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Vasopressin eliminates the expression of familiar odor bias in neonatal female mice through V1aR

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Summary

V1aR has a well established role in the neural regulation of adult mammalian social behavior. The role of V1aR in developmentally emerging social behavior is less well understood. We mapped V1aR at post-natal day 8 (P8) and demonstrate developmentally-specific expression in the neocortex and hippocampus. We tested the ability of male and female C57BL/6J mice to show orienting bias to a familiar odor at this age. We demonstrate that females, but not males, show an orienting bias for odors previously paired with the mother, which is eliminated by V1aR signaling.

Arginine-vasopressin (AVP) and the vasopressin V1a receptor (V1aR) acting within the forebrain are involved in social behavior in adult animals. Much less is known about the function of V1aR in neurobehavioral development. In the present study, at post-natal day 8 (P8) in neonatal C57BL/6J mice, we map V1aR and use an olfactory exposure paradigm to assess a role for V1aR on olfactory preferences. In addition to V1aR in the lateral septum and ventral tegmental area, we observe V1aR in the neocortex and hippocampus, not typically observed in adult mice, implicating a developmental sensitive period for V1aR to modulate these brain areas in an experience-dependent manner. Males and females were tested on P8 for orienting preferences after exposure to a non-social odor, presented either when the mother was in the home cage (contingent) or when the mother had been removed from the home cage (not contingent). Wild-type female mice show a selective orienting bias toward the exposed odor, but only in the contingent condition. Males did not show orienting bias after either training condition. Female *Avpr1a*^{-/-} mice showed strong familiar odor bias, regardless of the training condition. This finding led us to test the ability of AVP to diminish odor bias in females. Central application of AVP eliminated odor bias in *Avpr1a*^{+/+}, but not *Avpr1a*^{-/-} female mice. Together, these data indicate that AVP acting at V1aR eliminates the expression of familiar odor bias in neonatal mice. This suggests a developmental

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role for AVP on familiarity bias, which has implications for species-typical life history trajectories of social learning and natal dispersal.

Keywords

C57BL/6J; neurodevelopment; sex differences; neonatal olfactory behavior; anti-diuretic hormone; *Avpr1a*; V1aR KO; one-trial associative odor learning

Introduction

Arginine-vasopressin (AVP) acting at the vasopressin V1a receptor (V1aR) contributes to arousal, anxiety, social recognition behavior, and species-typical social behavior. Our knowledge of the behavioral contribution of central AVP and V1aR in mammals comes from a variety of genera, including rats, mice, voles, and hamsters (reviewed in (Donaldson and Young 2008; Sigling, Wolterink-Donselaar et al. 2009; Goodson and Thompson 2010; Meyer-Lindenberg, Domes et al. 2011; Albers 2012; Young and Flanagan-Cato 2012)). V1aR has been studied extensively in adult animals. In adults, AVP activation of V1aR at distinct brain sites can contribute to the memory of, orientation toward, and/or defense of resources (e.g. food/water, territory, mates, and offspring). For example, adult male *Avpr1a*^{-/-} mice show deficits on the eight-arm radial maze (Egashira, Tanoue et al. 2004), in social recognition behavior, and also show reduced anxiety (Bielsky, Hu et al. 2004; Egashira, Tanoue et al. 2007); but see also (Wersinger, Caldwell et al. 2007).

Despite our increased knowledge of AVP and V1aR in adult social and emotional behaviors, our understanding of the potential contribution of V1aR in early neurobehavioral development remains poorly understood. Given that AVP and V1aR, the latter encoded by the *AVPR1A* gene, have been implicated in neurodevelopmental disorders such as schizophrenia (Ohsawa, Kishimoto et al. 1993; Goldman, Robertson et al. 1997; Ryan, Sharifi et al. 2004; Teltsh, Kanyas-Sarner et al. 2011) and autism (Kim, Young et al. 2002; Wassink, Piven et al. 2004; Yirmiya, Rosenberg et al. 2006; Yang, Cho et al. 2010; Yang, Cho et al. 2010), there is an increased interest to understand the role of V1aR signaling in the ontogeny of the social brain. In a variety of species, V1aR distribution patterns in the brain display a robust transient developmental peak during post-natal development, peaking in some areas between the first and second post-natal weeks (Tribollet, Goumaz et al. 1991; Kuehl-Kovarik, Iqbal et al. 1997; Wang, Liu et al. 1997; Wang and Young 1997; Wang, Young et al. 1997; Hammock and Levitt 2012). V1aRs are present in infant rat brains and AVP elicits physiological activity (Tribollet, Goumaz et al. 1991; Tribollet, Goumaz et al. 1991). In neonatal rats, brain injections of high doses (greater than 100ng) of AVP cause a decrease in ultrasonic vocalizations as well as a reduction in core body temperature (Winslow and Insel 1993). The effects on USV and core temperature appear to be mediated through separate AVP receptors: V1aR appears to mediate the AVP-induced reduction in ultrasonic vocalizations and the AVPR2 seems to be responsible for AVP- induced hypothermia (Winslow and Insel 1993). Additionally, central AVP injection acting at V1aR decreases the latency for righting in an inclined surface assay (Winslow and Insel 1993), indicating a possible developmental role for V1aR in sensory-motor integration (Motz and

Alberts 2005). Further, central AVP decreases motor activity in infant rats, although the receptor mediating this response is unclear (Winslow and Insel 1993). AVP-deficient neonatal Brattleboro rats are hyperactive and this hyperactivity is maintained even in the presence of littermates, which normally promote contact-dependent quiescence (Schank 2009). The inability of social contact to reduce the hyperactivity phenotype suggests impaired social responsiveness by 10 days of age in the Brattleboro rat (Schank 2009), although the contributing receptor is unclear. With the exception of altered USV, very little is known about AVP acting at V1aR in primordial mammalian social behavior. A recent study by Sigling and colleagues (Sigling, Wolterink-Donselaar et al. 2009) suggests that AVP may act at V1aR to reduce the preference for familiar individuals in pre-weaning behavioral development, but secondary effects on activity complicate the interpretation.

