019; Inoue et al., 2022; Powell et al., 2022).

Arguably the most studied nonapeptide receptor site is that of OXTRs in the NAcc. The socially monogamous prairie vole exhibits very dense OXTR binding in the NAcc, particularly compared to their non-monogamous relative, the montane vole (Insel and Shapiro, 1992). This finding led to the hypothesis that accumbal OXTRs are important for facilitating pair bonding. Indeed, subsequent studies showed that blockade of OXTRs prevents the formation of a partner preference (Liu and Wang, 2003) and overexpression of OXTRs in the NAcc accelerates partner preference formation in female prairie voles (Ross et al., 2009). Although species-typical OXTR binding patterns may relate to mating structure in voles, accumbal OXTRs do not distinguish mating system in monogamous and promiscuous mice (Insel et al., 1991). Further, although Mongolian gerbils

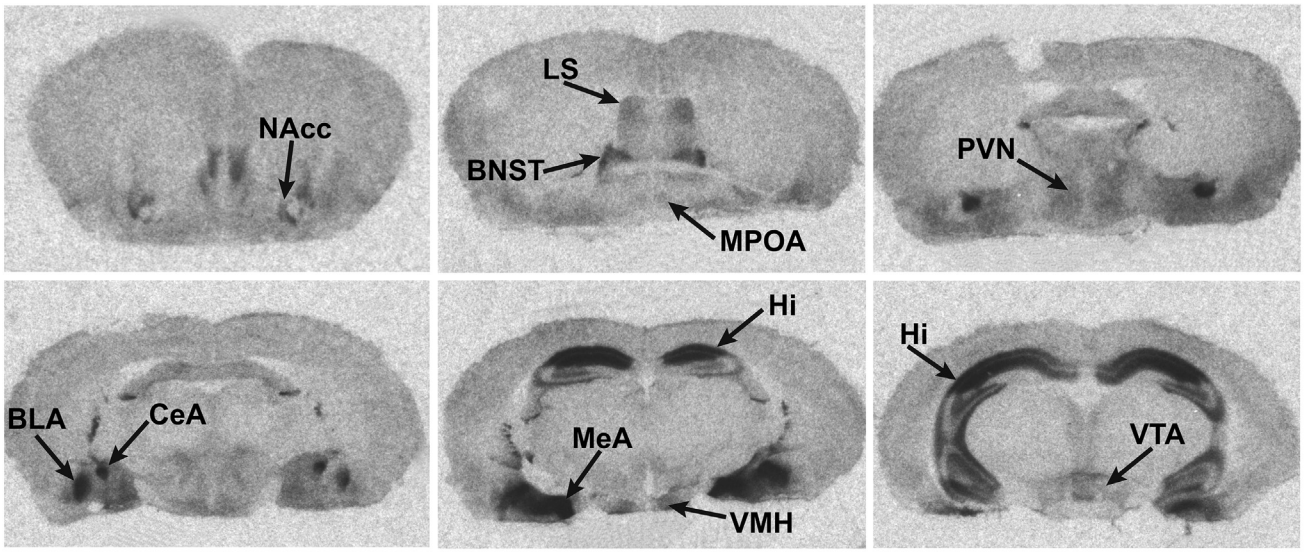

Fig. 2. OXTR binding. Representative autoradiograms of OXTR binding in coronal sections from female Mongolian gerbil tissue.

