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Distribution of nonapeptide receptors in the forebrain and midbrain of the spiny mouse (*Acomys cahirinus*)

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Abstract

Distribution of nonapeptide receptors in the forebrain and midbrain
of the spiny mouse (*Acomys cahirinus*)
By Jeanne M. Powell

The nonapeptides oxytocin (OT) and vasopressin (AVP) play key roles in modulating social behaviors across taxa via the activation of the OT receptor (OTR) and AVP V1a receptor (V1aR). Differences in the distributions and densities of these receptors have been linked to differences in social phenotype both within and across species. However, much of what we know about these systems have been learned using rodent models that do not generally display prosocial behaviors outside of reproductive contexts. The gregarious and communally breeding spiny mouse (*Acomys cahirinus*) presents a unique opportunity to explore nonapeptide-mediated social behavior because they exhibit high degrees of prosociality in both reproductive *and* non-reproductive contexts. Here, we provide a basic characterization of neuronal OTR and V1aR binding in spiny mice using receptor binding autoradiography. Across sexes, we observed the highest density of OTR binding in the ventral pallidum (VP), as well as a moderate amount of OTR binding within the subiculum, bed nucleus of the stria terminalis (BNST), and amygdalar nuclei. Robust V1aR binding was observed throughout the brain, with moderate to high binding observed in many olfactory, striatal, amygdalar, thalamic, hypothalamic and midbrain nuclei, as well as the lateral septum, BNST, and VP. This characterization lays a basic foundation for future studies that seek to examine the relationship between nonapeptide receptor density and phenotypic differences in behavior and identifies target regions for causal manipulation to determine direct contributions of nonapeptide circuitry to social behavior in spiny mice.

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Introduction

The nonapeptides, oxytocin (OT) and vasopressin (AVP), and their nonmammalian homologs, have been found to modulate social behavior across vertebrates (Caldwell, 2017). These peptides are primarily produced by magnocellular and parvocellular nuclei within the hypothalamus and are widely distributed throughout the brain via parvocellular axonal projections, as well as via magnocellular paracrine signaling (Baribeau & Anagnostou, 2015; Ludwig & Leng, 2006). At target brain regions, they bind to their receptors, including the OT receptor (OTR) and AVP receptor 1a (V1aR). It is through the activation of these receptors that OT and AVP have been shown to modulate a diverse range of social behaviors, including pair bonding (Young & Wang, 2004), parental care (Kelly & Adkins-Regan, 2020), flocking (Kelly et al., 2011), and aggression (Lischinsky & Lin, 2020).

OTR and V1aR regional distributions and densities therein have been characterized across several rodent species (Beery et al., 2008; Freeman et al., 2019; Göldner, 2016; Insel et al., 1994, 1994; Kalamatianos et al., 2010). These studies have revealed both commonalities and variability in the distribution of these receptors across the order *Rodentia*. For example, OTR, V1aR, or both are often found in brain regions known to be important for the regulation of social behaviors, including the nucleus accumbens (NAcc), lateral septum (LS), ventral pallidum (VP), bed nucleus of the stria terminalis (BNST), amygdala, and olfactory nuclei (Caldwell, 2017; Freeman et al., 2020). However, there is still ample variability observed in the density of receptors within those regions across species. Even closely related rodent species have highly variable distributions of V1aR and OTR (Freeman et al., 2019).

Some of the variability observed in V1aR and OTR distributions has functional significance. Comparative studies have revealed that these differences in receptor binding

locations and densities can explain differences in social phenotypes observed across species (Caldwell, 2017; Young & Wang, 2004). For example, within the genus *Microtus*, differences in receptor densities have been causally linked to differences in monogamy-related behaviors, such as pair bonding or a lack thereof, across monogamous and non-monogamous voles (Barrett et al., 2013; Lim, Wang, et al., 2004; Nair & Young, 2006; Wallum & Young, 2018). While studies such as these have advanced our understanding of how the nonapeptide system modulates social behavior, the majority of these studies examine social behavior in reproductive contexts, specifically pair bonding and parental care. In order to determine how nonapeptides may differentially regulate reproductive versus non-reproductive social behaviors, it is imperative to use a species that naturally exhibits such behaviors.

Spiny mice are cooperative breeders that live in large colonies of related and unrelated individuals nested within rocky outcroppings in Africa, the Middle East, and southern Asia (Deacon, 2009; Frynta et al., 2011; Nowak, n.d.). They are prosocial in both reproductive and nonreproductive contexts. In reproductive contexts, they provide alloparental care to unrelated neonates at an equal rate to that which they provide to their own offspring (Porter et al., 1980; Tučková et al., 2016). In non-reproductive contexts, they exhibit low rates of aggression towards novel and familiar individuals alike (Fricker et al., *Under Review at Journal of Mammalogy*). Further, they are socially bold, rapidly approaching novel and familiar animals, and exhibit a preference to affiliate with larger groups (i.e., referred to as gregariousness).

Not only is their social phenotype of key interest given that relatively few non-eusocial rodents display non-reproductive, prosocial behavior, but spiny mice are also highly amenable to lab use. They are already used in labs to study diabetes (Gonet et al., 1966; Shafrir, 2000),

complex tissue regeneration (Gawriluk et al., 2016; Seifert et al., 2012), and menstruation (Bellofiore et al., 2017), and they readily reproduce in captivity.

For these reasons, the spiny mouse (*Acomys cahirinus*) holds immense potential to contribute towards our understanding of how the mammalian brain drives prosocial behavior in *both* reproductive and nonreproductive contexts.

In order for spiny mice to be a valuable organism for social neuroscience studies, we need to first lay a foundation characterizing social neural circuitry given how variable it can be across species. We previously mapped the distribution of nonapeptide-producing neuronal populations in spiny mice (Kelly and Seifert, *In Revision at Neuroscience*), however, there remains a need to characterize distributions of nonapeptide receptors. Here, we performed receptor-binding autoradiography for OTR and V1aR to qualitatively map the distribution of nonapeptide receptors throughout the forebrain and midbrain of male and female spiny mice.