Neonates will learn to orient towards odors that were previously paired with the mother, with somatosensory stimulation or even with a mild foot-shock (Bouslama, Durand et al. 2005; Honeycutt and Alberts 2005; Armstrong, DeVito et al. 2006; Moriceau, Wilson et al. 2006; Roth and Sullivan 2006). The timing of the ability of odors to elicit orienting responses indicate that there is a sensitive period for social imprinting, where neural representations of stimuli natively associated with the mother (odors, nest material, food textures) wire together with and come to define social neural circuitry (Hammock and Levitt 2006). There are several laboratory assays in rodent neonates that have been used to explore the neurobiology of this behavior, including odor-shock (Moriceau and Sullivan 2006), odor-stroke (Roth and Sullivan 2006), homing (Honeycutt and Alberts 2005), and odor-mom (Armstrong, DeVito et al. 2006) paradigms. The consensus view is that neonates have a special context-dependent learning mechanism for odors to which they are exposed early in life. Because AVP and V1aR play a modulatory role in social orienting behaviors in adult, we hypothesized that they would play a similar role during behavioral development.

In an effort to understand a role for AVP signaling in cardinal social orienting behaviors during postnatal development, we undertook a series of studies to address these questions. First, we mapped V1aR by ligand binding at the time of testing, postnatal day 8 (P8) to assess potential sites of action. Second, we adapted a one-trial associative odor conditioning paradigm to test C57BL/6J wild-type and *Avpr1a*^{-/-} neonates for familiar odor bias. Finally, we assessed the behavioral response to pharmacological manipulation of V1aR in neonates receiving a central AVP injection.

Materials and Methods

Mice

All experiments were approved under the oversight of the Institutional Animal Care and Use Committee of Vanderbilt University in accordance with local, state and federal regulations to minimize pain and discomfort. *Avpr1a* mice on a mixed C57/129 background were provided by Dr. Larry Young at Emory University (*Avpr1a*^{tm1Sbhu} (Hu, Zhao et al. 2003)). The *Avpr1a* mice used in this report were fully backcrossed to the C57BL/6J strain at Vanderbilt University confirmed at 99.9% by strain-specific SNP genotyping (Genome Scanning Service, The Jackson Laboratory, Bar Harbor, ME). Heterozygous matings were used to control for potential effects of parental genotype. All mice were kept on a 12:12 L:D

cycle and provided access to food and water ad libitum. The genotypes of *Avpr1a* mice were determined by PCR from tail DNA using the following primers: *Avpr1a_WT_For* 5'-GGGCTGAGTTTCGTTCTGAGCATAC, *Avpr1a_WT_Rev* 5'-GGTCATCTTCACTGTGCGGATCTTG, *Avpr1a_KO_For* 5'-AGAGGCTATTCGGCTATGACTG, *Avpr1a_KO_Rev* 5'-TTCGTCCAGATCATCCTGATC, generating the following PCR amplicons: upper band (430bp) = KO, lower band (369bp) = WT.

Wild-type C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, Maine, USA) and bred in-house for the developmental mapping study and a portion of the neonatal behavior and injection studies. Timed pregnancies were checked daily for litters. The first appearance of a litter was designated as P0.

Receptor autoradiography

Three mice per sex from at least two litters were used for the analysis of V1aR ligand binding at postnatal day (P) 8. At the time of harvest, mice were deeply anesthetized with isoflurane vapors and euthanized. Brains were removed and snap frozen on dry ice and stored at -80°C until the brains were cut. Frozen brains were sliced to a thickness of 20 µm in six series and thaw mounted to Superfrost plus microscope slides. Slides were stored at -80°C until the receptor binding study was performed (as described previously (Hammock and Young 2005; Hammock and Levitt 2012)). Briefly, slides were allowed to thaw and air dry. Once dry, slides were lightly fixed for 2 minutes in 0.1% formaldehyde in 1×PBS. Slides were washed 2 × 10 minutes in 50mM Tris (pH 7.4), then slides were incubated at room temperature for one hour in tracer buffer containing 50mM Tris (pH 7.4), 10mM MgCl₂, 0.1% BSA, 0.05% bacitracin, 50pM ¹²⁵I linear-vasopressin antagonist (NEX 310, NEN/Perkin-Elmer, Waltham, MA, USA). After the tracer incubation, slides were washed 4 × 5 minutes in 50mM Tris (pH 7.4), 10mM MgCl₂ at 4°C, then at room temperature in fresh 50mM Tris (pH 7.4), 10mM MgCl₂ for 30 minutes. The slides were dipped in water and dried with a blow dryer on a cool setting. 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 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) connected to a PC. 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, Hammock et al. 2004). Densitometry measurements were converted to µCi/g by comparison to ¹⁴C autoradiographic standards (Miller and Zahniser 1987) (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.

One-Trial Associative Odor Learning in Neonates

Timed pregnancies for neonatal learning studies were generated with 3 consecutive overnight pairings of a heterozygous male and 3-4 heterozygous female *Avpr1a* mice. At E14.5-E16.5, females were visually inspected for pregnancies. Pregnant dams were then single-housed in a clean cage and moved to an incubator (Model no. RIT33SD, Powers Scientific, Inc., Pipersville, PA) maintained at 27°C +/- 2°C, 30-40% humidity, 12:12 L/D, where they remained until after their litters were born (postnatal day 0, P0) and reached P8.

1) Training via odor-mammary pairing—On P8, litters were assigned to either the experimental condition or the control condition using a method (Armstrong, DeVito et al. 2006) that generates one-trial olfactory conditioning in CD-1 mice on P2, P4, and P9, but only when mice had a 2 hour period of separation from the dam prior to training. In the experimental condition, dams were removed from the home cage for 2 hours in the morning. Just prior to returning the dam to the home cage, a cotton swab saturated with 14.8% *n*-hexanoic acid (CAS 142-62-1) or 16.6% citronellal (CAS 106-23-0) in mineral oil (CAS 8042-47-5) was applied to each mammary of the dam. Odorant presentation was counterbalanced across litters. The scented dam remained in the cage for 1 hour, after which she was removed. Testing began 2 hours after the final removal of the scented dam. In the control condition, the dam was briefly removed, scented as above and immediately returned to the pups, which had access to the scented dam for 1 hour. As with the experimental condition, the scented dam was removed after 1 hour and the pups were tested two hours later (see below). A total of 17 litters were used in these experiments, with an average litter size of 6.7 pups (46% male).

