Sex Differences in Oxytocin and Vasopressin V1a Receptor Binding Densities in the Mouse Brain: Focus on the Social Behavior Neural Network

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ABBREVIATIONS

AH= anterior hypothalamus
AIp= agranular insular area
ARH= arcuate hypothalamic nucleus
AVPV= anteroventral periventricular nucleus
ASD= autism spectrum disorder
AVP = vasopressin
BNST= bed nucleus of the stria terminals
BNSTa= anterior bed nucleus of the stria terminalis
BNSTmv= medial ventral bed nucleus of the stria terminalis
BNSTp= posterior bed nucleus of the stria terminalis
CA3= hippocampus CA3
CeA= central amygdala
CPu= caudate putamen
LHA= lateral hypothalamic area
LS= lateral septum
LSa= anterior lateral septum
LSr= rostral lateral septum
LSv= ventral lateral septum
MeA= medial amygdala
MeApd= posterodorsal medial amygdala
MPN= medial preoptic nucleus
MPOA= medial preoptic area
NAc= nucleus accumbens
OT= oxytocin
OTR= oxytocin receptor
PAG= periaqueductal gray
PVN= paraventricular nucleus
SBNN = social behavior neural network
SCN= suprachiasmatic nucleus
SON = supraoptic nuclei
VMH= ventromedial hypothalamus
V1aR= vasopressin V1a receptor

1. ABSTRACT

Oxytocin (OT) and vasopressin (AVP) often regulate social behaviors in sex-specific ways. We hypothesized that this could be mediated by sex differences in the OT receptor (OTR) and AVP V1a receptor (V1aR) in the brain. Here, we determined whether there are sex differences in OTR and V1aR binding densities in nodes of the social behavior neural network in the mouse brain. We also compared sex differences in the OTR and V1aR in the mouse brain with those found previously found in the rat brain. Although mice and rats are closely related species, they also display differences in social behavior. Therefore, we predicted to find similar as well as unique sex differences in OTR and V1aR in mice compared to rats. Generally, we found that sex differences in OTR and V1aR binding densities are brain region-specific and species-specific. In detail, male mice showed higher OTR binding density than female mice in the medial amygdala, anterior lateral septum, and posterior bed nucleus of the stria terminals. This is consistent with findings in rats. Furthermore, female mice displayed higher OTR binding density in the anteroventral periventricular nucleus and ventromedial hypothalamus. This is in contrast to rats, where males showed higher OTR binding densities in these regions. Lastly, females showed higher V1aR binding density in the anterior bed nucleus of the stria terminalis. However, this sex difference was not measured in rats due to low receptor expression in this region. Overall, these findings demonstrate the importance to determine sex differences in OTR and V1aR across species in order to gain a better understanding of the sex-specific behavioral functions of the OT and AVP systems.

2. INTRODUCTION

Rats (*Rattus norvegicus*) and mice (*Mus musculus*) have been widely used as model organisms in the investigation of neurobiological processes underlying neuropsychiatric disorders. While rats and mice may superficially appear very similar, their most recent common ancestor lived about 15-20 million years ago; consequently, there exists substantial differences between rats and mice on the molecular and thus behavioral level (Adkins et al., 2001). The brain oxytocin (OT) and vasopressin (AVP) systems, which have important implications in the regulation of social behaviors in mammals, are unique between the two rodent species, and this seems to significantly determine their behavioral patterns. Such differences between rats and mice must be considered as they can have possible bearings on the rodents' phenotypic responses in experimental procedures. Recently, studies done on adult rats have shown that OT and AVP regulate social behaviors in sex-specific ways (Bluthe & Dantzer 1990; Dantzer et al. 1987), and this may be mediated by sex differences in the OT receptor (OTR) and AVP receptor (V1aR) expression in various brain regions (Dumais et al. 2013; Lukas & Neumann, 2014; Dumais & Veenema, 2016b). Interestingly, the direction of sex differences across brain regions seem receptor specific, that is OTR binding density is higher in males than females (Dumais et al. 2013; Dumais & Veenema 2016a; Smith et al., 2017) and V1aR binding density is higher in females than males (Smith et al., 2017). However, the characterization of sex differences in OTR and V1aR binding density is lacking in mice and it is unclear whether these studies done on rats can be generalized to other species. Therefore, I determined whether there are sex differences in the OTR and V1aR binding densities in brain regions encompassing the so called social behavior neural network (SBNN) in mice, and I compared these findings with the previously reported sex differences in OTR and V1aR in rats. Together, these findings will provide an important

framework for testing species- and sex-specific roles of OTR and V1aR in regulating social behavior.

Oxytocin and Vasopressin

OT and AVP are evolutionarily conserved nonapeptides that differ from one another by two amino acids at the third and eighth positions (see Figure 1). OT and AVP are primarily synthesized in the paraventricular nucleus (PVN) and the supraoptic nuclei (SON) of the hypothalamus. Additionally, AVP is synthesized in the bed nucleus of the stria terminalis (BNST), medial amygdala (MeA), and suprachiasmatic nucleus (SCN) (Sofroniew & Weindl, 1978; De Vries & Buijs, 1983; Caffe et al., 1987). The OT and AVP synthesizing magnocellular cells of the hypothalamic SON and PVN project to the posterior pituitary, where OT and AVP are released into the bloodstream to regulate peripheral activities, including maternal responses (lactation and uterine contractions) and male reproductive responses (ejaculation) in the case of OT, and antidiuretic properties in the case of AVP (Fuchs & Poblete, 1970; Swanson & Sawchenko, 1983; Gimpl & Fahrenholz, 2001; Caldwell et al., 2007). Moreover, populations of OT synthesizing magnocellular neurons in the SON and PVN and AVP synthesizing parvocellular neurons located in the PVN, BNST, MeA, and SCN contain projections that remain in the brain (Buijs, 1978; De Vries & Buijs, 1983; De Vries et al.,1985; Gimpl & Fahrenholz, 2001; Knobloch et al., 2012). Through these central projections along with some dendritic release, OT and AVP reach their widely-distributed receptors across different brain

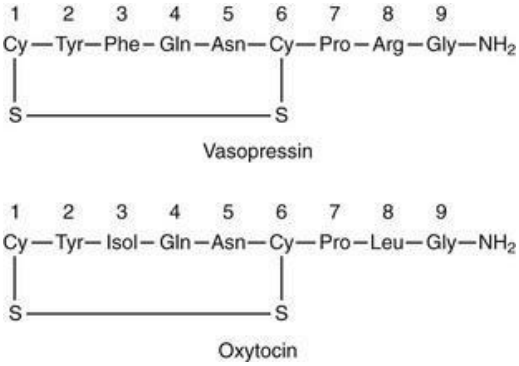

Figure 1: Neuropeptide OT and AVP Structure
Oxytocin and vasopressin are evolutionarily conserved nonapeptides that differ in the third and eighth positions. The neuropeptides have been implicated in regulating social behaviors. *(www.clinicalgate.com)*

regions (Jard et al., 1987; Tribollet et al., 1990; Gimpl & Fahrenholz, 2001; Ludwig & Leng, 2008). Via activation of these receptors, OT and AVP regulate a wide range of social, emotional, and cognitive behaviors, including aggression, maternal behavior, and social recognition (Veenema & Neumann, 2008; Goodson & Kabelik, 2009, Albers, 2015).

Oxytocin and Vasopressin Receptors

The behavioral effects of OT and AVP are mediated by their respective receptors, the OTR, V1aR, and V1bR, which are all seven-transmembrane domain G protein-coupled receptors (Gimpl & Fahrenholz, 2001; Barberis & Tribollet, 1996; Hasunuma et al., 2013). V1aR and V1b receptors (V1bR) are both expressed in the brain, although the V1bRs are more restricted in distribution (Young et al., 2006). However, most of the behavioral effects of AVP are mediated by the V1aR (Wang et al., 1994).