Methods

Animals

All spiny mice in this study were derived from breeders obtained from the Seifert Lab at the University of Kentucky and maintained at Emory University. Animals were housed in groups of 2-5 same-sex siblings in either a GR1800 Double Decker for Rats (Tecniplast, West Chester, PA, USA) or a standard polycarbonate rat cage. They were provided *ad libitum* water and Purina Prolab RMH 1000 (Lab Diet, St. Louis, MO, USA). The colony room was maintained at an ambient temperature of 24°C +/- 1°C and kept on a 14 h:10 h light/dark cycle (lights on at 7:00 AM). All procedures were approved by the Institutional Animal Care and Use Committee of Emory University (Protocol #201900126).

Tissue Preparation

All subjects were adults (female n = 8; male n = 8) aged between PND 60 and 270. Subjects were euthanized by CO_2 inhalation, and their brains were dissected and immediately frozen on powdered dry ice. The brains were then wrapped in tinfoil and stored in a -80°C freezer.

Brains were thawed to -20°C and coronally cryosectioned at 20 µm thickness onto two sets of Superfrost Plus slides (Fisher Scientific Co., Pittsburgh, PA, USA) at 200 µm intervals. Brain-mounted slides were again stored in the -80°C freezer until further processing.

Receptor autoradiography

Labeling

Receptor binding autoradiography for OTR and V1aR was performed on two sets of tissue from each brain. Notably, spiny mouse brain tissue is extremely fragile. During the OTR

assay, much of the tissue was damaged during washes and therefore the V1aR assay was performed using the bare minimum amount of tissue agitation needed for each wash.

Briefly, slides were thawed and then post-fixed in 0.1% paraformaldehyde for 2 minutes. They were washed in two changes of 50 mM Tris Buffer for 10 minutes each and transferred to binding chambers where they were immersed in I-125 Tracer Buffer for 1 hour. Slides labeled for OTR and V1AR were exposed to I-125 OVTA and I-125 LVA, respectively. Slides were then washed at 4°C in four changes of Tris and Magnesium Chloride Buffer for 5 minutes each. They were then dipped in sterile ultra-pure deionized water and dried completely. Slides were apposed to film in the dark along with a standard curve. Unfortunately, the standard curve used was expired and could not be used further in this study. Slides labeled for V1aR binding were exposed for 4 days. Slides labeled for OTR binding were exposed for 9 days.

Imaging

Films were digitized using an Epson Perfection V700 photo scanner (Epson, Suwa, Nagano, Japan) with the home mode document type text/line art setting at 2500 dpi. Each image was saved as an 8-bit grayscale image, with pixel gray values ranging from 0 to 255, where a value of 0 represents a black pixel and a value of 255 represents a white pixel. Therefore, gray values are negatively correlated with receptor binding densities.

Qualitative mapping

The receptor binding density for each region was determined in a semi-quantitative manner that allowed for a qualitative assessment of density variability across brain regions and subjects. Radioactivity transfers onto film in a non-linear fashion (Zilles et al., 2002). While a darker/lower gray value qualitatively represents a greater density of ligand binding in a region, the magnitude of difference in radioactivity represented by two gray values is difficult to

determine in the absence of a radioactive standard curve. Because we lacked a standard curve and best practice could not be followed, we opted to qualitatively measure the density of binding observed in each region and subject, as opposed to a more traditional quantitative approach. Notably, this approach was employed to ensure consistency in what was and was not considered receptor binding across regions and individuals and is only a place holder until we are able to perform this assay optimally.

Images were pseudo-colored using a customized lookup table in FIJI that allowed for consistent assessment of relative binding densities across regions and brains (Figure 1a; Schindelin et al., 2012). FIJI's measurement tool was used to determine the gray values associated with background noise and the densest observed binding. Pixels with an intensity greater than 98 were considered background, and the darkest binding was found to have a gray value of 18. This 80-point difference was used to make four gray value bins of functionally equal size that represented high (0-50; red), medium (51-66; yellow), low (67-82; green), and barely detectable (83-98; blue) receptor binding (Figure 1b). Background pixels with gray values ranging from 99 to 255 were pseudo-colored as a spectrum from black to white to allow for visualization of the surrounding tissue morphology. Then, a gaussian blur with a radius of 5 was applied both to minimize noise in the images and also to have a region be more represented by its average gray value. Again, while this qualitative method allowed for a more objective mapping, the bin values themselves are fairly arbitrary and more work is required to determine whether areas that express "barely detectable" receptor binding are indeed showing extremely low levels of binding or nonspecific binding.

Brain regions were identified using the Allen Mouse Brain Atlas (Lein et al., 2007), as well as previous mapping studies using receptor ligand autoradiography in rodents (Chappell et

al., 2016; Freeman et al., 2019; Lim, Murphy, et al., 2004; Smith et al., 2017). Brain region binding densities were marked as high, medium, low, or barely detectable based on the highest amount of expression present in the region (e.g. a region that shows yellow [medium] and green [low] will be marked as medium) and variability was determined by comparing this qualitative value across all subjects. Only regions that showed at least low binding densities in at least one brain were considered here.

Results

I-125 OVTA and I-125 LVA bound to several regions in the forebrain and midbrain of the spiny mouse, described in more detail below.

OTR binding

We observed I-125 OVTA binding across several brain regions, namely olfactory areas, cortical areas, hippocampal areas, striatal areas, the pallidum, the amygdala, the hypothalamus, the thalamus, and the midbrain (Table 1).

Binding was observed in both sexes in the accessory olfactory bulbs (AOB), anterior olfactory nucleus (AON), medial prefrontal cortex (mPFC), piriform area (PIR), pyramidal layer of the field CA1 (CA1sp), subiculum (SUB), nucleus accumbens (NAcc), bed nucleus of the stria terminalis (BNST), globus pallidus (GP), ventral pallidum (VP), basolateral amygdala (BLA), central amygdala (CeA), medial amygdala (MeA), posterior amygdalar nucleus (PA), posterior hypothalamic area (PHA), ventralmedial hypothalamus (VMH), paraventricular nucleus of the thalamus (PVT), reticular nucleus of the thalamus (RT), and periaqueductal gray (PAG). Though not further described, a low density of binding was observed in the molecular layers of the cerebellum and a medium density of binding was observed in the nucleus raphe pallidus in the hindbrain. Notably, binding in the lateral septum (LS) or caudate putamen (CP) were only observed in some individuals and only at “barely detectable” gray values. The only region to be observed in one sex was the claustrum (Cl), which was only observed in females.

