Published in final edited form as:

Neuroscience. 2012 October 11; 222: 20–28. doi:10.1016/j.neuroscience.2012.07.025.

Modulation of Parvalbumin Interneuron Number by Developmentally Transient Neocortical Vasopressin Receptor 1a (V1aR)

Elizabeth A.D. Hammock^a and Pat Levitt^b

^aDepartment of Pediatrics, Vanderbilt University School of Medicine & Vanderbilt Kennedy Center, Vanderbilt University, Nashville TN, 37232

^bZilkha Neurogenetic Institute, Department of Cell and Neurobiology, Keck School of Medicine of the University of Southern California, Los Angeles, CA 90089

Abstract

Arginine-vasopressin (AVP) and the vasopressin 1a receptor (V1aR) modulate social behavior and learning and memory in adult animals. Both functions depend upon the normal emergence of the balance of excitation and inhibition (E/I balance) in the neocortex. Here, we tested the hypothesis that V1aR signaling and E/I balance converge through the influence of the neuropeptide on interneuron number achieved in the neocortex. Postnatal mapping of forebrain V1aR binding in male and female mice revealed a transient expression of high levels of receptor in the neocortex and hippocampus in the second and third post-natal week. Receptor binding levels in these cortical structures fell dramatically in the adult, maintaining high expression subcortically. Surprisingly, we observed sex differences in the number of calbindin interneurons, and a contribution of V1aR to the number of parvalbumin immunoreactive neurons in the adult mouse neocortex. These data suggest that individual differences in developmentally transient V1aR signaling and even sex may alter the development of E/I balance in the neocortex, with long- lasting influence on information processing.

Keywords

C57BL/6J; post-natal development; sex difference; parvalbumin; calbindin; vasopressin 1a receptor

Two neural systems, neocortical GABAergic interneurons and the neuropeptide arginine-vasopressin (AVP), have been implicated in social behavior, and disruptions of these same systems have been reported in neurodevelopmental disorders in which there are atypical

© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

***Correspondence:** Elizabeth A.D. Hammock, Ph.D., Dept. Pediatrics, Vanderbilt University, 2215 Garland Ave, Light Hall, Rm 1115, Nashville, TN 37232, liz.hammock@vanderbilt.edu, Ph: 615-322-5913, F: 615-343-2093. Pat Levitt, Ph.D., Zilkha Neurogenetic Institute, Keck School of Medicine of University of Southern California, 1501 San Pablo Street, Room 105, Los Angeles, CA 90089-2821, plevitt@med.usc.edu, Ph: 323-442-1509, F: 323-442-2145.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author contributions

EADH co-conceived of the idea, performed and/or directly supervised all experiments, and co-wrote the manuscript. PL co-conceived of the idea, supervised, secured funding, and co-wrote the manuscript.

social interactions (reviewed in (Hammock and Young, 2006, Eagleson et al., 2010)). Growing evidence supports the hypothesis that disturbance in cerebral cortical excitation/inhibition (E/I) balance underlies the pathology of disorders of social information processing including autism spectrum disorders (ASD) and schizophrenia (Rubenstein and Merzenich, 2003, Belmonte et al., 2004, Levitt et al., 2004, Lewis et al., 2005, Chao et al., 2010, Eagleson et al., 2010). Typically, however, the neuropeptide and cortical interneuron networks have been studied separately, particularly because V1aR has a relatively unimpressive neocortical presence in adult mammals, such as rats, mice, voles, and hamsters (Petracca et al., 1986, Ostrowski et al., 1994, Dubois-Dauphin et al., 1996, Wang et al., 1997a, Wang and Young, 1997, Wang et al., 1997b, Campbell et al., 2009), with some exception (Rabhi et al., 1999, Beery et al., 2008).

Recently, through optogenetics, experimental disruption in the typical E/I balance has been functionally linked to social behavior changes (Yizhar et al., 2011). Parvalbumin (PV) interneurons have been a particular focus because of their role in influencing experience-dependent circuit refinement (Hensch, 2005, Kuhlman et al., 2010). Alterations in PV interneuron number or synapses have been reported in schizophrenia (Hashimoto et al., 2003, Lewis et al., 2005) and in mouse models of ASD (Gogolla et al., 2009).

Across mammalian species, AVP acts within the brain to modulate social-emotional behavior (reviewed in (Donaldson and Young, 2008, Frank and Landgraf, 2008)). Depending on the species, sex, and brain area, AVP increases anxiety-like behavior, social recognition memory, aggression, and mated partner preference behavior. Much of this behavioral work has been performed in adults and suggests that AVP acts through the central AVP receptor 1a (V1aR) and in the anterior pituitary through V1bR (Tanoue et al., 2004). Though likely to be small in effect size, genetic association studies implicate *AVPR1A* in ASD risk (Kim et al., 2002, Wassink et al., 2004, Yirmiya et al., 2006, Yang et al., 2010a, Yang et al., 2010b). Additionally, altered AVP signaling has been implicated in schizophrenia, especially in a sub-population with co-morbid polydipsia and hyponatremia (Kishimoto et al., 1989, Delva et al., 1990, Ohsawa et al., 1993, de Leon et al., 1994, Goldman et al., 1997, Ryan et al., 2004).

The independent relationships between each of these neurochemical systems and socialemotional behavior prompted us to explore a potential relationship between the central AVP system and neocortical interneurons. Because the number of interneurons in the neocortex is established developmentally (Wonders and Anderson, 2006), we mapped V1aR by ligand binding postnatally in male and female C57BL/6J mice. We then assessed the long-term consequences of constitutive V1aR loss on neocortical interneuron populations.