Within the central nervous system (CNS), OTR and V1aR are abundantly localized within the hypothalamic SON and PVN as well as in extrahypothalamic regions, including the hippocampus, amygdala subregions, BNST, medial preoptic area (MPOA), lateral septum (LS), ventral pallidum, nucleus accumbens (NAc), and caudate putamen (CPu; Tribollet et al., 1992; Barberis & Tribollet 1996; Tribollet et al., 1999; Gimpl & Fahrenholz, 2001). Comparisons of the neuroanatomical distribution of OTR and V1aR among various mammalian species reveal species-specific expression levels of receptor binding in the brain (Barberis & Tribollet, 1996; Young, 1999). Moreover, the species-specific expression of the brain OTR and V1aR seems to underlie the unique behavioral patterns of a given species, as seen in the territorial versus gregarious finches (Goodson & Wang, 2006), low versus high vocalizing singing mice (*Scotinomys*; Campbell et al., 2009), and monogamous prairie voles versus non-monogamous montane voles (Insel & Shapiro,

1992).

Gain and loss function studies in rodents provide evidence for the involvement of the OTR and V1aR in the regulation of basic social behaviors that are dysregulated in neuropsychiatric disorders, including social cognition, affiliative behavior, and social approach (Harony & Wagner, 2010). For instance, intracerebroventricular administration of OTR antagonist after exposure to the first conspecific have been shown to block social memory in male and female rats (Engelmann et al., 1998). In parallel, mice with a null mutation in the OTR gene show deficits in social discrimination (Takayanagi et al., 2005). In the case of AVP receptor studies, injecting V1aR antagonist into the lateral septum impaired social memory in adult male and female rats (Veenema et al., 2012), and this is further confirmed by a lack of social memory in male V1aR knockout mice (Bielsky et al., 2004).

Overall, there is substantial evidence for the involvement of OTR and V1aR as mediators of pro-social behaviors in rodents. While receptor expression has been shown to be unique among rodent species, a direct comparison of the OTR and V1aR expression patterns in different rodents, their behavioral implications, and how they are modulated in a species-specific manner is lacking. By investigating and comparing brain mechanisms underlying essential behavior in different rodent models, scientists can potentially discover better and more reliable animal models to study the etiology and neurobiological mechanisms underlying neuropsychiatric disorders characterized by social dysfunction.

Rats and Mice in Neuroscience Research

Rats and mice have been the leading model organisms used in biomedical research to advance the understanding of human diseases. However, over the past two decades, there has

been a notable shift in rodent-based research towards a higher usage of mice compared to rats (Ellenbroek & Youn, 2016). The increase in popularity of mice research was primarily attributed to the availability of superior molecular genetic techniques in mice, especially with the engineering of the knockout mouse starting in 1987 (Thomas & Capecci, 1987). This leads to an inevitable question of which rodent species would be a more appropriate model for neuroscience research. A careful, holistic examination of both species reveal that while rats and mice share many similar features, there are fundamental differences that must be accounted for before selecting the suitable model organism for an effective experiment.

There are specific techniques that are clearly better performed on one species than the other (see Figure 2). While greater size and weight of adult rats prove more practical for performing laboratory procedures such as surgical (Feduccia & Duvauchelle, 2017) and brain imaging techniques (Bartelle et al., 2016, Zimmer et al., 2014, Kim et al., 2014), there are great advantages of using mice as model organisms as well. The readily available transgenic and knockout mice models would allow scientists to determine the function of a gene in a particular system. Furthermore, the smaller size of mice provides better use for optogenetics, which is used for stimulating or inhibiting defined events in specific cells in moving, behaving animals. By transfecting neurons with specific light-sensitive proteins, cellular changes such as excitation or inhibition, or precise intracellular changes such as increases in Ca^{2+} or cyclic AMP, can be induced by illumination (Deisseroth, 2015). Because the brains of mice are smaller, it is easier for light to transmit into the deeper brain regions to study the neural

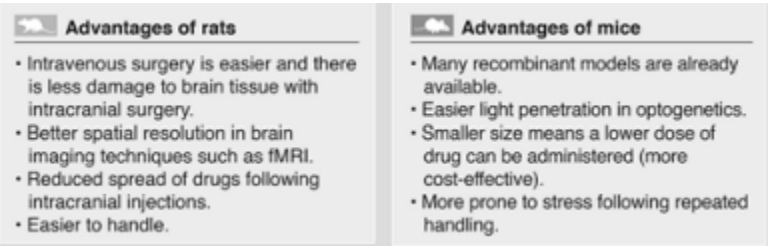

Figure 2: Advantages of rats and mice in neuroscience research
(Ellenbroek & Youn, 2016)

circuitry underlying behavior. Another distinct advantage of the lighter weight of mice is in the development of drugs; because doses are given to animals relative to their body weight, only a small quantity of compounds is needed, which would be more cost-effective in experiments (Ellenbroek & Youn, 2016).

Aside from techniques, there are substantial behavioral differences between the two rodents. Social cognition, which includes all processes elicited by or directed towards another subject, is a pertinent behavior when studying a variety of diseases, including bipolar disorder, schizophrenia, ASD. Rats and mice display differences in social cognition, which is likely attributed to their contrasting social structures. For instance, in nature, rats are much less territorial, and the hierarchical relationships between male rats are less definite compared to those of mice (Ellenbroek & Youn, 2016). This behavior likely reduces aggression and infanticide in the rats, whereas mice, which live in more territorial structures founded by a single male mating with multiple female, tend to be more aggressive (van Zegeren, 1980; Ellenbroek & Youn, 2016). Their social structures found in nature influence their social behaviors in the laboratory; studies have demonstrated that while most rats found social interaction rewarding, mice would spend less time interacting with each other and may actually find the behavior aversive (Kummer et al., 2014).

Overall, both rats and mice play an important role in neuroscience research, especially in understanding the etiology, pathophysiology and pharmacology of neuropsychiatric disorders (Feduccia & Duvauchelle, 2017). Therefore, it is important to consider the differences between rats and mice to test for species-specific regulation of social behavior, which would overall help scientists gain insights about neuropsychiatric disorders. Additionally, findings made in both rodent species would open the use of plethora of laboratory techniques that would help scientists to develop additional experiments to deepen their understanding of systems of their interests.

Social Behavior Neural Network

A substantial amount of evidence reveal that OT and AVP systems can elicit powerful effects on social behavior via acting on specific sites in a neural network within the CNS, now termed the "social behavior neural network" (SBNN) (see Figure 2). Over the past 30 years, scientists have ascribed specific behaviors to the activity of discrete brain regions. However, the large body of data accumulated revealed an overlap in the circuitry responsible for modulating a wide range of different social behaviors, including sexual behavior, communication, aggression, maternal behavior, and reproductive behavior (Newman, 1999). The data transformed the way scientists looked at neural mechanisms controlling social behavior as it led them to consider the possibility that the various brain structures form an integrated social behavior circuit, specifically a subcortical limbic network that regulates sex-steroid mediated social behavior (Newman 1999).