All regions with detectable binding in both sexes displayed variable receptor binding densities across individuals, except for the PVT in males which only showed a low density of binding across all male subjects. Though both sexes displayed binding in the PA, males

displayed higher and less variable binding densities than females, with all but one male showing a high density of binding in that region.

High density binding was observed in at least one individual within the BNST, VP, and PA in both sexes. Additionally, at least one female displayed high density binding in the SUB, GP, CeA, and MeA.

The only regions to display high density binding generally across individuals, as defined by the modal density level, were the VP in both sexes and PA in males.

V1aR binding

We observed I-125 LVA robust binding across several brain regions, namely olfactory areas, cortical areas, hippocampal areas, striatal areas, the pallidum, the amygdala, the hypothalamus, the thalamus, and the midbrain (Table 2). Of note, a low density of binding or higher was observed throughout the hypothalamus and midbrain, though only a few nuclei within were assessed for variability across subjects.

Binding was observed in both sexes in the agranular insular area (AI), AOB, AON, main olfactory bulb, glomerular layer (MOBgl), olfactory tubercle (OT), mPFC, PIR, induseum griseum (IG), LS, NAcc, BNST, VP, BLA, CeA, MeA, PA, lateral hypothalamic area (LHA), medial mammillary nucleus (MM), medial preoptic area (MPOA), PHA, periventricular hypothalamic nucleus, posterior part (PVp), VMH, zona incerta (ZI), lateral dorsal nucleus of thalamus (LD), ventral part of the lateral geniculate complex (LGv), medial geniculate complex (MG), paraventricular nucleus of the thalamus (PVT), anterior pretectal nucleus (APN), PAG, superior colliculus (SC), substantia nigra, reticular part (SNr), and ventral tegmental area (VTA). Though not further described, medium to high densities of binding were observed in the cochlear

nuclei and medulla, motor region (ME-mot) within the hindbrain. There were no regions observed that only displayed binding in one sex.

Most regions with detectable binding in both sexes displayed variable receptor binding densities across individuals, except for the LS (high density in both sexes), AOB (high density in males), NAcc (high density in males), and LD (medium density in females).

High density binding was observed in at least one individual within all measured regions except for the LD and SNr females and the LGv in both sexes. Further, numerous regions in the, namely in the olfactory, striatal, and amygdalar areas had high modal densities across both sexes. binding, +++: high density receptor binding, *: low levels of binding or higher were observed throughout the entire region and only a subset of nuclei are described. The modal density represents the density most often observed within a sex. Two symbols indicate a bimodal distribution. The density range represents the full range of densities seen within a sex.

Discussion

The present study mapped OTR and V1aR binding densities in the forebrain and midbrain of the spiny mouse and made qualitative comparisons of receptor binding densities across regions and individuals. We observed receptor binding in regions known to play important roles in modulating social behavior across species, such as OTR binding in the LS, NAcc, BNST, VP, MeA, BLA, and VMH, as well as V1aR binding in the LS, NAcc, BNST, VP, MeA, BLA, VMH, PAG, and VTA.

We observed variability in receptor binding densities across most brain regions, which is consistent with the level of variability seen in other genetically diverse rodent species, such as the prairie vole (Phelps & Young, 2003). This naturally occurring variability will allow for the exploration of how naturally occurring variation in nonapeptide receptor distributions link to differences observed in social phenotypes across individuals within this species.

Previous studies have observed higher OTR and V1aR binding densities in males compared to females (Dumais & Veenema, 2016). While we did observe denser OTR binding in the PA in males compared females, the qualitative metric used to compare binding densities across individuals did not allow for statistical comparison across the sexes. Therefore, it is possible that this difference is not significant and also that there may be sex differences that we have not yet been able to detect.

OTR binding in the spiny mouse

We observed OTR binding across the forebrain and midbrain in regions consistent with that of other rodents (see Table 3). Notably, we observed relatively high-density OTR binding in the VP, a moderate level of binding in the NAcc, and an apparent absence of OTR binding in the LS, and will therefore focus our discussion on these brain regions.

To our knowledge, the spiny mouse is only one of three rodents reported to have a high density of OTR binding in the VP, and only one of four to have any observed OTR binding in this region (see Table 3). The Norway rat (*Rattus norvegicus*) and the colonial ice rat (*Otomys sloggetti robertsi*) both have high density OTR binding in the VP and are notably both group-living species, though the Norway rat lives in smaller groups whereas the ice rat lives in larger groups (Beery et al., 2008; Chappell et al., 2016; Göldner, 2016; Modlinska & Pisula, 2020; Willan, 1990). Of particular interest is the colonial ice rat, a group-living and communally-breeding rodent in the mountains of South Africa (Göldner, 2016; Willan, 1990). Like the spiny mouse, the colonial ice rat does not discriminate between novel and familiar conspecifics, which may facilitate group living. Interestingly, the solitary vlei rat (*Otomys auratus*), a close relative to the colonial ice rat, has no observed OTR binding in this region, suggesting that species-typical group size may influence OTR expression in the VP (Göldner, 2016). Unfortunately, no studies have directly or indirectly assessed functions of VP OTRs. However, future study of OTRs in the VP of spiny mice may provide insight into the mechanisms that drive group living in mammals.

We observed a moderate amount of binding of OTR in the NAcc of both male and female spiny mice. The role of OTR in the NAcc has been well studied in relation to social attachment. For example, Olazábal and Young (2006a) found in prairie voles (*Mircrotus ochrogaster*) that higher OTR densities were correlated with higher expressions of spontaneous maternal care by virgin females and that blocking OTR in the NAcc blocked maternal behavior. Although spiny mice are non-monogamous, as communal breeders, both virgin and sexually-experienced males and females indiscriminately care for young, perhaps suggesting that NAcc OTR may play a primary role in caregiving (i.e., parental and alloparental) across species.

OTR in the NAcc has also been studied in relation to social stress and social approach, whereby activation of OTR in the NAcc facilitates social approach and reduction of OTR following social stressors inhibits social approach (Williams et al., 2020). We have found that spiny mice readily approach both novel and familiar conspecifics at rapid rates (Fricker et al., *Under Review*). Modulation of OTR in the NAcc may provide insights into the mechanism that drive social boldness in spiny mice.