1. Experimental Procedures

1.1 Mice

All experiments were performed with the approval and oversight of the Institutional Animal Care and Use Committee of Vanderbilt University in accordance with local, state and federal regulations. Wild type C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA) and bred in-house for the developmental mapping study. Breeders were checked daily for litters (day of birth=P0; weaning=P21). *Avpr1a* mice on a mixed C57/129 background were a generous gift from Dr. Larry Young at Emory University descended from mice generated by Shuang-Bao Hu and colleagues (Hu et al., 2003) (*Avpr1a^{tm1Sbhu}*). The *Avpr1a* mice used in this report were fully backcrossed to the C57BL/6J strain at Vanderbilt University: backcrossing was confirmed at 99.9% by strain-specific SNP genotyping (Genome Scanning Service, The Jackson Laboratory, Bar Harbor, ME). All experimental *Avpr1a* mice were offspring of heterozygous matings to control for the effects

of parental genotype. Mice were housed on a 12:12 L:D cycle with *ad libitum* access to food and water. Genotypes were determined by PCR from tail DNA (Avpr1a_WT_For_5'-GGGCTGAGTTTCGTTCTGAGCATAC, Avpr1a_WT_Rev_5'-GGTCATCTTCACTGTGCGGATCTTG; Avpr1a_KO_For_5'-AGAGGCTATTCGGCTATGACTG, Avpr1a_KO_Rev_5'-TTCGTCCAGATCATCCTGATC).

1.2 Receptor autoradiography

Three mice per sex from at least two litters per age were used for the developmental analysis of V1aR ligand binding. Mice were deeply anesthetized with isoflurane vapors and euthanized. Brains were removed and snap frozen on dry ice and stored at -80°C until cryosectioned at $20\text{ }\mu\text{m}$ in six series and thaw mounted to Superfrost plus microscope slides. Slides were stored at -80°C until the receptor binding study was performed using established methods (Hammock and Young, 2005) using 50pM I^{125} linear-vasopressin antagonist (Manning et al., 1988, Schmidt et al., 1991) (NEX 310, NEN/Perkin-Elmer, Waltham, MA, USA). Kodak Biomax MR film (Carestream Health, Inc., Rochester, NY, USA) was exposed to the slides for an initial film development of 70 hours, immediately followed by another film exposure of exactly 24 hours. The experiment was performed three separate times with one male and one female from each age included in each experiment. Films from the 24 hour exposure were used for quantification and were scanned at high resolution (1200 DPI) on a flatbed scanner (Epson Expression 1680, Seiko Epson Corporation, Suwa, Nagano, Japan). Quantification was performed in IMAGE J (NIH, Bethesda, MD, USA) with three consecutive samples obtained per brain area. ROI were selected by comparison to an atlas (Paxinos and Franklin, 2001) and by post-processing the slides for acetylcholinesterase (Lim et al., 2004) or Nissl substance by cresyl violet. Densitometry measurements were converted to $\mu\text{Ci/g}$ by comparison to C^{14} autoradiographic standards (American Radiolabeled Chemicals, St. Louis, MO, USA) co-exposed with the slides on the same film. Interpolation was performed in Matlab 7.0.4 (The Mathworks, Natick, MA, USA) using the 'interp1' function with the standards that fell within the linear range of the film. Specific activity was determined by subtraction of tissue background sampled from the dorsal striatum for each specimen at each age.

1.3 Immunohistochemistry

Interneuron marker analysis was done essentially as described previously (Powell et al., 2003, Eagleson et al., 2005). At 3 months of age ($n=7$ per sex per genotype), littermate *Avpr1a* wild type and knockout mice were lightly anesthetized with isoflurane vapors, weighed, deeply anesthetized with Nembutal (120 mg/kg IP, Abbott Laboratories, Abbott Park, IL) and transcardially perfused with a freshly prepared room temperature solution of formaldehyde-lysine-periodate fixative. Following cryoprotection and sectioning at $40\text{ }\mu\text{m}$, the tissue was stored in cryoprotectant at -20°C until immunostaining with 1:500 mouse anti-parvalbumin, clone PARV-19 (Sigma-Aldrich, St. Louis, MO, USA) or 1:10,000 anti-calbindin D-28k, CB38a (Swant, Bellinzona, Switzerland) in 4% non-fat dry milk, 0.2% Triton-X100 in PBS at 4°C with gentle rotation. Sections were washed and then incubated in secondary antibody of 1:1,000 Biotin-SP donkey anti-mouse IgG or 1:1,000 Biotin-SP-donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) for 1 hour at room temperature, then washed, incubated in ABC reagent (VectaStain Standard ABC kit, Vector Laboratories, Inc., Burlingame, CA, USA) for 1 hour at room temperature, washed again and incubated in DAB peroxide for color detection.