2) Training via odor-nest pairing—On P8, litters were randomly assigned to one of two odor exposure conditions: contingent or not contingent (Figure 2a). In the contingent condition, a tissue wipe was saturated with 500uL odor (14.8% *n*-hexanoic acid or 16.6% citronellal in mineral oil, counterbalanced across training trials) and placed in a Histo Prep Tissue Capsule (Fisher Brand, Pittsburgh, PA) taped inside a plastic petri dish lid. This was secured to the home cage wall directly above the home nest for one hour. At the end of the hour, both the odor source and the mother were removed from the home cage, such that these two stimuli co-terminated. After two hours, the pups were tested for familiar odor bias (see below). In the not contingent condition, the dam was removed from the home cage and the odor was added, exactly as above. After an hour, the odor source was removed and the mom was replaced to the cage. At the end of the next hour, the mother was removed again and after another hour (two hours after the odor presentation ended, as with the contingent condition) the pups were tested (see below). The contingent and not contingent groups both had a total of two hours of separation from their mother. Additionally, for both training paradigms, neonates were tested beginning two hours after the end of the odor exposure. A total of 22 litters were used in these experiments, with an average litter size of 7.5 pups (55% male).

3) Testing—Testing occurred just outside of the incubator in a dedicated room with ambient temperature of 26.5°C +/- 1.5°C. The pups were tested for their orienting behavior and activity level in a chamber (30.5 cm × 20.5 cm × 15.5 cm) on a nylon mesh floor

suspended above two-compartments (each 15cm × 15.5cm × 5cm), divided by a solid barrier under the nylon floor (Figure 2b). A tissue wipe containing freshly applied 500 uL of the odor used during training was placed under the nylon floor in one compartment, while 500 uL of the other novel odor was similarly placed in the second compartment (Figure 2b). The neonate was grasped with minimal litter disturbance in the middle of its back and placed along the midline in the test chamber. The neonate was replaced to the midline in the opposite orientation when any of the following occurred: the pup became quiescent with no head movement for more than 3 consecutive seconds, failed to maintain prone posture, reached one of the four corners of the test chamber, or rotated in place 2 full turns off of the midline. The testing procedure lasted for 180 seconds and was video recorded for later scoring (See Supplemental Video). After testing, each pup was marked, weighed, and returned to the home cage, but at the opposite end of the cage from the nest, so as not to disturb untested pups. At the end of behavioral testing for the entire litter, tail samples were obtained for later genotyping as described above.

4) Scoring—All behaviors were scored from video recordings by a trained observer blind to sex, genotype, drug treatment, training parameters, and trained odor position in the test apparatus. Videos were scored with Stopwatch+ (Center for Behavioral Neuroscience, Atlanta, GA) for the following variables: counts, duration, and latency to enter the trained and novel odor compartments; counts and duration along the midline; counts, duration, and latency to initiate ambulation (all four limbs in motion), the number of times the pup lost its upright posture, and the number of times the pup had to be returned to the midline. The minimum criterion for pup location was based on the position of the entire head. Based on the literature of odor orienting behavior in neonates, we chose duration in each odor compartment as our primary variables of interest. Results of other variables are summarized in the Supplementary Table.

Intracerebroventricular injections

P8 animals were trained in the odor-nest pairing contingent paradigm as above. Fifteen minutes prior to testing, the pups were sexed. For a given litter, half the females and half the males were injected intracerebroventricularly (ICV) with 20 ng of arginine-vasopressin (Sigma V9879, St Louis, MO) in 2 uL vehicle and the other half were injected with vehicle (bicarbonate-free artificial CSF with 0.05% fast green FCF dye). This dose was chosen in a range that had previously been shown to have effects on behavior without impacting peripheral blood pressure (Sodersten, Henning et al. 1983) or motor activity (Winslow and Insel 1993). Injections were performed without anesthesia using a handheld Hamilton syringe with a beveled 33 gauge needle aimed at the left lateral ventricle (approximately halfway between the eyes and the ears, off the midline by 1mm) at a depth of 2mm, achieved with a distance guide which stopped the insertion of the needle to depths beyond 2mm. After injections, pups were coded on their ventrum and returned to their nest with all of their littermates, so that at testing the experimenter was again naïve to the sex and drug treatment of the animals. Testing and scoring were performed exactly as described above. After testing, brains were dissected to visually confirm loading of the ventricles with dye. A total of 12 litters were used in these experiments, with an average litter size of 5.6 pups (53% male).

Statistical analyses

Statistical analyses for receptor density at P8 were performed in Matlab 7.0.4 using a one-way ANOVA (for brain area) and a sample size of 6 (3 per sex). All quantitated behavior data are presented as the mean $\pm$ S.E.M. Our *a priori* hypothesis was that neonates would show a familiar odor preference (rather than preference and/or aversion) for the pre-exposed odor. A test for preference is a one-tailed test to determine if time with the trained odor is greater than time spent with the novel odor. Thus, time spent with trained versus novel odors was tested with paired one-tailed student's t-tests. Effect sizes are reported as Cohen's d. P values are reported to 2 significant digits. Percent Difference Scores for odor bias in the AVP injection study in females were assessed with a two-way ANOVA (genotype, AVP dose). Justified least significant difference post-hoc tests were used to identify group differences. Excepting heterozygotes, no data were discarded. We currently do not know if heterozygotes show intermediate or binary (e.g. imprinted) expression of *Avpr1a* at this age. Without an *a priori* hypothesis about the biology of the heterozygous state, including them in the analysis dilutes the statistical power and is outside of the scope of this study.

Results

V1aR binding sites are present in P8 C57BL/6J neocortex, CA1 hippocampus, septum, VTA

V1aR density by receptor autoradiography in the P8 mouse appears strikingly different from reports detailing adult patterns of expression (Dubois-Dauphin, Barberis et al. 1996; Bielsky, Hu et al. 2004; Hammock and Levitt 2012). There were significant levels of binding above background at P8 (Figure 1a), including dense receptor binding in the hippocampus and neocortex. Overall one-way ANOVA demonstrated a main effect of brain area (Figure 1b; d.f. 3, $F = 18.0$, $P < 0.01$). Post-hoc comparison of means using the Scheffe procedure indicated that CA1 and septal areas were both more densely populated with receptor than the VTA and cortex ($P < 0.05$). While our study is underpowered to detect sex differences, the expression seemed qualitatively similar between males and females at this age.