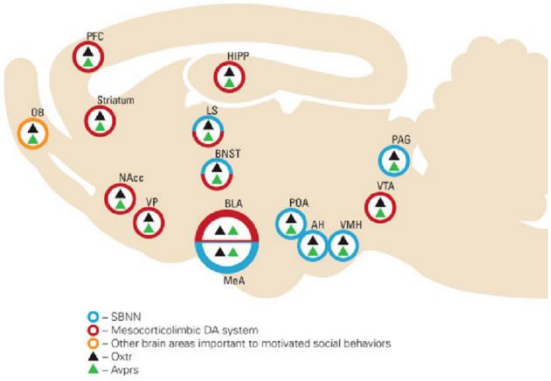

Figure 3: Social Behavior Neural Network
The SBNN is comprised of interconnected "nodes" or brain regions that are responsible for modulating a wide range of social behaviors.
(Caldwell & Albers, 2016)

The SBNN is comprised of "nodes" or brain regions that include the LS, periaqueductal gray (PAG), MPOA, anterior hypothalamus (AH), ventromedial hypothalamus (VMH), MeA, and the BNST (Newman 1999). All these nodes fulfill specific criteria: (1) each node is connected to all other nodes (Coolen & Wood, 1998), (2) each node serves as an important site of regulation of multiple social behaviors, (3) each node has neurons that contain gonadal hormone receptors (Simerly et al, 1990; Wood & Newman, 1995; Commins & Yahr, 1985). A specific social behavior, for instance, male sexual behavior, is elicited from the activity across the network; rather than the

previous hypothesis of an action produced by an "on" or "off" state of the nodes, the action (male sexual behavior) is a product of a sequence of several related behaviors (mounting, sniffing, grooming) that arises from a temporal and dynamic pattern of activity across the SBNN (Newman, 1999).

Comparative analysis across species, including mammals, reptiles, birds, amphibians, and teleost fish support the idea that the SBNN is evolutionarily conserved and that there may be potential homologous nodes in SBNN (Goodson, 2005; Goodson & Kingsbury, 2013; Albers, 2015). It is likely that the specific nodes within the SBNN and their functional activities may vary across species, given that the network is found in a diverse group of animals that expresses behaviors differently based on the relative importance of varying sensory input routes (Maney et al., 2008, Albers, 2015). However, while there may be substantial differences in structures providing the sensory input, the core nodes of the SBNN and their functions are rather similar across species (Albers, 2015).

While the concept of the SBNN is a transformative way of viewing neural mechanisms mediating social behavior, further knowledge is needed about how social behavior emerges from such a complex neural network. OT, AVP, and their receptors are localized throughout the nodes of the SBNN (Albers, 2015) and display the typical properties expected of a signaling system that controls social behavior. However, the mechanism of how the nonapeptides and their receptors interact across the network as well as how the systems are modulated in different species, ages, sexes, and individuals are incompletely understood.

Sex differences in the OTR and V1aR binding densities in the rat brain

Studies have shown that OT and AVP often regulate social behaviors differently in male and female adult rats, and this may be due to sex differences in OTR and V1aR expression in the brain (Bluthe & Dantzer, 1990; Dantzer et al., 1987; Engelmann et al., 1998; Dumais et al., 2013; Lukas & Neumann, 2014; Dumais & Veenema, 2016a, b). Additionally, sex differences in the brain OT and AVP systems may potentially underlie sex-biases in neuropsychiatric disorders such as schizophrenia (Jobst et al., 2014), ASD (Yang et al., 2010; Xu et al., 2013) depression (Yueng et al., 2014), and borderline personality disorder (Bertsch et al., 2013) in terms of prevalence, symptom severity, and treatment responses. Therefore, it is important to understand the sex differences in the OT and AVP systems as well as how OT and AVP mediate social behavior in a sex-specific way to gain insight into potential sex-specific therapeutic treatments for men and women diagnosed with neuropsychiatric disorders.

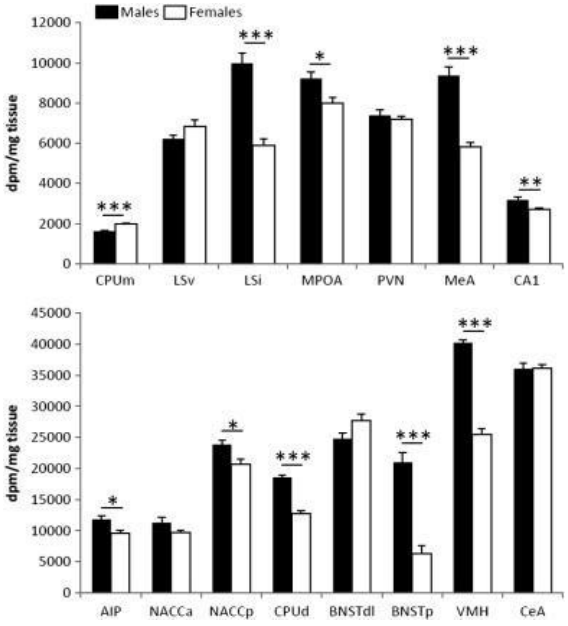

Figure 4: OTR binding in forebrain regions of male and female rats
Males have higher OTR binding densities than females in 9 out of the 15 forebrain regions analyzed: interior lateral septum, medial preoptic area, medial amygdala, CA1, agranular insular cortex, anterior nucleus accumbens, dorsal caudate putamen, posterior BNST, and ventromedial hypothalamus. Females have a higher OTR binding density in 1 out of the 15 forebrain regions analyzed: medial caudate putamen.
(Dumais et al., 2013)

Our lab recently performed comprehensive analyses on the sex differences in OTR and V1aR binding density in adult male and female rats and found sex differences in several regions analyzed (see Figure 3 and 4; Dumais et al., 2013; Dumais & Veenema 2016b; Smith et al, 2017). Among the studies, males consistently showed higher OTR binding density in three regions:

BNSTp, VMH, and MeA (Dumais et al., 2013; Smith et al., 2017). Sex differences in OTR binding were found in other regions, including CPu and LS; however, the direction of the sex differences was inconsistent (Dumais et al., 2013; Smith et al., 2017). In the case of V1aR, females showed higher binding densities in the arcuate nucleus, dorsal LS, ventromedial thalamus (Smith et al., 2017), while males showed higher binding in the somatosensory cortex, piriform cortex, nucleus of the lateral olfactory tract, medial posterior BNST, anteroventral thalamus, tuberal lateral hypothalamus, stigmoid hypothalamus, and dentate gyrus (Dumais & Veenema 2016b); however, the sex differences reported were also inconsistent between the two experiments. These inconsistencies are likely due to differences in experimental design as one study focused on the effects of age and sex for each receptor (Smith et al., 2017), and the other considered the effects of the estrus cycle phase and material experience on the receptors and social interest (Dumais et al., 2013; Dumais & Veenema, 2016b).

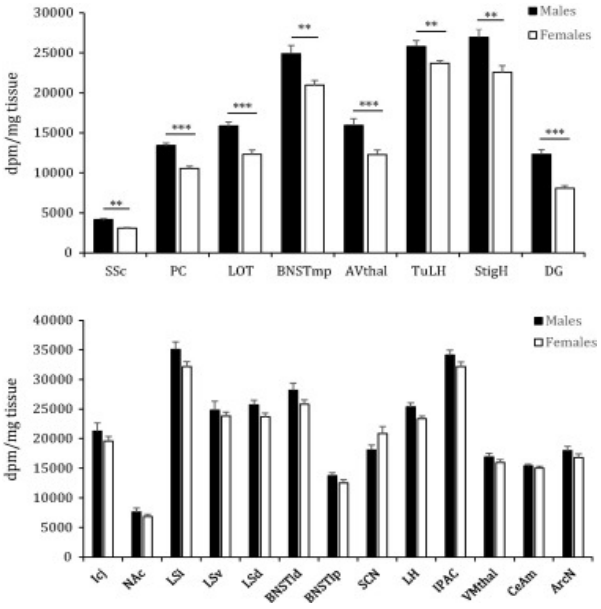

Figure 5: V1aR binding in forebrain regions of male and female rats
Males have higher V1aR binding densities than females in 8 out of the 21 forebrain regions analyzed (top): somatosensory cortex, piriform cortex, nucleus of the lateral olfactory tract, medial posterior BNST, ateroventral thalamus, tuberal lateral hypothalamus, stigmoid hypothalamus, and dentate gyrus. No sex differences were found in 13 regions analyzed (bottom).
(Dumais & Veenema., 2016b)

These sex differences were speculated to be implicated in regulating sex-specific social behaviors in rats. Indeed, our lab also demonstrated that sex differences in OTR binding densities could be involved in a neural circuitry regulating social interest in a sex-specific way (Dumais et al., 2013). Results showed that male social interest positively correlated with OTR binding densities in the MeA, and female social interest correlated negatively with OTR binding in the

central amygdala (CeA; Dumais et al., 2013).