1.4 Microscopy and quantitation

Microscope images were obtained at 10X on a Zeiss Axioplan with a high resolution color camera. All image capturing and quantitation was performed on coded slides by an

experimenter blinded to sex and genotype. Three sections per animal, each spaced 240 μm apart, were captured between -0.4 and -1.0 mm Bregma, and included medial aspects of the barrel field representation, S1 dysgranular region, shoulder and neck representation, forelimb representation and lateral aspects of hindlimb representation. All specimens were captured at the same microscope session with the same lighting conditions and microscope settings. Images were converted to gray scale 16 bit images and analyzed with IMAGE J (NIH, Bethesda, MD, USA). Debris was digitally removed and a constant threshold was chosen that was sufficient to detect cell bodies for all images. After thresholding, watershedding was used to individuate closely spaced cells. “Analyze particles” was then used to count cells and measure IR area meeting pre-defined size and circularity criteria, applied equally to all images. IR area in pixels was converted to cell diameter in microns by comparison to a slide micrometer. “Show outlines” was used to visually confirm that the automated counting matched the overall impression of the cells in the original microscope image. Raw cell counts were systematically corrected with the Abercrombie formula (Abercrombie, 1946).

1.5 Statistical Analysis

Data are presented as the mean $\pm$ S.E.M. Statistical analysis of receptor autoradiography was performed in Matlab 7.0.4 using two-way ANOVA (‘anova2’) with a sample size of 3 per age per sex. Each brain region was analyzed separately. Interneuron markers were also analyzed by two-way ANOVA with a sample size of 7 per sex per genotype in Matlab 7.0.4 with sex and genotype as independent variables. Minimum sample sizes were chosen for approximately 80% power to detect sex and genotype effect sizes greater than 1. An alpha level of 0.05 was used for significance for all experiments. Data were checked for normal distribution by kurtosis and skewness and when not meeting criteria for a normal distribution, the data were square root transformed.

2. Results

2.1 V1aR expression in neocortex and hippocampus is transient

V1aR binding was evident at all ages examined, with complex spatiotemporal patterns of expression (Figure 1). We observed no binding in V1aR KO tissue (data not shown). Although the selective ligand for V1aR showed increased binding to certain brain regions in developing tissue, this does not necessarily lead to a linear increase in the capacity for AVP to signal. GPCRs, including AVP receptors, potentially dimerize, altering the signaling kinetics of the receptor (Albizu et al 2006). Thus, specific ligand binding here is interpreted as receptor protein expression.

High levels of detectable binding were transient in the neocortex and hippocampus, which peaked at the beginning of the second post-natal week. Labeling in the hippocampus, as measured in CA1, was relatively intense through P21, and decreased substantially by P35. In the neocortex, layer IV was most densely labeled at P7, but decreased substantially by P14. We observed very low/no receptor binding in the neocortex at weaning (P21), adolescence (P35) and into adulthood (P60). Subcortically, regions that exhibit receptor expression in the adult, such as the septum, diagonal band, amygdala, paraventricular thalamic nucleus, lateral hypothalamus, ventral tegmental area and superior colliculus also showed binding developmentally (Figure 1).

Statistical analysis was performed to examine the variables of age and sex on receptor binding. In the CA1 subfield of the hippocampus, there was a significant main effect of age (d.f. 6, $F = 61.541$, $p = 7.77 \times 10^{-15}$) with no main effect of sex (d.f. 1, $F = 0.77$, $p = 0.387$), nor an interaction (d.f. 6, $F = 1.14$, $p = 0.365$) (Figure 2A). In the neocortex (sampled at S1

somatosensory cortex), the pattern was similar: there was an abrupt increase in receptor binding at the end of the first post-natal week, which declined with age. There was a main effect of age (d.f. 6, $F = 26.0$, $p = 3.12 \times 10^{-10}$), no main effect of sex (d.f. 1, $F = 0.0917$, $p = 0.764$), and a trend toward an age $\times$ sex interaction (d.f. 6, $F = 2.17$, $p = 0.077$) (Figure 2B).

In contrast, there was no statistically significant developmental shift in V1aR ligand binding in the septum. While there was a trend toward age as a significant factor (d.f. 6, $F = 2.17$, $p = 0.079$), it appears that this main effect represented only a subtle transient profile, with a peak at the end of the first post-natal week (Figure 2C). There was no main effect of sex (d.f. 1, $F = 2.15$, $p = 0.154$) nor an interaction between sex and age in the septum (d.f. 6, $F = 0.669$, $p = 0.675$). Similarly, the VTA showed only a trend toward a main effect of age (d.f. 6, $F = 2.30$, $p = 0.062$) and sex (d.f. 1, $F = 2.82$, $p = 0.104$) and no interaction (d.f. 6, $F = 0.752$, $p = 0.613$) (Figure 2D).

2.2 Constitutive loss of *Avpr1a* increases parvalbumin immunoreactivity in adult S1 cortex

Constitutive deletion of *Avpr1a* did not yield any obvious changes in mouse health or size (overall ANOVA for weight: genotype $p = 0.61$, sex $p = 5.8 \times 10^{-08}$, interaction $p = 0.75$). Histological examination of the brains of null mice did not reveal gross differences in morphology, such as nuclear organization subcortically and lamination in the neocortex.