NAcc OTR expression also differentiates social phenotype in some species. For example, group-living rodents, such as the colonial ice rat and naked mole rat (*Heterocephalus glaber*), have higher levels of OTR binding in the NAcc compared to their solitary counterparts, the vlei rat and cape mole rat (*Georychus capensis*), respectively (Table 3; Beery et al., 2008; Freeman et al., 2020; Kalamatianos et al., 2010). NAcc OTR expression also distinguishes mating system in voles, such that the socially monogamous prairie vole has higher densities of OTR in the NAcc compared to the non-monogamous meadow vole (*Microtus pennsylvanicus*; Ross et al., 2009). Therefore, OTR expression in this region may play a general role in social attachment rather than specifically promoting grouping behavior or monogamy. Given that spiny mice exhibit a diversity of prosocial behaviors in reproductive and non-reproductive contexts, future studies can seek to determine potential multidimensional roles of NAcc OTR in behavior.

Perhaps the most striking finding is the absence of OTR binding in the LS. OTR in the LS has been studied in rodents in relation to maternal and allomaternal behaviors, as well as social dominance (Curley et al., 2012; Lee et al., 2019; Olazábal & Young, 2006a). In mice, OTR binding densities in the LS positively correlate with the frequency of nursing bouts (Curley et al., 2012). However, in vole species, OTR binding densities in the LS negatively correlate with allomaternal behavior (Olazábal & Young, 2006b). As discussed above, spiny mice show robust

maternal and allomaternal care to young, and therefore future studies of OTR in this region may provide insights into the neural mechanisms that underly maternal and allomaternal behavior.

There is conflicting evidence across species as to whether this difference in OTR binding in the LS amounts to differences in social phenotypes. The social tuco-tuco has less OTR binding in the LS than its solitary relative, Haig's tuco-tuco (Beery et al., 2008). Similarly, the monogamous and family-group living prairie vole has less OTR binding in the LS as compared to the promiscuous and solitary montane vole (*Microtus montanus*; Freeman et al., 2020) These data suggest that LS OTR may negatively correlate with prosocial behaviors. However, this finding has not been replicated in mole rats, where neither the eusocial naked mole rat nor the solitary cape mole rat have observed OTR binding in the LS (Kalamatianos et al., 2010). This finding also has not been replicated in *Otomys*, but in the opposite direction where both the group-living and solitary species show high levels of OTR binding in the LS. Future studies may shed light on the role OTR in the LS plays in group living among mammals.

V1aR binding in the spiny mouse

We observed V1aR binding across the forebrain and midbrain in regions consistent with that of other rodents (see Table 4). Here, we will focus our discussion on two regions that displayed high density binding to V1aR, the LS and BNST, as well as the olfactory bulbs.

V1aR in the LS is known to modulate social recognition, anxiety, and aggression (Bielsky et al., 2005). For example, Bielsky et al. found in mice that injection of a V1aR antagonist impairs social recognition, whereas overexpression of V1aR in the LS increases social recognition, but also increased anxiety. V1aR in the LS may facilitate group living by increasing social recognition and decreasing aggression. Though generally spiny mice show low levels of aggression, there is variability across individuals with some acting highly aggressively towards

their familiar cage mates (unpublished observation). Further, social recognition may be important for maintaining relationships within a large group. Future studies in spiny mouse V1aR can explore the role the receptor plays in modulating aggression and social recognition within the LS.

V1aR binding in the LS has been observed in every rodent in which the receptor distribution has been characterized (Freeman et al., 2020). LS V1aR expression is thought to distinguish mating system in voles, given that the socially monogamous prairie vole has lower densities of V1aR in the LS compared to the non-monogamous montane vole (Sadino & Donaldson, 2018). However, comparison between the spiny mouse and Mongolian gerbil (*Meriones unguiculatus*) is not consistent with this observation (see Table 4). The Mongolian gerbil is a monogamous close relative to the communal spiny mouse in the *Muridae* family. Both the spiny mouse and the Mongolian gerbil have high density V1aR binding in the LS (Vallet et al., 1995). Further studies are needed to disentangle the role V1aR plays in mating systems versus group living.

BNST V1aR is thought to promote prosocial behavior by facilitating social interaction (Duque-Wilckens et al., 2016). It is also associated with modulating social valence and reward as it is a part of the mesolimbic reward system and Social Decision-Making Network (O'Connell & Hofmann, 2011). Given that spiny mice readily approach and positively interact with novel and familiar animals alike, it would be interesting to manipulate BNST V1aR in this species to see whether it alters gregariousness.

BNST V1aR binding has been observed in many rodents, though it is interestingly absent in the *Ctenomys* genus. A recent meta-analysis by Freeman et al. (2020) determined that V1aR binding distributions are more consistent within than across genae in rodents, suggesting that

within-genus comparison of V1aR distributions is more appropriate than comparing across distant species.

We observed high density V1aR binding in the MOBgl of the spiny mouse. Olfactory neural circuits that include both the olfactory regions and reward areas are known to modulate pair bonding in prairie voles (Young & Wang, 2004). It has been proposed that prairie voles may associate the smell of a partner with reward, which facilitates pair bonding. Indeed, olfaction is a key sensory modality for rodents to integrate social information, and further V1aR binding tends to exist within regions that reflect an animal's primary sensory modality (Grebe et al., 2021). Therefore, it is unsurprising that we observed high density binding of V1aR in the MOBgl, as well as the AOB.

Consistent with Freeman et al.'s (2020) finding that V1aR is more consistently distributed between closely related species as opposed to more distant ones, the spiny mouse's V1aR distribution was more like that of the Mongolian gerbil as compared to the social tuco-tuco even though their social phenotypes are more similar (see Table 4). Many regions with observed receptor binding in the spiny mouse were also observed in its close relative, the Mongolian gerbil (Vallet et al., 1995). Notably, while there was robust binding observed throughout all nuclei in the hypothalamus and midbrain in the spiny mouse, the Mongolian gerbil only had binding in some hypothalamic and midbrain nuclei. The spiny mouse also appears to have more binding within the thalamus as compared to the Mongolian gerbil. In contrast to the spiny mouse who lives in large groups of related and unrelated individuals and communally breeds, the Mongolian gerbil lives in family groups and is monogamous (Gromov, 2011). Given their close phylogenic relationship and differing social phenotypes, future studies could explore V1aR functional significance within regions that differ in their densities between these two species.