Further research is needed to determine the behavioral significance of sex differences in OTR and V1aR binding densities as well as the molecular mechanisms that underlie such sex differences. However, it is also important to recognize that social behavior may be species-specific, and studies done on rats may not always generalize to other species.

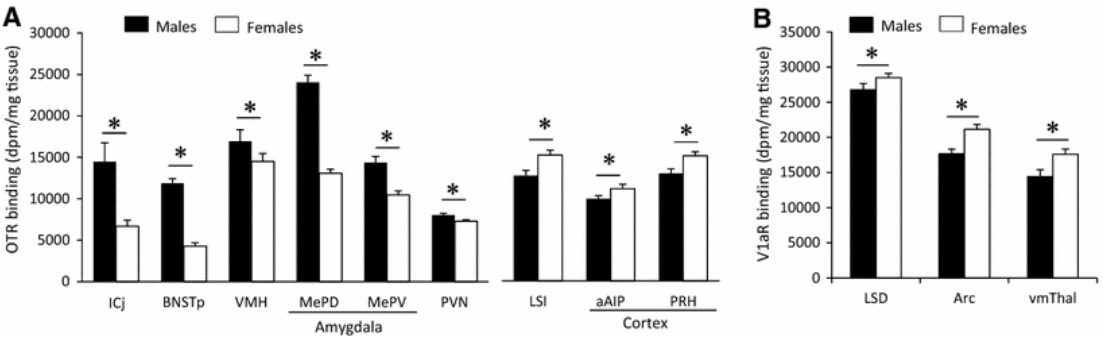

Figure 6: Brain regions in which (A) OTR and (B) V1aR binding densities demonstrate sex differences
Males have higher OTR binding densities than females in 9 out of the 35 regions analyzed: the islands of Calleja, posterior BNST, ventromedial hypothalamus, posterior-dorsal and posterior-ventral medial amygdala, and paraventricular nucleus of the hypothalamus. Females have higher OTR binding in three brain regions: the anterior insular cortex, perirhinal cortex, and intermediate lateral septum. Sex differences in V1aR binding densities were found in 3 of the 29 regions analyzed, where females had higher binding in the arcuate nucleus, dorsal lateral septum, and ventromedial thalamus.
(Smith et al., 2017)

Aims

As summarized above, OTR and V1aR binding densities have been characterized in adult male and female rats revealing sex differences in OTR and V1aR in certain brain regions which could underlie sex-specific social behaviors in rats. However, it is important to recognize that studies done on rats may not always generalize to other species. In addition to rats, mice are important model organism in particular because of the ability to manipulate its genome directly. Therefore, the aims of this research project were (1) to determine whether there are sex differences in OTR and V1aR binding densities in nodes of the SBNN in the mouse brain and (2) to compare

these sex differences with those found previously in rats. With the novel findings in mice, we can use the readily available genetic tools in mice to determine the possible function of a gene in the development of the sexual dimorphic OT and AVP system and its implications in behavioral differences in a sex-specific way. Because rats and mice are closely-related species but also display behavioral differences in nature, we predict that they would demonstrate similar as well as unique sex differences in OTR and V1aR binding densities compared to rats. These findings would provide a useful reference for future experiments involved in understanding the behavioral function of the OT and AVP systems in both sexes and across species.

3. MATERIAL AND METHODS

Animals

C57BL/6 mice were obtained from Charles River Laboratories (Raleigh, NC, USA) and maintained under standard laboratory conditions (12 h light/dark cycle, lights on at 7:00 am, food and water available ad libitum, 22 °C, 60 % humidity). Upon arrival at our facility, mice were housed in standard mouse cages (7.5 × 11.5 × 5 in.). Findings were compared to resultss using Wistar rats (Dumais et al., 2013; Dumais & Veenema, 2016b, Smith et al., 2017). All experiments were conducted in accordance with the guidelines of the NIH and approved by the Boston College Institutional Animal Care and Use Committee.

Receptor autoradiography

Coronal Sectioning

Mice (adult males: *n=16*; adult females: *n=16*) were killed using CO_2 inhalation and brains were removed, rapidly frozen in methylbutane on dry ice, and stored at -45°C. Brains were cut on

a cryostat into 16-µm coronal sections and mounted onto slides in eight adjacent series. Collection began at approximately 1.18 mm anterior to bregma and ended at approximately 2.80 posterior to bregma (Allen Brain Atlas; Franklin & Paxinos 2007). Sections were then frozen -45°C until receptor autoradiography was performed. Receptor autoradiography was conducted for OTR using [^{125}I]-Ornithine Vasotocin Analog (d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Orn^8,[^{125}I]Tyr^9-NH_2]-OVTA; Perkin Elmer, Boston, MA, USA) as tracer and for V1aR using [^{125}I]-d(CH_2)$_5$(Tyr[Me])-AVP (Perkin Elmer, Boston, MA, USA) as tracer on adjacent series. Specificity of these tracers to bind OTR and V1aR, respectively, has been demonstrated previously (Beery et al. 2008; Campbell et al. 2009; Anacker et al. 2016). Receptor autoradiography was conducted in accordance with Lukas et al. (2010). In brief, slides were thawed and dried at room temperature followed by a short fixation in 0.1% paraformaldehyde. The slides were then washed twice in 50mM Tris (pH 7.4), exposed to trace buffer (50 p< tracer, 50mM Tris, 10mM $MgCl_2$, 0.01% BSA) for 60 min, and washed four times in Tris +10mM $MgCl_2$. Finally, slides were dipped in distilled water, air-dried, and exposed to Biomax MR films (WWR International, Pittsburgh, PA, USA). Brain sections of both sexes were processed together and balanced across incubation chambers and exposure films. A 7-day exposure times were used to analyze OTR binding density in brain regions with relatively high OTR binding density (6 regions); posterior agranular insular area (AIp), MeA, anterior LS (LSa), ventral LS (LSv), rostral LS (LSr), and medial preoptic nucleus (MPN). OTR binding density in additional regions with lower OTR binding density was analyzed using a 14-day exposure time (5 regions); CeA, hippocampus CA3, BNSTp, anteroventral periventricular nucleus (AVPV), VMH. A 7-day exposure time was used to analyze V1aR binding density in a total of 6 brain regions; anterior BNST (BNSTa), arcuate hypothalamic nucleus (ARH), CeA, lateral hypothalamic area (LHA), LSr, and posterodorsal MeA (MeApd). All abbreviations of brain regions are in

accordance with Allen Brain Atlas (http://mouse.brain-map.org/static/atlas), except for LSa and BNSTa, where the anterior portion was added to delineate the separate areas analyzed in each of these brain regions; the bed nucleus of the stria terminalis, medial amygdala, and central amygdala were abbreviated BNST, MeA, CeA, respectively (instead of BST, MEA, CEA) for easier comparison with the rat brain.

Image and data analysis

Autoradiography films were digitized using a Northern Light Illuminator (InterFocus Imaging, UK) and optical densities of OTR and V1aR were measured in coronal sections using ImageJ (NIH, http://imagej.nih.gov/ij). The data were converted to dpm/mg (disintegrations per minute/milligram tissue) using a [^{125}I] standard microscale (American Radiolabeled Chemicals Inc,. St. Louis, MO, USA). Each measurement was subtracted by film background and binding densities were calculated by taking the mean of bilateral measurements in a fixed number of sections per region of interest per mice. The total number of sections included depended on the size of the region of interest with a minimum of two sections. Regions of interest included those of the social behavior neural network as well as a few additional regions with dense OTR or V1aR binding (see Table 1 and 2) This resulted in 11 brain regions analyzed for OTR and 6 brain regions analyzed for V1aR binding.