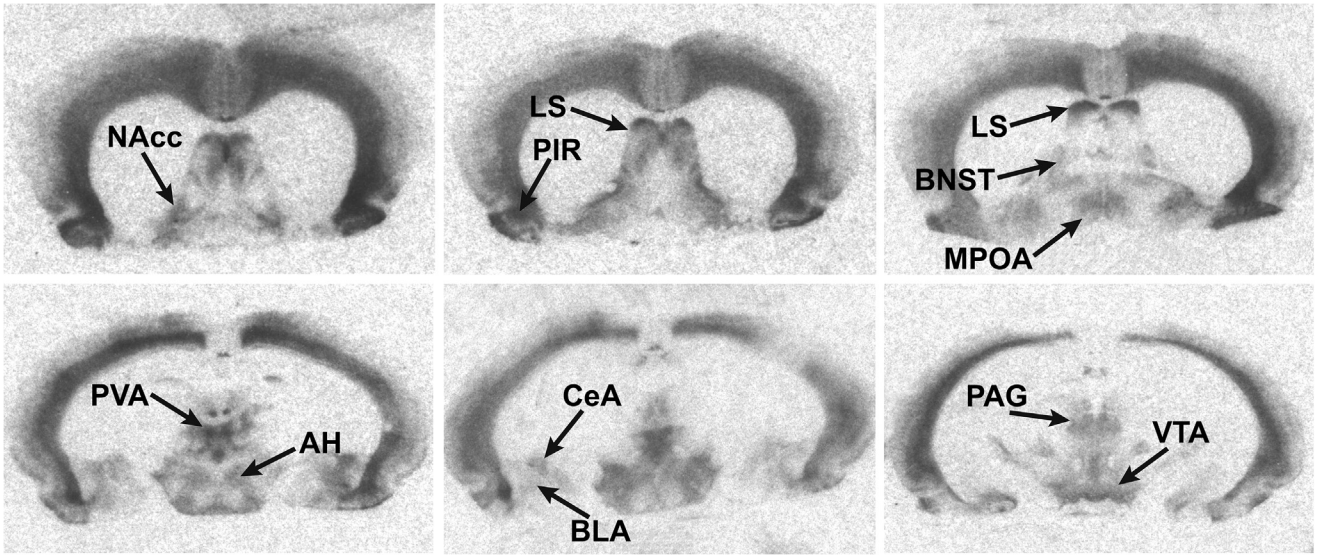

Fig. 3. AVPR1A binding. Representative autoradiograms of AVPR1A binding in coronal sections from female Mongolian gerbil tissue.

have a social system similar to prairie voles and form pair bonds and exhibit biparental care, here we found that both male and female gerbils have relatively light nonapeptide receptor binding in the NAcc. However, studies in California mice have demonstrated that NAcc OXTRs promote social approach in non-reproductive social contexts, demonstrating that OXT signaling in the NAcc is important for aspects of sociality other than pair bonding (Williams et al., 2020). Consistent with this, accumbal OXTRs are required for social reward conditioning in C56BL6J mice in non-reproductive contexts (Dolen et al., 2013). Importantly, though, NAcc OXTR is not necessarily required for the expression of sociality given that species like the colonial tuco-tuco, *C. sociabilis*, have no detectable OXTRs in this brain region (Beery et al., 2008).

The LS is becoming an increasingly popular brain region for studying the neural mechanisms of social behavior (Menon et al., 2022). Septal nonapeptide receptors are present in most rodents and are involved in a variety of behaviors. LS nonapeptide receptors promote alloparental care in prairie voles (Olazábal and Young, 2006), modulate aggression in rats (Oliveira et al., 2021), regulate reward and addiction in rats (Garate-Perez et al., 2021), and modulate social memory in C57BL6 mice (Horiai et al., 2020). Additionally, nonapeptide receptors in the LS may contribute toward distinguishing mating system in Peromyscus mice. The socially monogamous *P. californicus* exhibits dense OXTR and AVPR1A binding in the LS, whereas their promiscuous counterpart, *P. maniculatus*, exhibits rela-