Limitations

While these data serve as the first basic mapping of OTR and V1aR distributions in the spiny mouse brain, much remains to validate these findings. First, it is necessary to perform a competitive binding assay to ensure that the radioligands I-125 OVTA and I-125 LVA are indeed specifically binding to OTR and V1aR, respectively, and not non-specifically binding to other areas within this species. In the absence of this validation, it is difficult to draw any definitive conclusions from these data.

Secondly, as discussed in the methods, we were unable to use a standard curve to map grayscale values on to known amounts of radioactivity, and our resulting qualitative method did not allow for any statistical comparisons. We plan to redo these assays using standard curves and properly measure the amount of radioactivity in each region such that we will not only be able to be more quantitative about what we do and do not call high, medium, or low receptor binding, but also be able to make comparisons across sexes.

Additionally, brain regions were identified using only the autoradiograms. Between the lack of tissue markers displayed on autoradiograms and the lack of a standard spiny mouse brain atlas, it is possible that brain regions were inaccurately identified. Therefore, in future iterations we will stain adjacent slides using Nissl which will allow us to better visualize the tissue morphology and more confidently identify nuclei.

Lastly, it was not terribly apparent what gray value distinguished true background from extremely low levels of receptor binding. In order to determine whether a region is indeed displaying some receptor binding, we will label mRNA in adjacent slides using fluorescent in situ hybridization.

Conclusion

The findings here provide the first steps toward a basic characterization of distributions of OTR and V1aR binding in the forebrain and midbrain of the spiny mouse. Notably, we observed dense OTR binding in the VP and NAcc, and an absence of OTR binding in the LS. Additionally, we observed robust V1aR binding throughout the hypothalamus and midbrain, with the densest binding occurring in the LS. Future studies are needed to determine whether individual regions modulate aspects of the spiny mouse's social phenotype via modulation of the OT and AVP systems.

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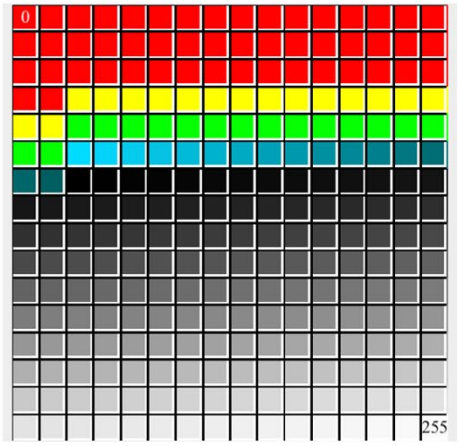
Figures