C57BL/6J neonates show modest evidence of odor preference in olfactory conditioning at P8

We adapted a paradigm to eventually probe the potential for an early role for V1aR in social behavior. There is a significant body of literature describing neonatal olfactory conditioning in rodents (primarily rats) which is an appropriate behavioral target at this age, arguably due to its relevance for social orienting skills (Hammock and Levitt 2006), in the context of an otherwise limited behavioral repertoire. In developing this task in C57BL/6J mice, we modeled our approach on a prior report in CD-1 mice (Armstrong, DeVito et al. 2006). This method was chosen because of its potential for high throughput screening of many mice and because of the robust effect size as presented in the initial report on CD-1 mice. In the Armstrong *et al* study, olfactory conditioning in neonatal CD-1 mice was achieved when an odorant was paired with the mother, but only when the neonates first had a 2 hour period of separation away from their mother. We found that this method does not induce a similar trained odor preference in C57BL/6J mice (Supplemental Figure 1a, 1b). In particular, the requisite two hours of separation needed to show odor preferences in CD-1 mice induced

significant immobility in both male and female C57BL/6J mice (Supplemental Figure 1c). While we failed to replicate the Armstrong study in C57BL/6J mice, we did observe significant odor bias in females when the odor and dam were presented simultaneously, but only when there was not prolonged separation prior to odor-dam pairing (one-tailed paired student's t-test, $P=0.05$; Cohen's $d=0.58$; See Supplemental Figure 1). In an effort to devise a functionally related protocol that would include a relevant control, we modified the training procedure and retained the original testing procedure. For the training period, half the litters were trained in a not-contingent fashion, such that the odor was presented for an hour, but with the mom out of the cage. The other litters were trained in the contingent condition, where the odor was presented in the cage with the mother present. In both groups, the total amount of time the mom was out of the cage was identical, as well as the elapsed time between odor exposure and testing. For experimental consistency, the odor was presented in the same way in both conditions. Instead of painting the odor on the dam, the odor source was secured to the wall of the home cage adjacent to the nest in both conditioning paradigms. In this paradigm, training did not influence mobility in C57BL/6J mice (two-way ANOVA on mobile duration scores for training, d.f. 1, $F=0.07$, $P=0.80$; and sex, d.f.1, $F=0.06$, $P=0.81$; interaction, d.f. 1, $F=0.05$, $P=0.82$). To maintain statistical power, heterozygotes, while tested, were not included in any analyses.

First, we analyzed the data from our wild-type mice to determine the sensitivity of the tasks in C57BL/6J. Our *a priori* prediction was that males and females in the contingent condition would spend more time with the trained odor than with the novel odor and animals in the not contingent condition would show no behavioral preference. Females trained in the contingent condition showed a bias for the trained odor (one-tailed paired t-test $P=0.05$; Cohen's $d = 0.99$); however, the males did not (one-tailed paired t-test $P=0.40$; Cohen's $d = 0.18$) (Figure 2c). In the not contingent condition, neither females (one-tailed paired t-test $P=0.48$; Cohen's $d = 0.04$) nor males (one-tailed paired t-test $P=0.30$; Cohen's $d = 0.28$) showed a preference for the trained odor. Although the odor conditioning effects were absent in wild type males and modest in wild-type females, we next analyzed effects of conditioning in mice lacking V1aR. As with wild type males, *Avpr1a*^{-/-} males did not exhibit a familiar odor preference in the not contingent (one-tailed paired t-test, $P=0.34$; Cohen's $d = 0.23$) or the contingent conditions (one-tailed paired t-test, $P=0.42$; Cohen's $d = 0.13$; Figure 2d). In contrast to the wild type females, *Avpr1a*^{-/-} females showed significant preference for the trained odor in both the not contingent (one-tailed paired t-test, $P<0.01$; Cohen's $d = 1.53$) and the contingent conditions (one-tailed paired t-test, $P=0.02$; Cohen's $d = 1.59$; Figure 2d). Female *Avpr1a*^{-/-} mice spent more time with a familiar odorant in both conditioning contexts, indicating that exposure alone enhances familiar odor preference when V1aR is incapable of signaling. This led us to the converse hypothesis that V1aR signaling would reduce familiar odor bias in females.

AVP eliminates the trained odor bias in WT females, but has no effect in KO

Because *Avpr1a*^{-/-} females showed a strong preference for the pre-exposed odorant, we hypothesized that activation of the receptor by injection with AVP would eliminate the contingent odor bias in *Avpr1a*^{+/+} females. Wild type females showed a familiar odor bias in the contingent paradigm only; thus, we trained females using that behavioral approach.

After training and just prior to testing, we injected 2 μ l of vehicle (bicarbonate free artificial cerebrospinal fluid, aCSF) or 20 ng of AVP in aCSF (Figure 3a). First, we confirmed that this dose of AVP does not alter mobility. The 20 ng dose used in this study did not induce a change in the duration of motor activity in females or males (Two-Way ANOVA, sex d.f. 1, F 1.10, $P=0.30$; drug d.f. 1, F 1.85, $P=0.19$; interaction d.f. 1, F 0.01, $P=0.91$).

In the neonates injected with 0 ng of AVP, we observed an independent replication of the data observed in our initial experiments. *Avpr1a*^{+/+} females showed a preference for the maternally-paired odor (one-tailed paired student's t-test for trained versus novel, $P<0.01$; Cohen's $d = 1.73$), *Avpr1a*^{-/-} females showed a preference for the maternally paired odor (one-tailed paired student's t-test for trained versus novel, $P=0.05$; Cohen's $d = 1.74$), and as before, males did not show a preference (one-tailed paired student's t-test for trained versus novel, $P=0.49$; Cohen's $d = 0.03$).

In order to directly compare the effects of AVP injection on odor bias across genotypes, we calculated a percent difference score for each neonate:

$$\text{Percent Difference Score} = 100 * (\text{time with trained odor} - \text{time with novel odor}) / (\text{time with trained odor} + \text{time with novel odor})$$

A score above zero represented a bias for the trained odor, and a score below zero represented a bias for the novel odor. Omnibus Two-Way ANOVA on Percent Difference Scores in females for genotype and AVP dose indicated a main effect for genotype (d.f. 1, $F=7.46$, $P=0.01$), no main effect for drug (d.f. 1, $F=0.22$, $P=0.65$), but a significant interaction (d.f. 1, $F=5.39$, $P=0.03$). Post-hoc analysis confirmed that the wild-type females that had been injected with 20ng of AVP displayed significantly lower Percent Difference Scores, or the lowest amount of familiarity bias, compared to all of the other females. *Avpr1a*^{+/+} and *Avpr1a*^{-/-} females injected with aCSF (0 ng AVP) showed a positive Percent Difference Score, indicating familiar odor bias. However, injections with AVP eliminated familiar odor bias in *Avpr1a*^{+/+} females (Figure 3a). Because AVP failed to reduce familiar odor bias in *Avpr1a*^{-/-} females, this suggests that AVP acts on V1aR signaling to eliminate familiar odor bias in *Avpr1a*^{+/+} females.