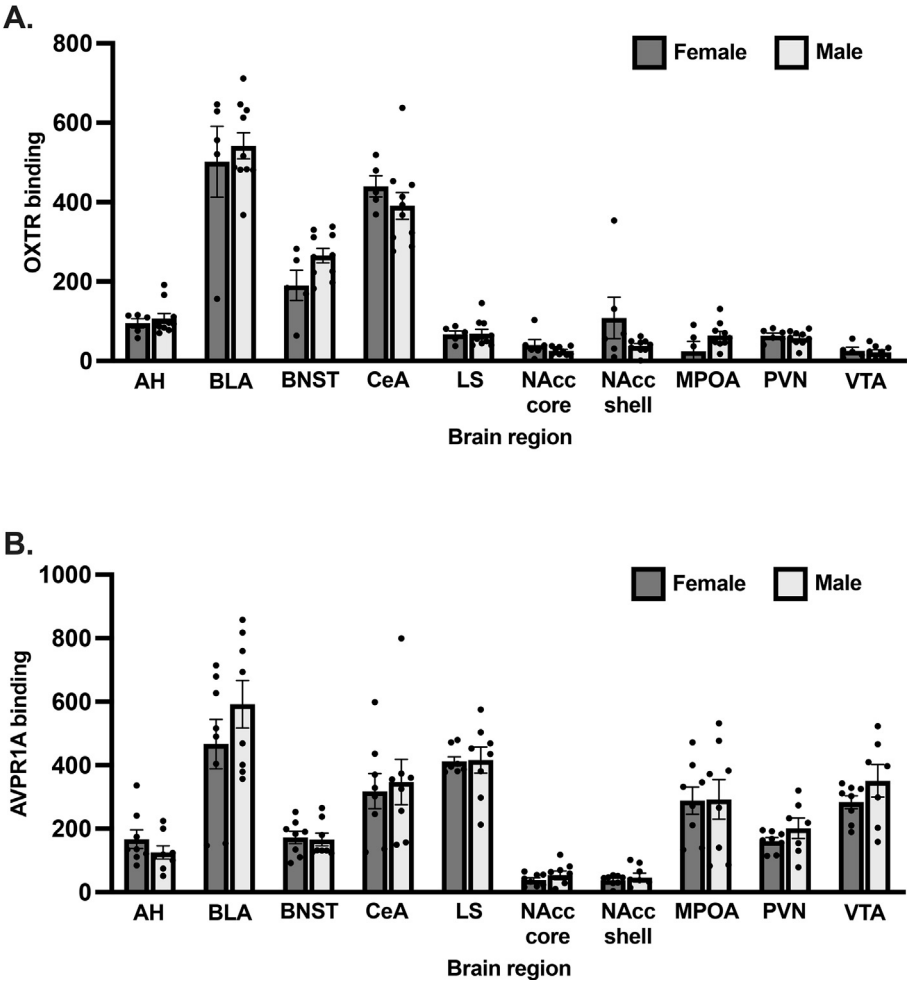

Fig. 4. Associations with gonadal sex on OXR and AVPR1A binding. Mean grayscale values (±SEM) for **(A)** OXTR and **(B)** AVPR1A binding in brain regions important for social behavior and reward.

tively light nonapeptide receptor binding in the LS, especially for AVPR1A (Insel et al., 1991). In the gerbils, we observed relatively light OXTR binding in the LS, reflecting binding densities of more social, large group-living species, such as colonial tuco-tucos, spiny mice, Norway rats, short-tailed singing mice, and guinea pigs (Tribollet et al., 1992a; 1992b; Beery et al., 2008; Campbell et al., 2009; Powell et al., 2022).

We observed moderate AVPR1A and light OXTR binding in the MPOA and moderate AVPR1A and OXTR binding in the AH of gerbils. The MPOA-AH is often referred to as the MPOA-AH continuum (Van De Poll and Van Dis, 1979; Harmon et al., 2002). AVP receptors in the MPOA-AH promote flank marking in Syrian hamsters (Albers et al., 1986), but are absent in the continuum in other species, such as Wister rats (Smith et al., 2017). However, OXTRs are present in the MPOA of most rodents, and are typically expressed in a sexually dimorphic manner (Beery et al., 2008; Dumais et al., 2013; Smith et al., 2017; Prounis et al., 2018; Freeman et al., 2019; Freeman et al., 2020; Inoue et al., 2022; Powell et al., 2022). MPOA OXTRs are more prevalent in C57BL6J uniparental mice and promote maternal behavior (Sharma et al., 2019), while in the biparental mandarin vole they facilitate paternal care, suggesting that there may be evolutionarily conserved functions of MPOA OXTR in modulating parental behavior in a species-specific manner (Yuan et al., 2019). Given that gerbils also exhibit biparental care (Agren, 1976, 1984; Deng et al., 2017), it is feasible that OXTRs in the MPOA may promote parental care in both males and females. Few studies report the presence of OXTRs specifically in the AH in rodents, however two recent studies identified *Oxtr* mRNA in the AH of prairie voles (Inoue et al., 2022) and OXTR binding and *Oxtr* mRNA in the AH of spiny mice (Powell et al., 2022). Infusion of OXT into the MPOA-AH continuum decreases aggression in female Syrian hamsters, suggesting that OXT binding in the AH could have anti-aggressive properties (Harmon et al., 2002); however, due to the promiscuous binding of nonapeptides (Liu et al., 2001; Taylor et al., 2020), whether these effects are due to OXT binding to OXTRs or AVPR1As remains unclear. Gerbils readily exhibit prosocial behavior with family members and pair bond partners, yet despite being territorial, in some contexts (i.e., not a resident-intruder paradigm) gerbils display moderate levels of prosociality with novel, same-sex conspecifics (Gonzalez Abreu et al., 2022). Future studies could explore the role of MPOA-AH nonapeptide receptors in gerbils and whether they play dichotomous roles in modulating behavior (i.e., prosocial vs. antisocial behavior).

Recent studies have begun exploring the role of nonapeptides in the modulation of social reward, with a greater focus on OXT. In hamsters, activation of OXTRs but not AVPR1As in the VTA is essential for the rewarding properties of social interactions (Song et al., 2016). In mice, OXT release in the VTA increases dopaminergic neural activity, and inhibition of PVN OXT neurons that project to the VTA reduces dopamine signaling and social approach (Hung et al., 2017; Xiao et al., 2017). In gerbils, we previously showed that PVN OXT neurons send axonal projections to the VTA, demonstrating that OXT has the potential to mediate reward circuitry in gerbils as it does in hamsters and mice (Gonzalez Abreu et al., 2022). Interestingly, in the present study we found that gerbils exhibit relatively light OXTR, but dense AVPR1A, binding in the VTA. To our knowledge, extremely few studies have examined the role of VTA

AVPR1As but given that there is substantial variation in how nonapeptide receptors modulate behavior across species, it is feasible that AVPR1As may play a greater role than OXTRs in mediating social reward in Mongolian gerbils. Consistent with this, a recent meta-analysis determined that AVPR1a and OXTR binding distributions are more consistent within than across genera in rodents, suggesting that comparisons of nonapeptide receptor distributions across distant species may not be appropriate (Freeman et al., 2020). This too may apply to nonapeptide receptor functions, and caution should be taken when attempting to generalize function across species.