Given the transient neocortical expression of V1aR during postnatal development, a study was undertaken to determine if there are long-term neuroanatomical consequences of loss of *Avpr1a*. Because E/I balance influences social behavior (Yizhar et al., 2011), and the parvalbumin population of interneurons have been implicated in this process, parvalbumin-immunoreactive (PV-IR) interneurons in adult neocortex were quantified (Figure 3). Loss of *Avpr1a* resulted in an increase in PV-IR cells (Figure 3B). Overall ANOVA on Abercrombie-corrected square root transformed cell counts indicated no main effect of sex (d.f. 1, $F = 1.8504$, $p = 0.19$), a main effect of genotype (d.f. 1, $F = 5.76$, $p = 0.025$) and no significant interaction (d.f. 1, $F = 1.24$, $p = 0.28$). Post-hoc analysis of the effect of loss of *Avpr1a* indicated that the increase in PV-IR neurons in the mutant mice was more dramatic in the females as it reached significance in females (post-hoc Tukey's least significant difference $p < 0.05$) but not in the males (post-hoc Tukey's least significant difference $p > 0.05$). Sex differences in PV-IR were not significant for either genotype (post-hoc Tukey's least significant difference $p > 0.05$). Statistical analysis of non-Abercrombie-corrected square root transformed cell counts yielded similar results. In addition to the increase in cell number, PV-immunopositive neurons exhibited a larger diameter per cell in the *Avpr1a*^{-/-} mice (Figure 3C): overall ANOVA indicated no main effect of sex (d.f. 1, $F = 0.908$, $p = 0.350$), a main effect of genotype (d.f. 1, $F = 5.74$, $p = 0.025$) and no significant interaction (d.f. 1, $F = 1.28$, $p = 0.268$).

2.3 Constitutive loss of *Avpr1a* does not alter calbindin-IR in S1 cortex

To determine whether *Avpr1a* loss impacts cortical interneurons in general, we also analyzed calbindin-IR. We observed no effect of *Avpr1a* loss on calbindin-IR cell number, although we identified a heretofore unknown significant main effect of sex across genotypes (Figure 3D). Overall ANOVA on Abercrombie-corrected square root transformed cell counts indicated a significant main effect of sex (d.f. 1, $F = 19.99$, $p = 0.00016$), with females having approximately twice as many CR-IR neurons in wild type and null mice. There was, however, no significant main effect of genotype (d.f. 1, $F = 3.59$, $p = 0.07$) and no significant interaction (d.f. 1, $F = 0.062$, $p = 0.805$). Post-hoc analysis indicated that females had more calbindin-IR cells in both the wild-type (post-hoc Tukey's least significant difference, $p < 0.05$) and knockout condition (post-hoc Tukey's least significant difference, $p < 0.05$). Post-hoc analysis indicated no significant group differences specific to genotype (post-hoc

Tukey's least significant difference, $p > 0.05$). Statistical analysis of square root transformed calbindin-IR cell counts with and without Abercrombie correction yielded identical results. In addition to increased cell number in females, the average calbindin-IR cell diameter also was larger in females (Figure 3E). Overall ANOVA of IR cell diameter indicated a significant main effect of sex (d.f. 1, $F = 8.76$, $p = 0.0068$), no main effect of genotype (d.f. 1, $F = 2.46$, $p = 0.129$) and no significant interaction (d.f. 1, $F = 0.039$, $p = 0.844$).

3. Discussion

In the present study, we mapped the postnatal trajectory of V1aR ligand binding in the C57BL/6J mouse forebrain, where we observe a transient expression of V1aR in the neocortex and hippocampus at the end of the first post-natal week. In addition to mapping the receptors, we determined the consequences of constitutive loss of V1aR on the expression of markers of interneurons in the neocortex. Loss of V1aR results in significantly more PV-IR cells in the neocortex. Also, we observed that females have significantly more calbindin-IR cells than males. These data indicate a novel finding that developmentally transient V1aR signaling and sex contribute to cortical interneurons. V1aR signaling appears to negatively regulate the complement of PV interneurons in the neocortex, while female factors positively regulate (or male factors negatively regulate) the complement of calbindin interneurons. Interneurons and AVP signaling have been implicated separately in species-typical mammalian social behavior, and could interact developmentally to affect stable E/I balance in adults.

Early attempts to define the developmental trajectory of AVP binding sites in the mammalian brain were performed using tritiated vasopressin ($[^3\text{H}]\text{-AVP}$) in rats by receptor autoradiography (Petracca et al., 1986, Snijdwint et al., 1989, Szot et al., 1989, Tribollet et al., 1991a, Tribollet et al., 1991b, Szot et al., 1992). These studies identified several brain regions with early and prolonged expression such as the lateral septum and the amygdala. Other brain areas demonstrated transient profiles of expression with peak AVP binding levels prior to weaning, such as the caudate, the hippocampus and the cingulate cortex. However, the transient $[^3\text{H}]\text{-AVP}$ binding in the cingulate cortex of the rat was later determined to be a transient oxytocin receptor profile (Tribollet et al., 1991b), highlighting the need for developmental mapping studies with highly selective V1aR ligands. While $[^3\text{H}]\text{-AVP}$ is not an ideal ligand for mapping V1aR, these studies were consistent with the intriguing hypothesis that vasopressin may play a role in brain development, as had been postulated by Boer and colleagues who reported brain malformations in the vasopressin deficient Brattleboro rat (Boer et al., 1982a, Boer et al., 1982b).