Consistent with our previous data in males, AVP had no effect in *Avpr1a*^{+/+} males: both vehicle (as described above, one-tailed paired student's t-test for trained versus novel, $P=0.49$; Cohen's $d = 0.03$) and AVP-injected *Avpr1a*^{+/+} males (one-tailed paired student's t-test for trained versus novel, $P=0.22$; Cohen's $d = 0.45$) showed no preference for the trained odor and their Percent Difference Scores were not different from each other (one-tailed t-test for Percent Difference Scores at 0 versus 20 ng AVP, $P=0.38$, Figure 3b). Given the lack of genotype effect and the lack of a response to AVP in *Avpr1a*^{+/+} males, drug injection studies were not pursued further in *Avpr1a*^{-/-} males.

Discussion

Olfactory stimuli during early postnatal development serve as key modulators of socially relevant behavioral development (e.g. (Denenberg, Hudgens et al. 1964; Brunjes and Alberts

1979; Rosenblatt 1983; Balogh and Porter 1986)). To further understand a role for AVP signaling in orienting behaviors to familiar odors during development, we performed a series of studies in neonatal mice. Mapping V1aR expression by ligand binding at postnatal day 8 (P8) revealed that there is AVP signaling potential in areas known to be involved in social recognition (e.g lateral septum) and in additional areas, such as the neocortex and hippocampus, that typically are not considered in adult behavioral functions of V1aR activation in mouse models. We then developed a modified one-trial olfactory conditioning paradigm that appears to be less taxing on P8 C57BL/6J neonates than established one-trial methods. We observed conditioned responses in female, but not male neonates, with a particularly strong familiar odor bias response in *Avpr1a*^{-/-} females. Finally, we observed that central AVP injection blocked odor bias in *Avpr1a*^{+/+}, but not *Avpr1a*^{-/-} females. Taken together, these data suggest that central AVP may antagonize familiar odor bias in C57BL/6J neonates, the influence of which, we speculate, could accumulate during a developmental sensitive period to influence life history traits.

Our observations indicate that AVP at V1aR may be a critical signaling pathway in the earliest experience-expectant wiring of the social brain (Sigling, Wolterink-Donselaar et al. 2009). In this context, neonatal mice initially rely on olfactory information, as hearing and vision are not functionally integrated at these early ages. These olfactory stimuli become coupled with interoceptive cues such as gut distension and feeding satiety as well as the exteroceptive cues of skin temperature and somatosensory stimulation. This early classical conditioning lays the foundation for a prolonged developmental period of both classical and operant conditioning to build a species-typical behavioral repertoire (Rosenblatt 1983; Hammock and Levitt 2006). Each orientation toward the nest and the dam is an opportunity for operant conditioning by primary reinforcers of temperature, food and a growing palette of secondary reinforcers. Therefore, it has been argued that olfactory-guided behaviors toward or away from familiar odors are critical components of mammalian social ontogeny, *ab ovo*.

To probe early olfactory orienting behaviors, we initially tested neonates by a high throughput assay (Armstrong, DeVito et al. 2006), but found that C57BL/6J neonates at P8 did not respond well to the two hour requisite separation from the dam. We observed familiar odor bias in females (but not males) when the odor was painted on the dam, but only when the pups were not previously separated from the dam (Supplemental Figure 1). These data are in contrast with the Armstrong method in CD-1 mice, which demonstrated that a 2 hour separation prior to odor-dam pairing was required to observe a preference. Armstrong *et al* suggested that this was evidence that CD-1 mice required an arousing stimulus (i.e. maternal separation) to show conditioned odor preferences. The data from the two studies suggest an interesting strain difference in tolerance to maternal separation between C57BL/6J and CD-1 mice, but this would need to be investigated directly. Because we observed significant familiar odor bias when the odor was presented on the mom, we modified the one-trial paradigm, shortening the amount of time that neonates are separated from the dam, while providing a valuable control for odor exposure and dam separation. This control measure addresses in a limited way the idea that the odor preference behavior of the neonate is specific to learning when the mom is present (contingent) versus when the

mom is absent (not-contingent). Our conditioning paradigm reveals sex differences in C57BL/6J neonates in the expression of orienting behavior as a result of exposure alone, particularly in females without intact V1aRs. Additionally, injection with AVP blocked the preference that females exhibited for the pre-trained odor; AVP had no effect in males. This blockade was absent in *Avpr1a*^{-/-} females. Together, these data indicate that AVP, acting via the V1aR, reduces the tendency of female neonatal C57BL/6J mice to show a behavioral bias towards a familiar odor previously paired with the mother.

Avpr1a^{-/-} females showed strong familiar odor bias even when the training odor was presented without the maternal conditioning stimulus. This could mean that odor exposure alone was sufficient to elicit orienting bias in *Avpr1a*^{-/-} females. However, the neonates in both the not contingent and contingent exposure groups were all exposed as litters and not as individuals outside of the home cage environment. This raises the possibility that the warm and moving litter in the home cage was a sufficient conditioning stimulus for *Avpr1a*^{-/-} females. This would mean that these neonates have a broadly tuned palate of secondary social reinforcers that has developed because of the absence of V1aR, while perhaps *Avpr1a*^{+/+} mice have a more narrowly tuned definition of social reinforcers, due to the observed action of AVP at V1aR on orienting behavior. This hypothesis remains to be explored.