Amygdalar nonapeptide receptors and social discrimination

The densest site for OXTR and AVPR1A binding in the gerbils was the BLA, yet surprisingly few studies have examined the functions of BLA nonapeptide receptors. Indeed, Wistar rats do not even express detectable AVPR1As in the BLA (Smith et al., 2017). However, converging lines of evidence support a potential role for amygdalar and extended amygdalar OXTRs in mediating social discrimination. Activation of BLA OXTRs via an OXT receptor agonist (TGOT) enhances learning and discrimination between visual and auditory cues in rats (Fam et al., 2018). However, this effect may not be specific to nonsocial cues given that lesioning the BLA in male Mongolian gerbils reduces aggressive behavior and increases social investigation and allogrooming of same-sex conspecifics in a social interaction task, suggesting that endogenous activation of BLA OXTRs in gerbils may increase discrimination, thereby decreasing social investigation and approach (Woolley et al., 2006). Similarly, a study in male rats found that inactivation of BLA neurons that project to the BNST reduces freezing behavior and increases social behavior during social interactions with a novel, same-sex conspecific, suggesting that the BLA-BNST circuit may promote social avoidance. (Vantrease et al., 2022). Lastly, OXTRs in the BNST promote stress-induced social avoidance in California mice (Duque-Wilckens et al., 2018). Together these findings suggest that activation of BLA OXTRs may enhance learning and increase social discrimination abilities and subsequently send signals to the BNST, where activation of OXTRs may facilitate social avoidance, particularly in species that are territorial, such as California mice and Mongolian gerbils. Future studies could explore not only the role of distinct nonapeptide receptor sites in behavior, but also how activation or inhibition of nonapeptide receptors in one region influence activity at up- or down-stream sites.

A lack of sex effects on OXTR and AVPR1A binding densities

Sex differences in nonapeptide receptor densities have been reported in numerous species including Wistar rats, Syrian hamsters, Siberian hamsters, prairie voles, spiny mice, and California mice (Dubois-Dauphin et al., 1991; Insel et al., 1991; Delville and Ferris, 1995; Dumais et al., 2013; Smith et al., 2017; Prounis et al., 2018; Powell et al., 2022). However, here we observed no effect of sex on OXTR or AVPR1A binding densities across the brain regions we selected for analysis. A similar lack of sex differences in binding densities was observed in juvenile Richardson's ground squirrels (Freeman et al., 2014). It is possible that our sample sizes were too small to detect effects of sex, however, similar sample sizes were used for a nonapeptide receptor characterization study in spiny mice, which found sex differences in OXTR, but not AVPR1A, binding densities (Powell et al., 2022). Although we did not observe an effect of gonadal sex on nonapeptide receptor distributions or binding densities, it is possible that sex effects may be observed in brain regions not examined here and/or that life history stage and sexual/pair bonding experience may differentially influence nonapeptide receptor densities across the sexes.

Here we characterized the distributions of OXTR and AVPR1A binding throughout the basal forebrain and midbrain of female and male Mongolian gerbils. We did not identify any effects of sex on binding densities in selected brain regions that are important for the modulation of social behavior and reward. Importantly, a lack of sex differences in the brain does not necessarily indicate a lack of sex differences in behavior (Vries, 2004), and future studies should explore the potential for effects of sex on nonapeptide-mediated behavior in gerbils. An increasing number of studies are using gerbils for studying the neural mechanisms of social behavior, such as social learning (Paraouty et al., 2020), nonapeptide-steroid interactions in the mediation of prosocial behavior (Kelly et al., 2022), and social development (Taylor et al., 2017). Further, gerbils are commonly used in the field of auditory neuroscience (Heeringa et al., 2020; Jeffers et al., 2021; Anbuhl et al., 2022). Given that the social presence of a conspecific can enhance learning of auditory discrimination tasks in gerbils (Paraouty et al., 2020) and that there is some evidence that AVP plays a role in auditory perception in other species (Campbell et al., 2009; Charlton et al., 2019), it is likely that nonapeptides play a role in auditory processing in gerbils. Thus, the results presented here lay a basic foundation for future studies that seek to manipulate the nonapeptide system in gerbils in fields such as social and auditory neuroscience as well as those interested in basic physiology.

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