Our results in mice at P60 (8.5 weeks) are identical to those reported by Dubois-Dauphin and colleagues (Dubois-Dauphin et al., 1996) in C57BL/6J mice at 12 weeks using this highly selective V1aR antagonist, ^{125}I -linear AVP (Schmidt et al., 1991). In particular, the adult C57BL/6J mouse shows no V1aR expression in the neocortex or hippocampus. In contrast, rats showed binding in the neocortex as well as the hippocampus in adulthood with this ligand, indicating species differences in the distribution of this receptor in adulthood (Johnson et al., 1993). No other developmental studies have been performed in mice or rats with the highly selective ^{125}I -linear AVP, which has only been used developmentally in voles (Wang et al., 1997a, Wang and Young, 1997, Wang et al., 1997b). Voles, like rats, have significant early transient expression of V1aR in some brain areas, although the areas differ between species. For example, as measured by $[^3\text{H}]\text{-AVP}$, rats show transient pre-weaning expression (Tribollet et al., 1991b) in the facial nucleus, hypoglossal nucleus, zona incerta, dorsal raphe and locus ceruleus. In contrast, prairie voles (as measured by the highly selective and potent V1a ligand, ^{125}I -linear AVP) show transient expression in the cingulate cortex and montane voles showed transient expression in other neocortical areas (Wang et

al., 1997b). Attempts to map AVP binding sites in developing brains of other species, such as golden hamsters (Delville et al., 1994) and Brazilian opossums (Kuehl-Kovarik et al., 1997), present a consistent picture that 1) there are some brain areas with transient appearance of AVP binding sites that wane before weaning and 2) those sites differ between species. Each of the species examined has a different developmental pattern from the mouse as presented in this report. The mouse appears to be unique regarding the exceptionally dense transient expression of V1aR in the hippocampus, but montane vole-like in the transient expression in the neocortex. Thus, there are species-specific developmental trajectories of receptor expression in distinct brain regions, which includes an early transient peak in some brain area(s) that wane precipitously into adulthood. As with the known anatomical variability in adult expression patterns of this receptor (Young, 1999), these unique developmental trajectories could reflect an early role for V1aR in species-specific socialemotional development. Individual differences in the regulation of V1aR (Hammock and Young, 2005, Knafo et al., 2008) during development might also play a role in individual differences in socialemotional traits, though this will require direct examination.

In addition to the transient developmental aspects of *Avpr1a* expression in the neocortex of the mouse, we observed stable effects of *Avpr1a* loss on the number of PV-IR neurons in the mouse neocortex. Specifically, adult knock-out mice displayed significantly more PV-IR cells. Whereas genotype did not significantly contribute to the number of calbindin-IR cells, we note an unexpected difference between male and female mice in the number of calbindin neurons in the neocortex. The functional significance of this sex bias is unknown, but would be important to examine electrophysiologically in terms of potential sex differences in the ontogeny of cortical E/I balance.

PV interneurons provide a “blanket of inhibition” and are connected to all nearby pyramidal cells (Packer and Yuste, 2011), serving in a broad fashion to dampen local excitatory activity. Our findings are consistent with the possibility that high AVP signaling during development, due to individual differences in AVP levels, *AVPR1A* regulation, osmotic challenges or other early stressors, contribute to reduction in PV interneuron connectivity. We observed peak expression in the neocortex and hippocampus at the beginning of the 2nd post-natal week, which waned by weaning. This period of postnatal mouse cortical development corresponds to the third trimester in pre-natal human cortical development (Clancy et al., 2007). The translational potential of these findings would be greatly enhanced if there were a similar transient developmental peak during pre-natal human brain development. Analysis of expression data in human development (BrainSpan, Allen Institute for Brain Science (61)(54) of *AVPR1A* mRNA in human somatosensory neocortex (Figure 4) reveals a dynamic expression pattern as well, with high neocortical expression prenatally, including substantial individual variation early in life. A regulatory polymorphism in the 5' region of *AVPR1A* has been associated with expression levels in adult human brain (Knafo et al., 2008). Based on our findings in mice, it would be of interest to determine whether individuals with a regulatory polymorphism of *AVPR1A*, conferring greater gene expression in the developing neocortex, would be more sensitive to stressors affecting AVP signaling, which in turn could impact neocortical PV interneuron development.

Acknowledgments

We thank Larry Young, PhD of Emory University for the founder mice we used to establish fully congenic *Avpr1a* mice on a C57BL/6J background. We also acknowledge the gifted technical support of Caitlin Law, Jennifer Fletcher, Lisa McFayden-Ketchum, Paula Woods, Deborah Gregory, Danielle Sganga, Donte Smith and Shelby Smith. Further, we would like to thank Louis Muglia, MD, PhD for facilitating the completion of the studies at Vanderbilt University. This work was supported by NIMH 080759 (P.L.), T32 MH075883 (E.A.D.H) and NICHD P30HD15052 to Vanderbilt University.