At P8, AVP has access to several brain sites with robust V1aR density as measured with I¹²⁵-linear AVP, including the lateral septum, a brain region that participates in social orienting behavior like social recognition (Landgraf, Gerstberger et al. 1995; Landgraf, Frank et al. 2003; Bielsky, Hu et al. 2005). V1aRs are present transiently in the neocortex and the hippocampus during development. It is possible that AVP signaling through V1aR at these developmental ages participates in experience-dependent wiring of those brain circuits, the effects of which may persist long after the transient peak of receptor expression has faded (Hammock and Levitt 2012). The V2 receptor also is expressed transiently in the brain during development (Kato, Igarashi et al. 1995), but the results of injections of AVP into *Avpr1a*^{-/-} are not consistent with V2R mediating the effect of AVP blocking odor bias. Based on our developmental mapping of the receptor and evidence from the literature, V1aR in the lateral septum is a prime candidate for a potential locus mediating the behavioral effects we observed. In addition to the lateral septum, we observed receptor ligand binding in the mouse hippocampus and neocortex, which is specific to pre-weaning development (Hammock and Levitt 2012). Events that cause a spike in endogenous AVP release will likely modulate the lateral septum and potentially these other brain areas through volume transmission (Landgraf 1995). If AVP reduces orienting toward familiar stimuli (via the lateral septum) this may lead to attention to and experience with other (novel) stimuli. We put forth the following testable hypothesis: experience with novel stimuli in combination with activation of neocortical or hippocampal V1aR may lead to alternate developmental trajectories and experience-dependent specialization.

AVP has been investigated extensively for its role in social recognition behavior in adults (reviewed in (Albers 2012; Wacker and Ludwig 2012)). The standard laboratory assay for assessing social recognition behavior as a proxy for memory in adult rodents relies on the exploratory behavior of rodents. When an adult rat or mouse is presented with a novel

juvenile conspecific, the level of initial investigation of this novel stimulus is very high. After a period of exploration, animals will show less exploration over time. This reduction in investigation of an individual is interpreted as evidence of a transient memory of the now familiar individual. In this task, AVP facilitates the reduction in investigation of the familiar stimulus rat in both males and females (Dantzer, Bluthé et al. 1987; Le Moal, Dantzer et al. 1987; Bluthé and Dantzer 1990), and V1aRs in the lateral septum participate in this social recognition behavior (Dantzer, Koob et al. 1988; Popik, Vos et al. 1992; Landgraf, Frank et al. 2003; Veenema, Bredewold et al. 2012). The standard behavioral task for social recognition behavior noted above requires engagement of both memory and motivational circuitry, as the individual adjusts their relative motivation to approach or avoid social stimuli.

This alternative concept of social recognition behavior is to suppose that active social recognition includes both memory and a malleable motivational state, especially influenced through the lateral septum (reviewed in (Sheehan, Chambers et al. 2004)). This, in turn, is consistent with the conclusion that AVP affects the behavioral readout of the task, which has been called a measure of social recognition memory. We suggest that the impact of AVP may be more accurately described as reduction in familiarity preference or reduced motivation to explore a familiar individual. If interpreted in this way, our findings are consistent with a role for AVP in the reduction of familiarity bias/ increase in novelty bias, rather than social recognition memory per se. In this way, our findings are consistent with adult rodent tasks of social recognition behavior, and the habituation/dishabituation tasks, in that in our study, AVP injection prompted the females to “habituate” to the exposed odor, albeit without an orienting preference towards the novel odor. It is unclear why this effect was absent in C57BL/6J male neonates, or was not modulated by increasing or decreasing AVP activity in males.

The unexpected finding of a sex difference points to the possibility of an enhancement of circuit activity related to odor bias, in an AVP-independent fashion that is positively regulated by as yet undetermined female factors. As indicated in our current working model (Figure 4), this activity is vulnerable to AVP signaling, especially in females (Figure 4a,b). Males may lack or perhaps actively inhibit this odor bias circuit activity, and therefore, show no further modulation by AVP. We were surprised that the male *Avpr1a*^{-/-} mice behaved identically to *Avpr1a*^{+/+}, given that the AVP system is sexually dimorphic and more robust in males (De Vries and Panzica 2006), and males express AVP in extrahypothalamic brain areas (i.e. bed nucleus of the stria terminalis, medial amygdala) earlier than females (Szot and Dorsa 1993). However, our results in mice are consistent with reported effects observed in pre-weanling rats at age P17-P18 that were injected with AVP (Sigling et al 2009). In that study, AVP increased the latency to “home” toward familiar nest odors, and AVP decreased familiarity bias. Females appeared to respond more robustly to AVP than the males. Further, our data are consistent with reported effects in juvenile rats (P33) demonstrating that AVP signaling diminishes the preference for familiar individuals (Veenema, Bredewold et al. 2012). Our data, in combination with data from Sigling *et al* (Sigling, Wolterink-Donselaar et al. 2009) and Veenema *et al* (Veenema, Bredewold et al. 2012), indicate that males and females may not follow the same developmental trajectory for the timing of influence of

AVP on primordial social orienting responses. The sex differences in developmental timing of expression of AVP in extrahypothalamic areas (Szot and Dorsa 1993) may serve to reinforce the sex differences in behavior.