Statistics

For all statistical analysis, a student's t-test was used to test for sex differences in OTR and V1aR binding density in each brain region. Significance was set at $p<0.05$ for all comparisons (11 regions for OTR; 6 regions for V1aR).

4. RESULTS

Sex differences in OTR and V1aR binding densities in the mouse brain

Sex differences in OTR binding density were found in 6 out of the 11 brain regions analyzed (See Table 1 for summary). Males demonstrated higher OTR binding densities than females in the MeA, LSa, and pBNST. Females displayed higher binding densities than males the AVPV, MPN, and VMH. Lastly, no sex differences in OTR binding were found in the LSr, LSv, CeA, CA3, and AIp.

One sex difference in V1aR binding density was found out of the six regions analyzed. Females showed higher V1aR binding density than males in the BNSTa. The CeA, MeApd, LSr, ARH, and LHA displayed no sex differences in V1aR binding density (see Table 2 for summary).

5. DISCUSSION

We first hypothesized that mice would display sex differences in OTR and V1aR binding density in brain regions within the SBNN. In accordance with this hypothesis, our data analysis demonstrated several brain regions within the SBNN in which OTR binding densities differed between males and females (6 out of 11 regions); MeA, LSa, BNSTp, AVPV, and VMH (See Figure 7a,b,c; Figure 8a,b; Table 1). However, this was not the case for V1aR binding in mice, where findings revealed far fewer sex differences in regions of SBNN (1 out of 6 regions); BNSTa (See Figure 10a; Table 2).

Moreover, given that rats and mice are closely related species that display some differences in social behavior, we hypothesized that mice and rats would display mostly similar but also some unique sex differences in the same brain regions. In line with this hypothesis, our analysis revealed mostly conservation of the prevalence and absence of sex differences in OTR and V1aR binding

Sex Differences in Oxytocin Receptor Binding Densities		
Brain Region	**Mice**	**Rats**
MeA	Males higher than females	Males higher than females
LSa	Males higher than females	-Region = interior LS -Males higher than females in (Dumais et al., 2013) - No sex difference (Smith et al., 2017)
BNSTp	Males higher than females	Males higher than females
AVPV	Females higher than males	Males higher than females
VMH	Females higher than males	Males higher than females
MPN	Females higher than males	Not Measured
CeA	No sex difference	No sex difference
AIp	No sex difference	No sex difference
CA3	No sex difference	-Not measured -But, hippocampal CA1 displayed sex difference where males higher than females
LSv	No sex difference	No sex difference
LSr	No sex difference	No sex difference

Table 1: Summary of Sex Differences in OTR Binding Densities in Mice and Rats
Sex differences in OTR binding densities were measured in 11 brain regions in mice; MeA, LSa, BNSTp, AVPV, VMH, MPN, CeA, AIp, CA3, LSv, Lr. These results were compared to findings previously seen in rats according to data in *Dumais et al., 2013* and *Smith et al., 2017*.

Sex Differences in Vasopressin V1a Receptor Binding Densities		
Brain Region	**Mice**	**Rats**
BNSTa	Females higher than males	Not measured
ARH	No sex difference	-Females higher than males (Smith et al., 2017) -No sex difference (Dumais & Veenema, 2016b)
CeA	No sex difference	No sex difference
LHA	No sex difference	No sex difference
LSr	No sex difference	No sex difference
MeApd	No sex difference	No sex difference

Table 2: Summary of Sex Differences in V1aR Binding Densities in Mice and Rats
Sex differences in V1aR binding densities were measured in 6 brain regions in mice; BNSTa, ARH, CeA, LHA, LSR, MeApd. These results were compared to findings previously seen in rats according to data in *Dumais & Veenema, 2016b* and *Smith et al., 2017*.

between the two species with some differences. For example, all of the brain regions that displayed no sex differences in mice also displayed no sex differences in rats: hippocampus CA3, CeA, AIp, LSr, LSv (in the case of OTR; see Figure 7d,e,f; Table 3), and MeApd, LSr, CeA, ARH, and LSA (in the case of V1aR; see Figure 9; Table 4). Additionally, the majority regions that have sex differences in OTR binding densities in mice were also found previously in rats (5 out of 6 regions); MeA, LSa, BNSTp, AVPV, and VMH. However, this was not the case for V1aR binding. A sex difference in the BNSTa was found in mice; however, the sex difference in this region was not measured in rats due to low receptor expression. Out of the regions that displayed sex differences in both species, however, the direction of the sex difference was not always consistent between mice and rats. Males displayed higher OTR binding in MeA, LSa, BNSTp in both species. However, the AVPV and VMH showed higher OTR binding in females in mice when males showed higher OTR binding in these regions in rats (see Figure 8).

In this discussion, we highlight sex- and species-specific patterns of OTR and V1aR binding density with potential relevance to sex and species differences in the regulation of social behavior. According to previous literature, several assumptions can be made regarding the OT and AVP system parameters and their roles in regulating social behavior. We make the assumption that higher receptor binding density suggests higher receptor activation (Young et al., 1999; Knobloch et al., 2012; Johnson et al., 2016). Additionally, if OTR or V1aR expressed in a given brain region has already been shown to be implicated in the facilitation of a particular social behavior, we assume that higher receptor binding density would enhance this facilitation (Popi &van Ree, 1991; Engelmann & Landgraf, 1994; Everts & Koolhaas, 1999). However, we also recognize that these assumptions regarding OT and AVP system functions may be oversimplified. For instance, OT and AVP system function is also inherently dependent on OT and AVP release

Figure 7.