References

- Allen Brain Atlas Resources [Internet]. Seattle (WA): Allen Institute for Brain Science; © 2009. Available from: <http://www.brain-map.org>.
- Abercrombie M. Estimation of nuclear population from microtome sections. *The Anatomical Record*. 1946; 94:239–247. [PubMed: 21015608]
- Beery AK, Lacey EA, Francis DD. Oxytocin and vasopressin receptor distributions in a solitary and a social species of tuco-tuco (*Ctenomys haigi* and *Ctenomys sociabilis*). *J Comp Neurol*. 2008; 507:1847–1859. [PubMed: 18271022]
- Belmonte MK, Cook EH Jr, Anderson GM, Rubenstein JL, Greenough WT, Beckel-Mitchener A, Courchesne E, Boulanger LM, Powell SB, Levitt PR, Perry EK, Jiang YH, DeLorey TM, Tierney E. Autism as a disorder of neural information processing: directions for research and targets for therapy. *Mol Psychiatry*. 2004; 9:646–663. [PubMed: 15037868]
- Boer GJ, Uylings HB, Patel AJ, Boer K, Kragten R. The regional impairment of brain development in the Brattleboro diabetes insipidus rat; some vasopressin supplementation studies. *Ann N Y Acad Sci*. 1982a; 394:703–717. [PubMed: 6758657]
- Boer GJ, Van Rheeën-Verberg CM, Uylings HB. Impaired brain development of the diabetes insipidus Brattleboro rat. *Brain Res*. 1982b; 255:557–575. [PubMed: 7074363]
- Campbell P, Ophir AG, Phelps SM. Central vasopressin and oxytocin receptor distributions in two species of singing mice. *J Comp Neurol*. 2009; 516:321–333. [PubMed: 19637308]
- Chao HT, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, Neul JL, Gong S, Lu HC, Heintz N, Ekker M, Rubenstein JL, Noebels JL, Rosenmund C, Zoghbi HY. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*. 2010; 468:263–269. [PubMed: 21068835]
- Clancy B, Kersh B, Hyde J, Darlington RB, Anand KJ, Finlay BL. Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics*. 2007; 5:79–94. [PubMed: 17426354]
- de Leon J, Verghese C, Tracy JJ, Josiassen RC, Simpson GM. Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. *Biol Psychiatry*. 1994; 35:408–419. [PubMed: 8018788]
- Delva NJ, Crammer JL, Lawson JS, Lightman SL, Sribney M, Weier BJ. Vasopressin in chronic psychiatric patients with primary polydipsia. *Br J Psychiatry*. 1990; 157:703–712. [PubMed: 2279208]
- Delville Y, Mansour KM, Quan EW, Yules BM, Ferris CF. Postnatal development of the vasopressinergic system in golden hamsters. *Brain Res Dev Brain Res*. 1994; 81:230–239.
- Donaldson ZR, Young LJ. Oxytocin, vasopressin, and the neurogenetics of sociality. *Science*. 2008; 322:900–904. [PubMed: 18988842]
- Dubois-Dauphin M, Barberis C, de Bilbao F. Vasopressin receptors in the mouse (*Mus musculus*) brain: sex-related expression in the medial preoptic area and hypothalamus. *Brain Res*. 1996; 743:32–39. [PubMed: 9017227]
- Eagleson K, Hammock EAD, Levitt P. Interneuron Pathophysiology: Paths to Neurodevelopmental Disorders. In: Pallas, SL., editor. *Developmental Plasticity of Inhibitory Circuitry*. New York: Springer; 2010.
- Eagleson KL, Bonnin A, Levitt P. Region- and age-specific deficits in gamma-aminobutyric acidergic neuron development in the telencephalon of the uPAR(–/–) mouse. *J Comp Neurol*. 2005; 489:449–466. [PubMed: 16025458]
- Frank E, Landgraf R. The vasopressin system—from antidiuresis to psychopathology. *Eur J Pharmacol*. 2008; 583:226–242. [PubMed: 18275951]
- Gogolla N, Leblanc JJ, Quast KB, Sudhof TC, Fagioli M, Hensch TK. Common circuit defect of excitatory-inhibitory balance in mouse models of autism. *J Neurodev Disord*. 2009; 1:172–181. [PubMed: 20664807]
- Goldman MB, Robertson GL, Luchins DJ, Hedeker D, Pandey GN. Psychotic exacerbations and enhanced vasopressin secretion in schizophrenic patients with hyponatremia and polydipsia. *Arch Gen Psychiatry*. 1997; 54:443–449. [PubMed: 9152098]