The life history implications of sex differences in early odor bias remain untested. Early sex differences in odor bias might underlie later sex differences in dispersal at weaning. For example, wild species of mice (including *Mus musculus*, from which C57BL/6J were derived) show sex differences in natal dispersal. Typically, males have higher rates of natal dispersal than females, which tend to remain in the birth territory (Krackow 2003). Our data lead to a novel hypothesis that AVP, acting at central V1aR sites, may contribute to the probability of natal dispersal in rodents, by counteracting a natal bias circuit which is active in females (Figure 4c). Of considerable interest in the framework of this hypothesis is that developmental manipulation of androgen increases home range size in females (Zielinski, Vomsaal et al. 1992) and masculinizes AVP content in the mammalian brain (de Vries, Buijs et al. 1981; De Vries and Panzica 2006). Both of these are consistent with a potential role for AVP within a broader network to increase the probability of natal dispersal, through elimination of an orienting preference to familiar nest odors during experience-dependent development (Hoset, Ferchaud et al. 2010). Further, this model suggests that neonatal C57BL/6J males might gain the capacity for odor bias if they were exposed to atypically low levels of androgens during development. These data indicate a testable mechanism by which environmental factors that influence AVP signaling, such as stress (Dent, Okimoto et al. 2000; Veenema, Blume et al. 2006), dietary fluctuation in phytoestrogens (Patisaul and Jefferson 2010), or individual differences in V1aR levels (Phelps and Young 2003; Hammock and Young 2005; Ophir, Wolff et al. 2008; Solomon, Richmond et al. 2009) during developmental sensitive periods can influence life history traits. Individual differences in AVP signaling affecting orienting behaviors may lead to differential attention and subsequent reinforcement during developmental sensitive periods which would alter later behavioral expertise, including social expertise. Our findings may help shed light on the neurobiological mechanisms of gene association studies implicating the AVP system in neurodevelopmental disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Albers HE. The regulation of social recognition, social communication and aggression: vasopressin in the social behavior neural network. *Horm Behav.* 2012; 61(3):283–292. [PubMed: 22079778]
- Armstrong CM, DeVito LM, et al. One-trial associative odor learning in neonatal mice. *Chem Senses.* 2006; 31(4):343–349. [PubMed: 16495436]
- Balogh R, Porter RH. Olfactory preferences resulting from mere exposure in human neonates. *Infant Behavior and Development.* 1986; 9(4):395–401.
- Bielsky IF, Hu SB, et al. The V1a vasopressin receptor is necessary and sufficient for normal social recognition: a gene replacement study. *Neuron.* 2005; 47(4):503–513. [PubMed: 16102534]
- Bielsky IF, Hu SB, et al. Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. *Neuropsychopharmacology.* 2004; 29(3):483–493. [PubMed: 14647484]
- Bluthe RM, Dantzer R. Social recognition does not involve vasopressinergic neurotransmission in female rats. *Brain Res.* 1990; 535(2):301–304. [PubMed: 1963571]
- Bouslama M, Durand E, et al. Olfactory classical conditioning in newborn mice. *Behav Brain Res.* 2005; 161(1):102–106. [PubMed: 15904716]
- Brunjes PC, Alberts JR. Olfactory stimulation induces filial preferences for huddling in rat pups. *J Comp Physiol Psychol.* 1979; 93(3):548–555. [PubMed: 479397]
- Dantzer R, Bluthe RM, et al. Modulation of social memory in male rats by neurohypophyseal peptides. *Psychopharmacology (Berl).* 1987; 91(3):363–368. [PubMed: 3104959]
- Dantzer R, Koob GF, et al. Septal vasopressin modulates social memory in male rats. *Brain Res.* 1988; 457(1):143–147. [PubMed: 3167559]
- de Vries GJ, Buijs RM, et al. Ontogeny of the vasopressinergic neurons of the suprachiasmatic nucleus and their extrahypothalamic projections in the rat brain--presence of a sex difference in the lateral septum. *Brain Res.* 1981; 218(1-2):67–78. [PubMed: 7023607]
- De Vries GJ, Panzica GC. Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: different mechanisms, similar endpoints. *Neuroscience.* 2006; 138(3):947–955. [PubMed: 16310321]
- Denenberg VH, Hudgens GA, et al. Mice Reared with Rats: Modification of Behavior by Early Experience with Another Species. *Science.* 1964; 143(3604):380–381. [PubMed: 14074860]
- Dent GW, Okimoto DK, et al. Stress-induced alterations in corticotropin-releasing hormone and vasopressin gene expression in the paraventricular nucleus during ontogeny. *Neuroendocrinology.* 2000; 71(6):333–342. [PubMed: 10878495]
- Donaldson ZR, Young LJ. Oxytocin, vasopressin, and the neurogenetics of sociality. *Science.* 2008; 322(5903):900–904. [PubMed: 18988842]
- Dubois-Dauphin M, Barberis C, et al. Vasopressin receptors in the mouse (*Mus musculus*) brain: sex-related expression in the medial preoptic area and hypothalamus. *Brain Res.* 1996; 743(1-2):32–39. [PubMed: 9017227]
- Egashira N, Tanoue A, et al. V1a receptor knockout mice exhibit impairment of spatial memory in an eight-arm radial maze. *Neurosci Lett.* 2004; 356(3):195–198. [PubMed: 15036628]
- Egashira N, Tanoue A, et al. Impaired social interaction and reduced anxiety-related behavior in vasopressin V1a receptor knockout mice. *Behav Brain Res.* 2007; 178(1):123–127. [PubMed: 17227684]
- Goldman MB, Robertson GL, et al. Psychotic exacerbations and enhanced vasopressin secretion in schizophrenic patients with hyponatremia and polydipsia. *Arch Gen Psychiatry.* 1997; 54(5):443–449. [PubMed: 9152098]
- Goodson JL, Thompson RR. Nonapeptide mechanisms of social cognition, behavior and species-specific social systems. *Curr Opin Neurobiol.* 2010; 20(6):784–794. [PubMed: 20850965]
- Hammock EA, Levitt P. Modulation of parvalbumin interneuron number by developmentally transient neocortical vasopressin receptor 1a (V1aR). *Neuroscience.* 2012; 222C:20–28. [PubMed: 22820266]