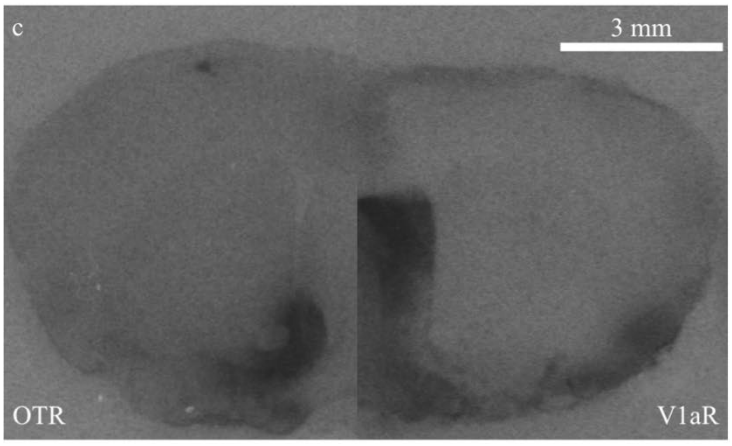
Figure 1

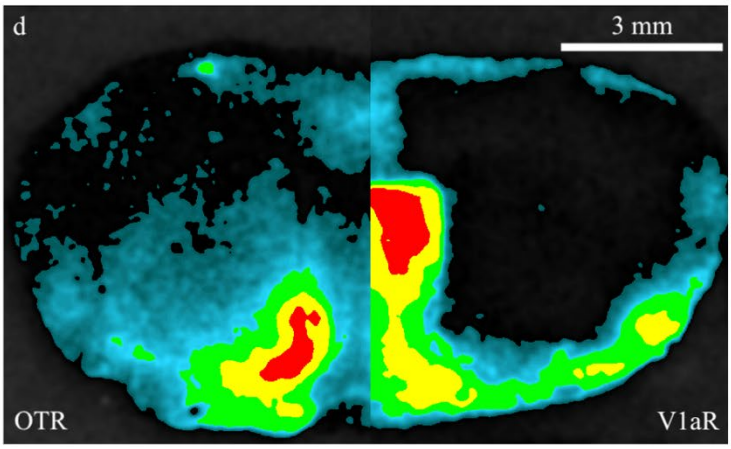
a



b

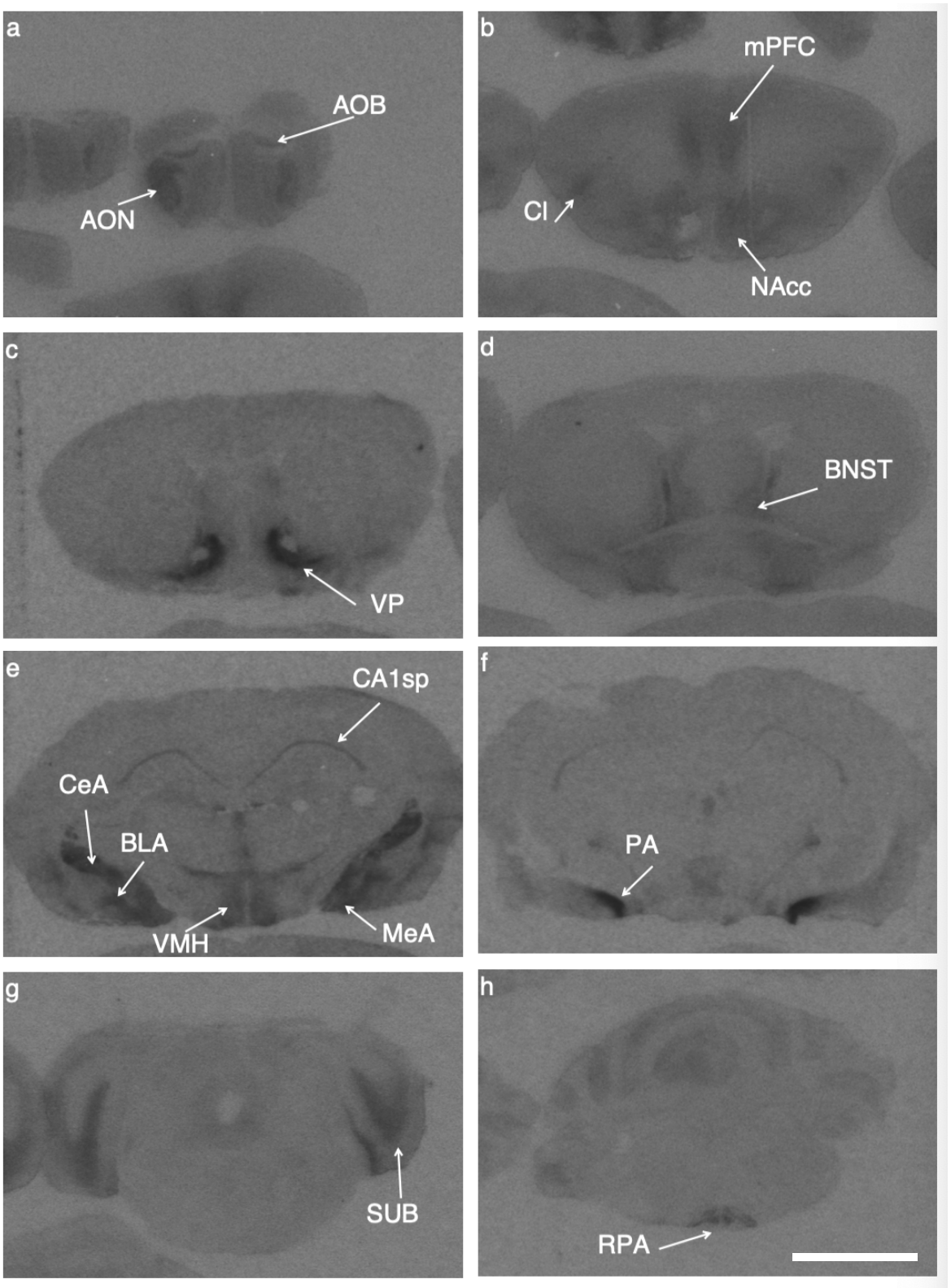
Gray Value Range	Color	Receptor Binding Density
0-50	Red	High
51-66	Yellow	Medium
67-82	Green	Low
83-98	Blue	Barely Detected





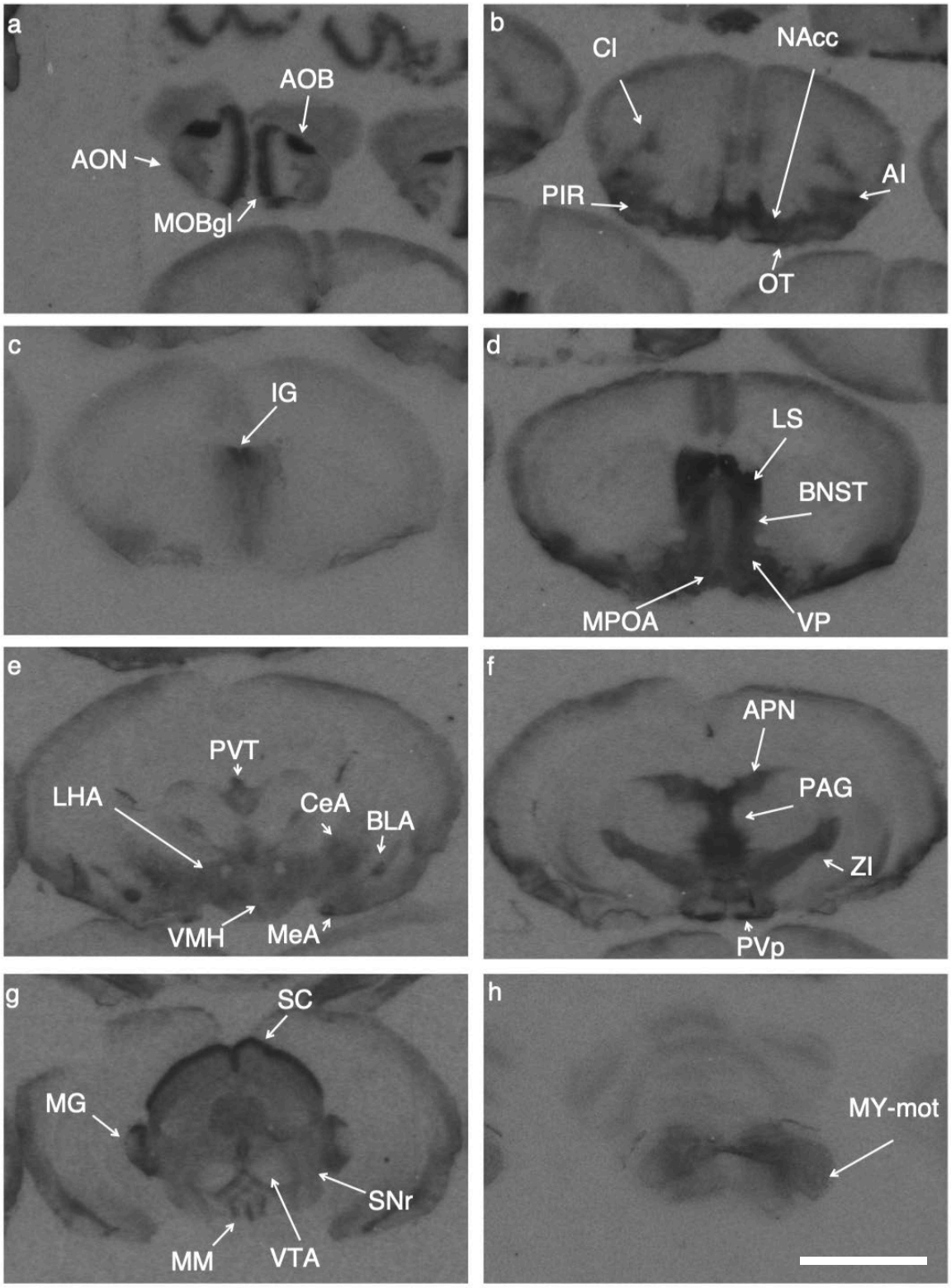
Pseudo-coloring of grayscale images to determine relative binding densities. (a) Customized lookup table with each box representing the color assigned to each pixel with grayscale intensities from 0 (black) to 1 (white); (b) assigned color and receptor binding density based on grayscale value; (c) representative grayscale image showing OTR binding on the left and V1aR binding on the right; (d) the pseudo-colored image with Gaussian blur.