Oxytocin Receptor Binding: Similar to Rats

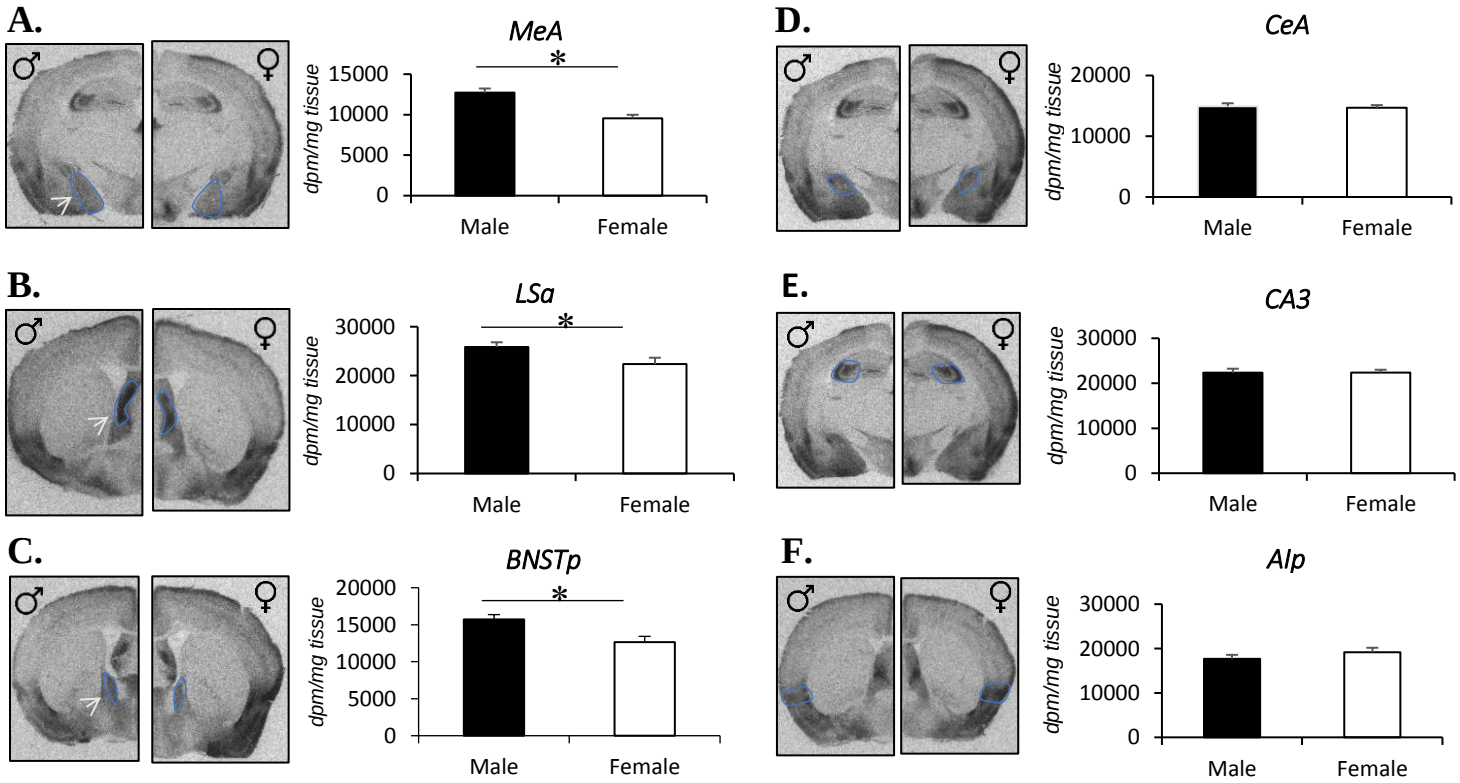

Figure 7: Oxytocin Receptor Binding: Similar to Rats
Representative coronal sections showing OTR binding densities in regions of the SBNN (outlined in blue) in male (left) and female (right) mice. White arrows indicate sex with higher OTR binding density. In both mice and rats, males demonstrated higher OTR binding densities in the MeA (A.), LSa (B.), and BNSTp (C.). Both rodent species also displayed no sex difference in the CeA (D.), CA3 (E.), and AIp (F.) Bars indicate means + SEM. *= $p<0.05$. Student's t-test. Rat data extrapolated from *Dumais et al., 2013* and *Smith et al., 2017*.

Figure 8.

Oxytocin Receptor Binding: In Contrast to Rats

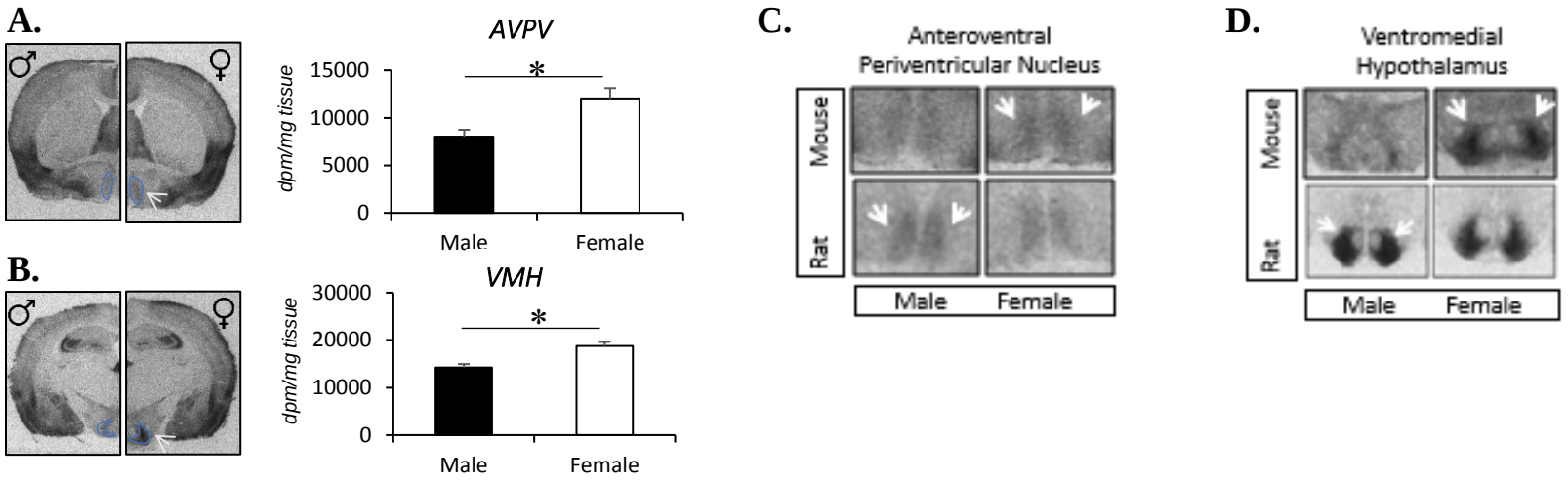

Figure 8: Oxytocin Receptor Binding: In Contrast to Rats
Representative coronal sections showing OTR binding densities in regions of the SBNN (outlined in blue in A. and B.) in male (left) and female (right) mice. White arrows in A.-D. indicate sex with higher OTR binding density. Female mice showed higher OTR binding than male mice in the AVPV (A.), and VMH (B.). This is in contrast to the sex difference in rats, where males showed higher OTR binding densities in these regions (C., D.). Bars indicate means + SEM. *= $p<0.05$. Student's t-test. Rat images and data were extrapolated from (*Dumais et al., 2013* and *Smith et al., 2017).*

Figure 9.