- Hammock EA, Young LJ. Microsatellite instability generates diversity in brain and sociobehavioral traits. *Science*. 2005; 308:1630–1634. [PubMed: 15947188]
- Hammock EA, Young LJ. Oxytocin, vasopressin and pair bonding: implications for autism. *Philos Trans R Soc Lond B Biol Sci*. 2006; 361:2187–2198. [PubMed: 17118932]
- Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci*. 2003; 23:6315–6326. [PubMed: 12867516]
- Hensch TK. Critical period plasticity in local cortical circuits. *Nat Rev Neurosci*. 2005; 6:877–888. [PubMed: 16261181]
- Hu SB, Zhao ZS, Yhap C, Grinberg A, Huang SP, Westphal H, Gold P. Vasopressin receptor 1a-mediated negative regulation of B cell receptor signaling. *J Neuroimmunol*. 2003; 135:72–81. [PubMed: 12576226]
- Johnson AE, Audigier S, Rossi F, Jard S, Tribollet E, Barberis C. Localization and characterization of vasopressin binding sites in the rat brain using an iodinated linear AVP antagonist. *Brain Res*. 1993; 622:9–16. [PubMed: 8242389]
- Kim SJ, Young LJ, Gonen D, Veenstra-VanderWeele J, Courchesne R, Courchesne E, Lord C, Leventhal BL, Cook EH Jr, Insel TR. Transmission disequilibrium testing of arginine vasopressin receptor 1A (AVPR1A) polymorphisms in autism. *Mol Psychiatry*. 2002; 7:503–507. [PubMed: 12082568]
- Kishimoto T, Hirai M, Ohsawa H, Terada M, Matsuoka I, Ikawa G. Manners of arginine vasopressin secretion in schizophrenic patients--with reference to the mechanism of water intoxication. *Jpn J Psychiatry Neurol*. 1989; 43:161–169. [PubMed: 2796026]
- Knafo A, Israel S, Darvasi A, Bachner-Melman R, Uzevsky F, Cohen L, Feldman E, Lerer E, Laiba E, Raz Y, Nemanov L, Gritsenko I, Dina C, Agam G, Dean B, Bornstein G, Ebstein RP. Individual differences in allocation of funds in the dictator game associated with length of the arginine vasopressin 1a receptor RS3 promoter region and correlation between RS3 length and hippocampal mRNA. *Genes Brain Behav*. 2008; 7:266–275. [PubMed: 17696996]
- Kuehl-Kovarik MC, Iqbal J, Jacobson CD. Autoradiographic localization of arginine vasopressin binding sites in the brain of adult and developing Brazilian opossums. *Brain Behav Evol*. 1997; 49:261–275. [PubMed: 9150896]
- Kuhlman SJ, Lu J, Lazarus MS, Huang ZJ. Maturation of GABAergic inhibition promotes strengthening of temporally coherent inputs among convergent pathways. *PLoS Comput Biol*. 2010; 6:e1000797. [PubMed: 20532211]
- Levitt P, Eagleson KL, Powell EM. Regulation of neocortical interneuron development and the implications for neurodevelopmental disorders. *Trends Neurosci*. 2004; 27:400–406. [PubMed: 15219739]
- Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci*. 2005; 6:312–324. [PubMed: 15803162]
- Lim M, Hammock E, Young L. A method for acetylcholinesterase staining of brain sections previously processed for receptor autoradiography. *Biotech Histochem*. 2004; 79:11–16. [PubMed: 15223749]
- Manning M, Klis WA, Kruszynski M, Przybylski JP, Olma A, Wo NC, Pelton GH, Sawyer WH. Novel linear antagonists of the antidiuretic (V2) and vasopressor (V1) responses to vasopressin. *Int J Pept Protein Res*. 1988; 32:455–467. [PubMed: 3246475]
- Ohsawa H, Kishimoto T, Shimayoshi N, Matsumura K, Tahara K, Kitera K, Higashiura N, Noriyama Y, Matsumoto H, Hirai M, et al. Atrial natriuretic peptide and arginine vasopressin secretion in schizophrenic patients. *Acta Psychiatr Scand*. 1993; 88:130–134. [PubMed: 8213206]
- Ostrowski NL, Lolait SJ, Young WS 3rd. Cellular localization of vasopressin V1a receptor messenger ribonucleic acid in adult male rat brain, pineal, and brain vasculature. *Endocrinology*. 1994; 135:1511–1528. [PubMed: 7925112]
- Packer AM, Yuste R. Dense, unspecific connectivity of neocortical parvalbumin-positive interneurons: a canonical microcircuit for inhibition? *J Neurosci*. 2011; 31:13260–13271. [PubMed: 21917809]
- Paxinos, G.; Franklin, KBJ. The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 2001.

- Petracca FM, Baskin DG, Diaz J, Dorsa DM. Ontogenetic changes in vasopressin binding site distribution in rat brain: an autoradiographic study. *Brain Res.* 1986; 393:63–68. [PubMed: 3730894]
- Powell EM, Campbell DB, Stanwood GD, Davis C, Noebels JL, Levitt P. Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction. *J Neurosci.* 2003; 23:622–631. [PubMed: 12533622]
- Rabhi M, Stoeckel ME, Calas A, Freund-Mercier MJ. Historadioautographic localisation of oxytocin and vasopressin binding sites in the central nervous system of the merione (*Meriones shawi*). *Brain Res Bull.* 1999; 48:147–163. [PubMed: 10230706]
- Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav.* 2003; 2:255–267. [PubMed: 14606691]
- Ryan MC, Sharifi N, Condren R, Thakore JH. Evidence of basal pituitary-adrenal overactivity in first episode, drug naive patients with schizophrenia. *Psychoneuroendocrinology.* 2004; 29:1065–1070. [PubMed: 15219658]
- Schmidt A, Audigier S, Barberis C, Jard S, Manning M, Kolodziejczyk AS, Sawyer WH. A radioiodinated linear vasopressin antagonist: a ligand with high affinity and specificity for V1a receptors. *FEBS Lett.* 1991; 282:77–81. [PubMed: 1827414]
- Snijdewint FG, Van Leeuwen FW, Boer GJ. Ontogeny of vasopressin and oxytocin binding sites in the brain of Wistar and Brattleboro rats as demonstrated by lightmicroscopical autoradiography. *J Chem Neuroanat.* 1989; 2:3–17. [PubMed: 2551339]
- Szot P, Myers KM, Dorsa DM. Effect of vasopressin administration and deficiency upon 3H-AVP binding sites in the CNS and periphery during development. *Peptides.* 1992; 13:389–394. [PubMed: 1409016]
- Szot P, Myers KM, Swank M, Dorsa DM. Characterization of a 3H-arginine8-vasopressin binding site in the cingulate gyrus of the rat pup. *Peptides.* 1989; 10:1231–1237. [PubMed: 2622802]
- Tanoue A, Ito S, Honda K, Oshikawa S, Kitagawa Y, Koshimizu TA, Mori T, Tsujimoto G. The vasopressin V1b receptor critically regulates hypothalamic-pituitary-adrenal axis activity under both stress and resting conditions. *J Clin Invest.* 2004; 113:302–309. [PubMed: 14722621]
- Tribollet E, Goumaz M, Raggenbass M, Dreifuss JJ. Appearance and transient expression of vasopressin and oxytocin receptors in the rat brain. *J Recept Res.* 1991a; 11:333–346. [PubMed: 1653339]
- Tribollet E, Goumaz M, Raggenbass M, Dubois-Dauphin M, Dreifuss JJ. Early appearance and transient expression of vasopressin receptors in the brain of rat fetus and infant. An autoradiographical and electrophysiological study. *Brain Res Dev Brain Res.* 1991b; 58:13–24.
- Wang Z, Liu Y, Young LJ, Insel TR. Developmental changes in forebrain vasopressin receptor binding in prairie voles (*Microtus ochrogaster*) and montane voles (*Microtus montanus*). *Ann N Y Acad Sci.* 1997a; 807:510–513. [PubMed: 9071385]
- Wang Z, Young LJ. Ontogeny of oxytocin and vasopressin receptor binding in the lateral septum in prairie and montane voles. *Brain Res Dev Brain Res.* 1997; 104:191–195.
- Wang Z, Young LJ, Liu Y, Insel TR. Species differences in vasopressin receptor binding are evident early in development: comparative anatomic studies in prairie and montane voles. *J Comp Neurol.* 1997b; 378:535–546. [PubMed: 9034909]
- Wassink TH, Piven J, Veland VJ, Pietila J, Goedken RJ, Folstein SE, Sheffield VC. Examination of AVPR1a as an autism susceptibility gene. *Mol Psychiatry.* 2004; 9:968–972. [PubMed: 15098001]
- Wonders CP, Anderson SA. The origin and specification of cortical interneurons. *Nat Rev Neurosci.* 2006; 7:687–696. [PubMed: 16883309]
- Yang SY, Cho SC, Yoo HJ, Cho IH, Park M, Kim BN, Kim JW, Shin MS, Park TW, Son JW, Chung US, Kim HW, Yang YH, Kang JO, Kim SA. Association study between single nucleotide polymorphisms in promoter region of AVPR1A and Korean autism spectrum disorders. *Neurosci Lett.* 2010a; 479:197–200. [PubMed: 20546835]
- Yang SY, Cho SC, Yoo HJ, Cho IH, Park M, Yoe J, Kim SA. Family-based association study of microsatellites in the 5' flanking region of AVPR1A with autism spectrum disorder in the Korean population. *Psychiatry Res.* 2010b; 178:199–201. [PubMed: 20452058]