Figure 2



Representative OTR binding across the spiny mouse brain. Scale bar represents 5 mm.

Figure 3



Representative V1aR binding across the spiny mouse brain. Scale bar represents 5 mm.

Tables

Table 1

	Female		Male	
Region	**Modal Density**	**Density Range**	**Modal Density**	**Density Range**
Olfactory areas				
AOB	+	-, +	-	-, +
AON	+	+, ++	+	-, +, ++
Cortical Areas				
Cl	+	-, +	-	-
mPFC	+	-, +	+	-, +
PIR	+	-, +	-, +	-, +
Hippocampal areas				
CA1sp	-	-, +	-	-, +
SUB	++	+, ++, +++	+, ++	+, ++
Striatal areas				
NAcc	++	+, ++	++	+, ++
Pallidum				
BNST	+	+, ++, +++	+, +++	+, ++, +++
GP	+	-, +, +++	+	-, +, ++
VP	+++	++, +++	+++	++, +++
Amygdala				
BLA	++	-, +, ++	+	-, +, ++
CeA	++	+, ++, +++	++	+, ++
MeA	++	+, ++, +++	++	+, ++
PA	+, ++	+, ++, +++	+++	++, +++
Hypothalamus				
PHA	+	-, +	-, +	-, +, ++
VMH	-	-, +, ++	+	-, +, ++
Thalamus				

PVT	+	-, +, ++	+	+
RT	+	-, +, ++	+	-, +, ++
Midbrain				
PAG	-	-, +, ++	-	-, +

Qualitative description of OTR binding in the spiny mouse brain. –: indicates barely detectable receptor binding, +: low density receptor binding, ++: medium density receptor binding, +++: high density receptor binding. The modal density represents the density most often observed within a sex. Two symbols indicate a bimodal distribution. The density range represents the full range of densities seen within a sex.

Table 2

	Female		Male	
Region	**Modal Density**	**Density Range**	**Modal Density**	**Density Range**
Olfactory areas				
AI	+++	-, ++, +++	+++	++, +++
AOB	+++	++, +++	+++	+++
AON	+++	-, ++, +++	++	+, ++, +++
MOBgl	+++	+, ++, +++	+++	++, +++
OT	++, +++	+, ++, +++	++	+, ++, +++
Cortical Areas				
mPFC	++	+, ++, +++	++	+, ++, +++
PIR	++	+, ++, +++	++	++, +++
Hippocampal areas				
IG	++	++, +++	+++	++, +++
Striatal areas				
LS	+++	+++	+++	+++
NAcc	+++	+, ++, +++	+++	+++
Pallidum				
BNST	++	++, +++	++	++, +++
VP	+++	++, +++	++	++, +++
Amygdala				
BLA	+++	+, ++, +++	+++	++, +++
CeA	++	++, +++	++	+, ++, +++
MeA	+++	++, +++	++, +++	++, +++
PA	++	++, +++	++, +++	++, +++

Hypothalamus*				
LHA	++	++, +++	++	++, +++
MM	++	++, +++	+++	+, ++, +++
MPOA	++	++, +++	++	+, ++, +++
PHA	++	++, +++	++	++, +++
PVp	+++	+, ++, +++	+++	+, ++, +++
VMH	++	++, +++	+	+, ++, +++
ZI	++	++, +++	++	+, ++, +++
Thalamus				
LD	++	++	++	+, ++, +++
LGv	+	+, ++	++	-, +, ++
MG	+++	++, +++	+++	+, ++, +++
PVT	+++	+, ++, +++	+++	++, +++
Midbrain*				
APN	++	++, +++	++	+, ++, +++
PAG	+++	++, +++	++	+, ++, +++
SC	+++	+++	+++	++, +++
SNr	++	++	+	+, ++, +++
VTA	++	+, ++	++	+, ++, +++

Qualitative description of V1aR binding in the spiny mouse brain. –: indicates barely detectable receptor binding, +: low density receptor binding, ++: medium density receptor

Table 3

	Spiny mouse	**Mouse**[1]	**Norway rat**[1,2]	**Prairie vole**[1,2,3]	**Montane vole**[1]	**Ice rat**[4]	**Vlei rat**[4]	**Social tuco-tuco**[1]	**Haig's tuco-tuco**[1]	**Naked mole rat**[5]	**Cape mole rat**[5]
Mating system	*Communal*[6]	*Promiscuous*[7]	*Promiscuous*[9]	*Monogamous*[11]	*Promiscuous*[12]	*Communal*[13]	*Promiscuous*[14]	*Communal*[15]	*Promiscuous*[16]	*Eusocial*[5]	*Promiscuous*[18]
Grouping	*Large*[6]	*Small*[8]	*Small*[10]	*Small*[11]	*Solitary*[12]	*Large*[13]	*Solitary*[14]	*Large*[15]	*Solitary*[16]	*Large*[17]	*Solitary*[18]
Olfactory areas											
AON	+	+++	++	ND	-	ND	+	-	-	ND	ND
Cortical Areas											
mPFC	+	-	+	+++	+	+++	+	++	++	ND	ND
PIR	+	-	-	+++	++	+	-	+	-	-	+++
Hippocampal areas											
CA1	-	-	+	-	-	+++	-	-	++	++	+
Striatal areas											
LS	-	+++	+	+	+++	+++	+++	+	++	-	-
NAcc	++	+	+	+++	+	+++	-	-	-	+++	-
Pallidum											
BNST	+	-	+++	+++	+	+++	++	+	++	+++	+
VP	+++	+	+++	-	-	+++	-	-	-	-	ND
Amygdala											
BLA	++	++	+	+	+	++	++	++	+++	ND	ND
CeA	++	-	+++	+++	+++	+++	++	++	+	+++	-
MeA	++	+++	+	+	ND	+	+++	+++	+++	++	-
PA	+++	++	++	++	+++	+	+	+++	+++	ND	ND
Hypothalamus											
VMH	-	+	+++	++	+++	-	+++	++	++	ND	ND
Thalamus											
PVT	+	+++	+	+++	+	+++	+++	+	-	ND	ND

A comparison of OTR binding densities across rodent species. Spiny mouse binding densities are represented by the modal density across sexes. –: indicates barely detectable receptor binding, +: low density receptor binding, ++: medium density receptor binding, +++: high density receptor binding, ND: no data. 1: Beery et al., 2008, 2: Freeman et al., 2020, 3: Lim, et al., 2004, 4: Göldner, 2016, 5: Kalamatianos et al., 2010, 6: Nowak, n.d., 7: Csanády et al., 2019, 8: Singleton, 1983, 9: Chu et al., 2015, 10: Modlinska & Pisula, 2020, 11: Getz et al., 1993, 12: McGuire & Novak, 1986, 13: Willan, 1990, 14: Davis & Meester, 1981, 15: Lacey, 2004, 16: Lacey et al., 1998, 17: O'Riain & Faulkes, 2008, 18: Bennett & Jarvis, 1988.