Vasopressin V1a Receptor Binding: Similar to Rats

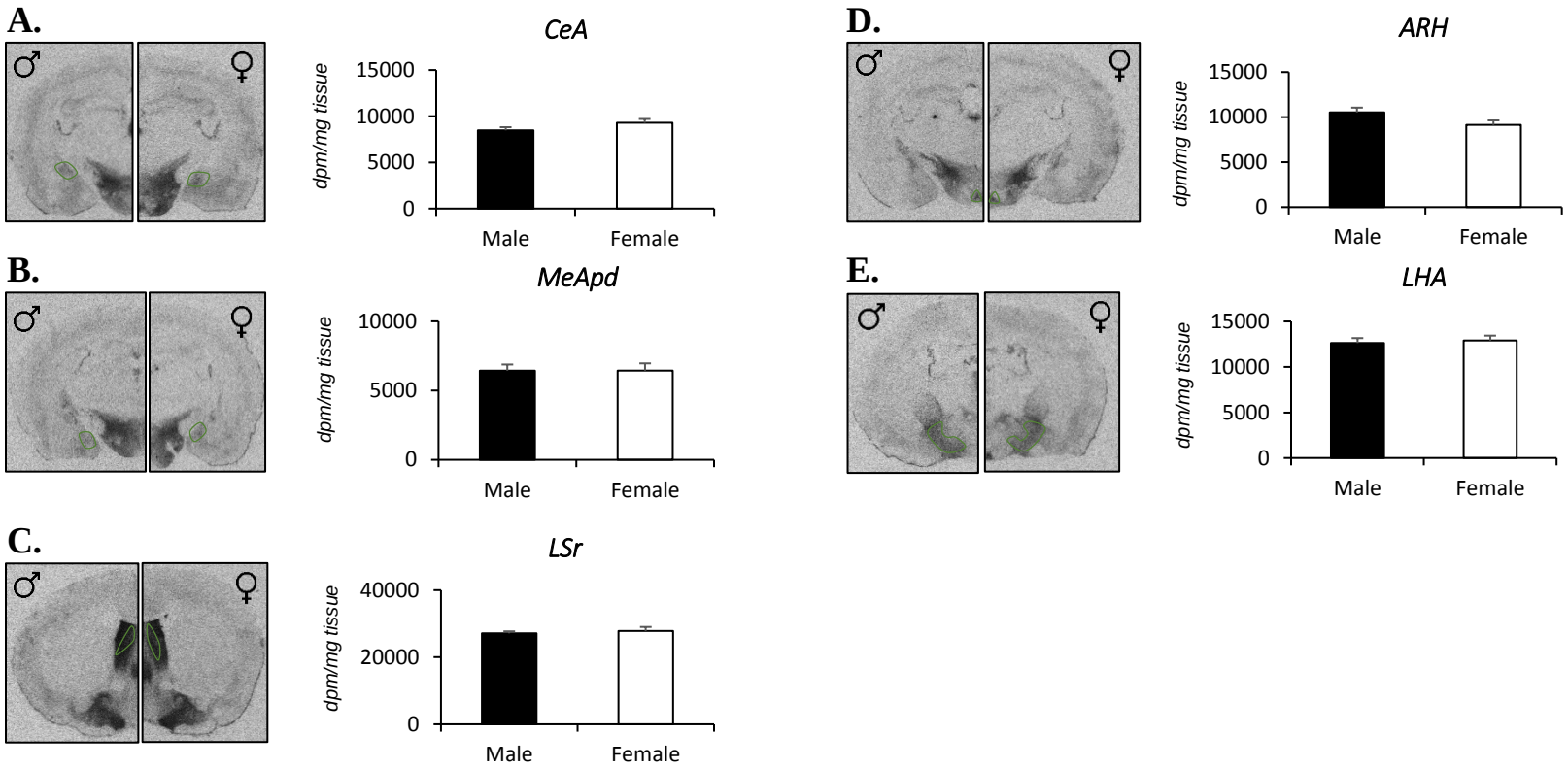

Figure 9: Vasopressin V1a Receptor Binding: Similar to Rats
Representative coronal sections showing V1aR binding densities in regions of the SBNN (outlined in green) in males (left) and female (right) mice. The CeA, MeApd, LSr, ARH, and LHA (A.-E.) all do not display sex differences in V1aR binding in mice. This is similar to results seen in rats. Bars indicate means + SEM. *= $p<0.05$. Student's t-test. Rat V1aR data were extrapolated from *Dumais & Veenema, 2016b* and *Smith et al., 2017.*

Figure 10.
Vasopressin V1a Receptor Binding: In Contrast to Rats

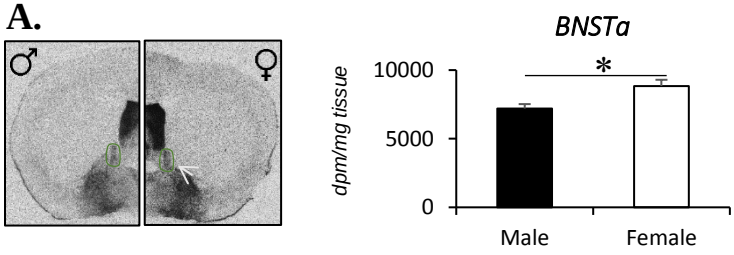

Figure 10: Vasopressin V1a Receptor Binding: In Contrast to Rats
Representative coronal section showing V1aR binding density in the BNSTa (outlined in green) in male (left) and female (right) mice. Female mice showed higher V1aR binding in the BNSTa. This sex difference was not measured in rats potentially due to low expression of receptors in this subregion. Bars indicate means + SEM. *= $p<0.05$. Student's t-test. Rat V1aR data were extrapolated from *Dumais & Veenema, 2016b* and *Smith et al., 2017.*

in the brain, and few studies have measured extracellular OT and AVP release from a given brain region (Bosch & Neumann, 2010; Bosch et al., 2010; Veenema et al., 2010; Lukas et al., 2011; Dumais et al., 2016). Additionally, although OTR and V1aR binding density patterns are unique among rodent species (Beery et al., 2008; Kelly & Ophir, 2015; Albers, 2015; Hammock, 2015), we will mainly be discussing relevant research in mice and rats. Keeping these limitations in mind, the overall main purpose of this discussion is to provide a framework for the sex-specific functions of OTR and V1aR in mice and rats.

Conservation of sex differences in OTR binding in the medial amygdala, anterior LS, posterior BNST in mice and rats: relevance to sex-specific social behaviors

OTR binding was denser in males than females in the MeA, BNSTp, and LSa in mice. The direction of the sex difference was also seen in previous studies in rats (Uhl-Bronner et al., 2005; Dumais et al., 2013; Smith et al., 2017), suggesting that these are highly conserved and robust sex differences. These brain regions represent core nodes of the SBNN (Newman 1999), and several

studies suggest that the OT promotes pro-social behaviors by acting on these nodes in a sex-specific manner in mice and rats. For example, OT facilitates social recognition in adult male mice by acting on OTR in MeA (Samuelsen & Meredith, 2011; Ferguson et al., 2001). In female mice, social recognition is not only mediated by OT, but also dependent on estrogen receptors (ER) α and β in the MeA (Choleris et al., 2003; Choleris et al, 2006). While male mice were not used as experimental models in these specific studies, another experiment demonstrated that ER α and β knockout male mice exhibit no behavioral deficits in detection of predator chemical signals (Kavaliers et al., 2008). This demonstrates that ER could potentially regulate olfactory-mediated social recognition differently in males and female mice. In parallel to mice, OT and OTR in the MeA are well known to play an important role in the regulation of social recognition in rats. Social recognition was impaired in male rats after OTR blockade in the MeA (Ferguson et al., 2001; Lukas et al., 2013). Furthermore, administration of OTR antagonist into the LS via retrodialysis impaired social memory in male rats (Lukas et al., 2013). In female rats, social investigation time correlated positively with OTR binding density in the MeA and not in males (Dumais et al., 2013), further confirming the sex-specific involvement of OT in processing social odor cues and mediating pro-social behaviors across species.

Besides the MeA and LS, OT regulates social behaviors differently in males and females in the BNSTp. For instance, OT administration into the BNSTp prolongs social recognition in male rats but not in females (Dumais et al., 2016). Unfortunately, to the best of our knowledge, the role of OTR in the BNSTp in mice are unknown. However, it is important to mention that the BNSTp is a highly sexually dimorphic brain region in terms of cell number, neurochemical expression, and neurocircuitry in both rats and mice (for review see Dumais et al., 2016). The BNSTp contains more cells in males than in females in both rats and mice (Del Abril et al., 1987;

Sex difference in OTR binding: Mice vs. Rats			
Similar to Rats		**In Contrast to Rats**	
MeA	Males higher than females	**AVPV**	Females higher than males
LSa	Males higher than females	**VMH**	Females higher than males
BNSTp	Males higher than females		
CeA	No sex difference		
AIp	No sex difference		
CA3	No sex difference		
LSv	No sex difference		
LSr	No sex difference		

Table 3: Sex Difference in OTR binding: Mice vs. Rats
The direction of the sex difference in OTR binding in the MeA, LSa, and BNSTp were similar to the direction previously seen in rats, where males demonstrated higher binding than females in these regions. Females showed higher OTR binding density in the AVPV and VMH. This is in contrast to findings in rats, where males showed higher binding in these regions. The regions that displayed no sex differences in OTR binding also showed no sex differences in rats. These results were compared to findings previously seen in rats according to data in *Dumais et al., 2013* and *Smith et al., 2017*.

Sex difference in V1aR binding: Mice vs. Rats			
Similar to Rats		**In Contrast to Rats**	
CeA	No sex difference	**BNSTa**	Females higher than males
LHA	No sex difference		
LSr	No sex difference		
MeApd	No sex difference		
ARH	No sex difference		

Table 4: Sex Differences in V1aR Binding: Mice vs. Rats
Female mice showed higher V1aR binding than male mice in the BNSTa. This sex difference was not measured in rats due to low receptor expression in this subregion. Both mice and rats showed no sex difference in V1aR binding in the CeA, LHA, LSr, and MeApd. Female rats showed higher binding in the ARH according to *Smith et al.*, 2017; however, no sex difference was found in this region according to *Dumais & Veenema, 2016b*. These results were compared to findings previously seen in rats according to data in *Dumais & Veenema, 2016b* and *Smith et al., 2017*.