- Yirmiya N, Rosenberg C, Levi S, Salomon S, Shulman C, Nemanov L, Dina C, Ebstein RP. Association between the arginine vasopressin 1a receptor (AVPR1a) gene and autism in a family-based study: mediation by socialization skills. *Mol Psychiatry*. 2006; 11:488–494. [PubMed: 16520824]
- Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K. Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature*. 2011; 477:171–178. [PubMed: 21796121]
- Young LJ, Frank A, Beach Award. Oxytocin and vasopressin receptors and species-typical social behaviors. *Horm Behav*. 1999; 36:212–221. [PubMed: 10603285]

Highlights

C57BL/6J mice show robust and transient pre-weaning V1aR in the cortex.

V1aR KO mice have more parvalbumin-IR cells in the neocortex than WT.

Female mice have more calbindin-IR cells in the neocortex than male mice.

Therefore, sex and V1aR modulate interneuron markers in the neocortex.

Figure 1. Developmental profile of V1aR ligand binding in wild-type C57BL/6J mouse forebrain. A) Representative autoradiographs showing the rostro-caudal ligand binding of I^{125} -labeled linear vasopressin antagonist (NEX310, Perkin Elmer, Waltham, MA, USA), a potent and selective ligand for V1aR.

Figure 2. Quantitation of relative ligand binding demonstrating transiently elevated ligand binding capacity in the second and third post-natal weeks for CA1 hippocampus (A) and S1 neocortex (B), compared to relatively stable ligand binding for the septum (C) and the VTA (D). Graphs represent average $\pm$ S.E.M.

Figure 3.

Cortical interneuron populations in adult mice are affected by loss of *Avpr1a*. A) Interneuron PV-IR in P90 *Avpr1a*^{+/+} and ^{-/-} S1 cortex, demonstrating increased IR cell number, size and neuropil staining in *Avpr1a*^{-/-}. Scale bar = 200 μm. B) *Avpr1a*^{-/-} mice show increased PV-IR cell number in S1 cortex compared to *Avpr1a*^{+/+} mice. C) *Avpr1a*^{-/-} mice show increased PV-IR cell diameter in S1 cortex compared to *Avpr1a*^{+/+} mice. D) Overall, females show significantly greater calbindin (CB)-IR cell number diameter in S1 cortex compared to males, with no main effect of genotype. E) CB-IR cell diameter is larger in females compared to males, with no significant differences between genotypes. Asterisks denote significant differences with post-hoc analysis at the alpha = 0.05 level. Graphs of cell

counts represent Abercrombie-corrected cell counts. Square-root transformed cell count data were used in the statistical analysis.

Figure 4.

Relative expression (Log₂ normalized reads per kilobase of exon model per million mapped reads, RPKM) of *AVPR1A* in human S1 neocortex by developmental epoch compiled from www.brainspan.org, accessed on September 29th, 2011. Multiple races, both sexes and at least 2 individuals per age were used to generate the dataset. The dataset does not include third trimester fetuses and newborns, and were therefore not available for analysis. Details of the transcription profiling can be viewed at the website. Mean $\pm$ S.D.