Table 4

	Spiny mouse	Mouse[1, 18]	Norway rat[1,2,3]	Prairie vole[1,2,4,5]	Montane vole[1,4]	Social tuco-tuco[1]	Haig's tuco-tuco[1]	Mongolian gerbil[17]
Mating system	*Communal*[6]	*Promiscuous*[7]	*Promiscuous*[9]	*Monogamous*[11]	*Promiscuous*[12]	*Communal*[13]	*Promiscuous*[14]	*Monogamous*[15]
Grouping	*Large*[6]	*Small*[8]	*Small*[10]	*Small*[11]	*Solitary*[12]	*Large*[13]	*Solitary*[14]	*Small*[16]
Olfactory areas								
AON	++	ND	++	[-]	[-]	-	-	[++]
MOB	+++	+	++	+++	+++	+++	-	++
Cortical Areas								
mPFC	++	-	-	[-]	ND	-	++	[+++]
PIR	++	-	+	+++	++	-	-	+++
Hippocampal areas								
IG	++, +++	ND	ND	ND	ND	-	+	ND
Striatal areas								
LS	+++	+++	+++	+	[+++]	++	++	+++
NAcc	+++	-	+	-	ND	+	++	ND
Pallidum								
BNST	++	+	++	+	+	-	-	++
VP	++	+++	-	+++	[+++]	+	++	++
Amygdala								
BLA	+++	-	-	-	-	ND	ND	++
CeA	++	++	++	+	+	ND	ND	+
MeA	+++	-	-	+	+	ND	ND	+
PA	++			[+++]	[-]	[+++]	[+++]	[+]
Hypothalamus								
LHA	++	[++]	[+++]	[-]	[-]	ND	ND	[-]
MM	++	[+++]	[+++]	[-]	[-]	ND	ND	[++]
MPOA	++	[-]	[+]	+	[-]	ND	ND	[+]
PHA	++	[+]	[-]	[+]	[-]	ND	ND	[+++]
PVp	+++	[++]	[+++]	ND	ND	ND	ND	[+++]
VMH	++	[-]	[-]	[-]	[-]	ND	ND	[-]
ZI	++	[+]	[++]	[-]	[-]	ND	ND	[-]
Thalamus								
LD	++	[+]	[+]	+++	+	ND	ND	[-]
LGv	+	[-]	ND	ND	ND	ND	ND	[-]
MG	+++	[++]	[-]	[+]	[-]	ND	ND	[-]
PVT	+++	[++]	[-]	[++]	[-]	ND	ND	[++]
Midbrain								
APN	++	[-]	[-]	[+++]	[-]	ND	ND	[+++]
PAG	+++	[+]	[++]	[-]	[++]	ND	ND	[++]
SC	+++	++	++	+++	+++	ND	ND	+
SNr	+	[++]	[++]	[-]	[-]	ND	ND	[-]
VTA	++	[+++]	[++]	[+]	[+]	ND	ND	[++]

A comparison of V1aR binding densities across rodent species. –: indicates barely detectable receptor binding, +: low density receptor binding, ++: medium density receptor binding, +++: high density receptor binding, ND: no data, []: density was assessed qualitatively using manuscript figures, multiple designations reflect a bimodal distribution of densities. 1: Beery et al., 2008, 2: Freeman et al., 2020, 3: Smith et al., 2017, 4: Lim, et al., 2004, 5: Insel et al., 1994, 6: Nowak, n.d., 7: Csanády et al., 2019, 8: Singleton, 1983, 9: Chu et al., 2015, 10: Modlinska & Pisula, 2020, 11: Getz et al., 1993, 12: McGuire & Novak, 1986, 13: Lacey, 2004, 14: Lacey et al., 1998, 15: Tchabovsky et al., 2019, 16: Gromov, 2011, 17: Vallet et al., 1995, 18: Dubois-Dauphin et al., 1996.

Appendix 1

Abbreviations

AI	Agranular insular area
AH	Anterior hypothalamus
AOB	Accessory olfactory bulb
AON	Anterior olfactory nucleus
APN	Anterior pretectal nucleus
BLA	Basolateral amygdala
BNST	Bed nucleus of the stria terminalis
CA1sp	Field CA1, pyramidal layer
CeA	Central amygdala
CL	Claustrum
CN	Cochlear nuclei
CP	Caudate putamen
GP	Globus pallidus
Hpc	Hippocampus
IG	Induseum griseum
LD	Lateral dorsal nucleus of thalamus
LGv	Ventral part of the lateral geniculate complex
LHA	Lateral hypothalamic area
LS	Lateral septum
MeA	Medial amygdala
MG	Medial geniculate complex
MM	Medial mammillary nucleus
MOBgl	Main olfactory bulb, glomerular layer
mPFC	Medial prefrontal cortex
MPOA	Medial preoptic area
MY-mot	Medulla, motor related
NAcc	Nucleus accumbens
OT	Olfactory tubercle
PA	Posterior amygdalar nucleus

PAG	Periaqueductal gray
PHA	Posterior hypothalamic area
PIR	Piriform area
PIR	Piriform area
PVp	Periventricular hypothalamic nucleus, posterior part
PVT	Paraventricular nucleus of the thalamus
RPA	Nucleus raphe pallidus
RT	Reticular nucleus of the thalamus
SC	Superior colliculus
SNr	Substantia nigra, reticular part
SUB	Subiculum
VMH	ventralmedial hypothalamus
VP	Ventral pallidum
VTA	Ventral tegmental area
ZI	Zona incerta