Guillamon et al., 1988; Forger et al., 2004). Male rats also show higher ER α (Kelly et al., 2013) and vasopressin (De Vries & Miller, 1998) immunoreactivity in the BNSTp. Importantly, compared to female rats, male rats have a three-fold higher OTR binding density in the BNSTp (Dumais et al., 2013). This is the first study to confirm the direction of the sex difference in OTR binding density in the BNSTp in mice, where males, again, exhibit denser OTR binding than

females. Because all these parameters are higher in males than in females, this suggests that the BNSTp in males is designed to potentially respond in a distinct way to incoming social olfactory cues from afferent brain regions (Halpern, 1987; Guillamon & Segovia, 1997), assess information, and mediate behavioral responses differently than in females. For instance, the sex differences in the BNSTp may potentially allow for the promotion of male and female-specific expression of social behaviors in rats, including male aggressive behavior (Calcagnoli et al., 2014), maternal behavior (Numan & Numan 1996), and male copulatory behavior (Claro et al., 1995). Because less is known about the function of OT and OTR in the BNSTp in mice, it would be of interest to determine the behavioral implications of a male-biased OTR expression in mice and compare findings to those seen in rats.

Taken together, our findings demonstrate that the male-biased sex differences in OTR binding in the MeA, LSa, and BNSTp are conserved across species, and studies suggest that the OT system acting in these regions may potentially regulate behavioral responses in a sex-specific manner. Importantly, because these differences are conserved across several mammalian species, they may have similar mechanisms which establish and maintain the differences in OTR binding in these regions. With novel information in mice, we could further explore the function of OT system in these brain-regions in mice and compare these findings to those previously seen in rats. Additionally, it would also be an interesting avenue of research to determine the genetic contributions to the sexual dimorphic OT system using the available knockout and transgenic mice.

Mice and rats display opposite sex difference in OTR binding in the AVPV and VMH

OTR binding was denser in females than in males in the AVPV and VMH in mice. This is in contrast to findings in rats, where males demonstrated higher OTR binding in these regions

(Uhl-Bronner et al., 2005; Dumais et al., 2013). Interestingly, the AVPV and VMH in both mice and rats are sexually dimorphic in terms of morphology and neurochemical expression, and studies have shown that the sexual dimorphic parameters may lead to sex differences in parental and reproductive behaviors (Bleier et al., 1982; Matsumoto & Arai, 1983; Simerly et al., 1985). In the AVPV, females have a higher cell density and volume than males in both rats and mice (Bleier et al., 1982). Moreover, female rats have a higher distribution of AVPV neurons containing dopamine (Simerly et al., 1985) and cholescystokinn (Micevych et al., 1987) than male rats. In the case of the VMH, the volume has been reported to be higher in males than females (Matsumoto & Arai, 1983), and masculinization of this region in rats involves the work of androgen receptors, which are also higher in concentration in males (Dugger et al., 2006).

Our findings in mice add to the sexual dimorphic parameters displayed by the AVPV and VMH, which may overall contribute to sex-specific modulation of social behaviors. For example, Scott et al., 2015 demonstrated that tyrosine hydroxylase (TH)-expressing neurons in the AVPV of mice are more numerous in females than in males, and this governs the maternal care and OT secretion in female mice while not having any effect on paternal care. Increased TH expression in the AVPV TH(+) neurons of female mice increased OT secretion, while ablation reduced OT circulation (Scott et al, 2015). Additionally, it was shown that the AVPV TH(+) neurons relay a monosynaptic input to OT-expressing neurons in the AVPV (Scott et al., 2015). These findings demonstrate a causal relationship between a sexual dimorphic neural circuit and sex differences in paternal behavior. It would be of interest to investigate whether the higher OTR binding in the AVPV in female mice would also play a role in the sexual dimorphic neural circuit that regulate sex differences in parental behavior. Additionally, the VMH in both mice and rats is established as a major site in the regulation of sexual behavior in a sex-typical way. OT has been shown to

stimulate lordosis in female rats (Arletti et al., 1985; Schulze & Gorzalka 1991) while improving copulatory behaviors in male rats (Arletti et al., 1985). This region in mice has also been implicated in the regulation of sexual behaviors as well (Olivier & Wiepkema 1974). The sex difference in progesterone receptor expressing neurons in the VMH controls distinct sex-typical behaviors in male and female mice, including lordosis in females and mating and aggression in males (Yang et al., 2013). It would be of interest to investigate whether OT or OTR would influence sexual behavior and aggression in a sex-specific way in mice and compare findings to those seen in rats.

Sex difference in V1aR binding in the anterior BNST: role in anxiety in mice and rats

The V1aR binding density was higher in female mice than in male mice in the BNSTa. This sex difference was not measured in rats due to low V1aR expression in this region Previous studies indicate that this subdivision of the BNST is a crucial region for modulating anxiety in rodents (Song & Knopfel, 2015; Lebow & Chen., 2016). For example, the BNSTa has been shown to exert anxiolysis in mice (Kim et al., 2013). Using optogenetics in mice, it has been elucidated that projections from the BNSTa to LH, parabrachial nucleus, and ventral tegmental area promote reduced risk-avoidance, reduced respiratory rate, and increased positive valence, respectively, while inhibition of these circuit elements induced opposing behavioral effects (Kim et al., 2013).

Unlike the BNSTp, the anterior division does not display neuroanatomical size differences (Campi et al., 2013); however, this subdivision has been implicated in regulating sex-specific anxiety-related behaviors in rodents. Evidence suggests that female rats, but not males, have higher neuronal activity in the BNSTa after restraint stress (Babb et al., 2013). Additionally, stress-induced increase in brain-derive neurotrophic factor protein in the BNSTa has been shown to contribute to social withdrawal in females but not in male mice (Greenberg et al., 2013).

Unfortunately, studies on the role of V1aR in the anterior division is limited; however, the V1aR in other subregions of the BNST has been shown to modulate anxiety in rodents in a sex-specific manner as well. While V1aR inhibition in the medial ventral BNST (BNSTmv) had anxiogenic effects in both social and nonsocial contexts in males; the effects of the inhibition in females were limited to social contexts (Duque-Wilckens et al., 2016). It would be of interest to investigate whether the V1aR in the BNSTa would also produce anxiolytic effects in a sex-specific way in mice and rats and also elucidate the potential sex-specific underlying neurocircuitry. Studying the sex differences in the anxiety-related neurocircuitries and behaviors may also help explain the vast discrepancy between the prevalence and treatment outcomes of men and women diagnosed with depressive episodes and anxiety disorders.

6. CONCLUSION

An advantage of using a comparative approach in neuroscience research is that discoveries made from different model organisms can be used to determine general rules relating to brain structure and function that may apply across many vertebrate species. Rats have been the traditional animal of choice because they are docile, have readily large brains, breed readily, and are practical to work with. However, as mice are gaining in popularity due to the availability of genetic tools, it is necessary to understand the similarities and differences between these two important model organisms. Our work demonstrates conserved as well as unique sex differences in OTR and V1aR binding in nodes of the SBNN in mice and rats. We found regions where the sex difference in OTR and V1aR binding are similar in direction in both rats and mice. We also found regions where the direction of the sex difference in OTR binding is contrasting between the two rodent species. We further discussed the potential relevance of our findings to sex-specific

and species-specific behaviors. Moving forward, researchers can attend to these species differences as they select the optimal model organism to investigate the sex differences in OTR and V1aR across species to gain a better understanding of the sex-specific behavioral functions of OT and AVP systems.

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