- Hammock EA, Young LJ. Microsatellite instability generates diversity in brain and sociobehavioral traits. *Science*. 2005; 308(5728):1630–1634. [PubMed: 15947188]
- Hammock EAD, Levitt P. The discipline of neurobehavioral development: The emerging interface of processes that build circuits and skills. *Human Development*. 2006; 49(5):294–309.
- Honeycutt H, Alberts JR. Housing pregnant mice (*Mus musculus*) in small groups facilitates the development of odor-based homing in offspring. *J Comp Psychol*. 2005; 119(4):418–429. [PubMed: 16366775]
- Hoset KS, Ferchaud AL, et al. Natal dispersal correlates with behavioral traits that are not consistent across early life stages. *Behavioral Ecology*. 2010
- Hu SB, Zhao ZS, et al. Vasopressin receptor 1a-mediated negative regulation of B cell receptor signaling. *J Neuroimmunol*. 2003; 135(1-2):72–81. [PubMed: 12576226]
- Kato Y, Igarashi N, et al. Distribution and developmental changes in vasopressin V2 receptor mRNA in rat brain. *Differentiation*. 1995; 59(3):163–169. [PubMed: 7589900]
- Kim SJ, Young LJ, et al. Transmission disequilibrium testing of arginine vasopressin receptor 1A (AVPR1A) polymorphisms in autism. *Mol Psychiatry*. 2002; 7(5):503–507. [PubMed: 12082568]
- Krackow S. Motivational and heritable determinants of dispersal latency in wild male house mice (*Mus musculus musculus*). *Ethology*. 2003; 109(8):671–689.
- Kuehl-Kovarik MC, Iqbal J, et al. Autoradiographic localization of arginine vasopressin binding sites in the brain of adult and developing Brazilian opossums. *Brain Behav Evol*. 1997; 49(5):261–275. [PubMed: 9150896]
- Landgraf R. Mortyn Jones Memorial Lecture. Intracerebrally released vasopressin and oxytocin: measurement, mechanisms and behavioural consequences. *J Neuroendocrinol*. 1995; 7(4):243–253. [PubMed: 7647766]
- Landgraf R, Frank E, et al. Viral vector-mediated gene transfer of the vole V1a vasopressin receptor in the rat septum: improved social discrimination and active social behaviour. *Eur J Neurosci*. 2003; 18(2):403–411. [PubMed: 12887422]
- Landgraf R, Gerstberger R, et al. V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities, and anxiety-related behavior in rats. *J Neurosci*. 1995; 15(6):4250–4258. [PubMed: 7790909]
- Le Moal M, Dantzer R, et al. Centrally injected arginine vasopressin (AVP) facilitates social memory in rats. *Neurosci Lett*. 1987; 77(3):353–359. [PubMed: 3614767]
- Lim M, Hammock E, et al. A method for acetylcholinesterase staining of brain sections previously processed for receptor autoradiography. *Biotech Histochem*. 2004; 79(1):11–16. [PubMed: 15223749]
- Meyer-Lindenberg A, Domes G, et al. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat Rev Neurosci*. 2011; 12(9):524–538. [PubMed: 21852800]
- Miller JA, Zahniser NR. The use of ¹⁴C-labeled tissue paste standards for the calibration of ¹²⁵I-labeled ligands in quantitative autoradiography. *Neurosci Lett*. 1987; 81(3):345–350. [PubMed: 3431749]
- Moriceau S, Sullivan RM. Maternal presence serves as a switch between learning fear and attraction in infancy. *Nat Neurosci*. 2006; 9(8):1004–1006. [PubMed: 16829957]
- Moriceau S, Wilson DA, et al. Dual circuitry for odor-shock conditioning during infancy: corticosterone switches between fear and attraction via amygdala. *J Neurosci*. 2006; 26(25):6737–6748. [PubMed: 16793881]
- Motz BA, Alberts JR. The validity and utility of geotaxis in young rodents. *Neurotoxicol Teratol*. 2005; 27(4):529–533. [PubMed: 16040231]
- Ohsawa H, Kishimoto T, et al. Atrial natriuretic peptide and arginine vasopressin secretion in schizophrenic patients. *Acta Psychiatr Scand*. 1993; 88(2):130–134. [PubMed: 8213206]
- Ophir AG, Wolff JO, et al. Variation in neural V1aR predicts sexual fidelity and space use among male prairie voles in semi-natural settings. *Proc Natl Acad Sci U S A*. 2008; 105(4):1249–1254. [PubMed: 18212120]
- Patisaul HB, Jefferson W. The pros and cons of phytoestrogens. *Front Neuroendocrinol*. 2010; 31(4):400–419. [PubMed: 20347861]

- Paxinos, G.; Franklin, KBJ. The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 2001.
- Phelps SM, Young LJ. Extraordinary diversity in vasopressin (V1a) receptor distributions among wild prairie voles (*Microtus ochrogaster*): patterns of variation and covariation. *J Comp Neurol*. 2003; 466(4):564–576. [PubMed: 14566950]
- Popik P, Vos PE, et al. Neurohypophyseal hormone receptors in the septum are implicated in social recognition in the rat. *Behav Pharmacol*. 1992; 3(4):351–358. [PubMed: 11224137]
- Rosenblatt JS. Olfaction mediates developmental transition in the altricial newborn of selected species of mammals. *Developmental Psychobiology*. 1983; 16(5):347–375. [PubMed: 6618012]
- Roth TL, Sullivan RM. Examining the role of endogenous opioids in learned odor-stroke associations in infant rats. *Dev Psychobiol*. 2006; 48(1):71–78. [PubMed: 16381030]
- Ryan MC, Sharifi N, et al. Evidence of basal pituitary-adrenal overactivity in first episode, drug naive patients with schizophrenia. *Psychoneuroendocrinology*. 2004; 29(8):1065–1070. [PubMed: 15219658]
- Schank JC. Early locomotor and social effects in vasopressin deficient neonatal rats. *Behav Brain Res*. 2009; 197(1):166–177. [PubMed: 18786571]
- Sheehan TP, Chambers RA, et al. Regulation of affect by the lateral septum: implications for neuropsychiatry. *Brain Res Brain Res Rev*. 2004; 46(1):71–117. [PubMed: 15297155]
- Sigling HO, Wolterink-Donselaar IG, et al. Home seeking behavior in rat pups: Attachment vs. kin selection, oxytocin vs. vasopressin. *Eur J Pharmacol*. 2009; 612(1-3):48–53. [PubMed: 19356730]
- Sodersten P, Henning M, et al. Vasopressin alters female sexual behaviour by acting on the brain independently of alterations in blood pressure. *Nature*. 1983; 301(5901):608–610. [PubMed: 6828140]
- Solomon NG, Richmond AR, et al. Polymorphism at the *avpr1a* locus in male prairie voles correlated with genetic but not social monogamy in field populations. *Mol Ecol*. 2009; 18(22):4680–4695. [PubMed: 19821904]
- Szot P, Dorsa DM. Differential timing and sexual dimorphism in the expression of the vasopressin gene in the developing rat brain. *Brain Res Dev Brain Res*. 1993; 73(2):177–183.
- Teltsh O, Kanyas-Sarner K, et al. Oxytocin and vasopressin genes are significantly associated with schizophrenia in a large Arab-Israeli pedigree. *Int J Neuropsychopharmacol*. 2011:1–11.
- Tribollet E, Goumaz M, et al. Appearance and transient expression of vasopressin and oxytocin receptors in the rat brain. *J Recept Res*. 1991; 11(1-4):333–346. [PubMed: 1653339]
- Tribollet E, Goumaz M, et al. 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*. 1991; 58(1):13–24.
- Veenema AH, Blume A, et al. Effects of early life stress on adult male aggression and hypothalamic vasopressin and serotonin. *Eur J Neurosci*. 2006; 24(6):1711–1720. [PubMed: 17004935]
- Veenema AH, Bredewold R, et al. Vasopressin regulates social recognition in juvenile and adult rats of both sexes, but in sex- and age-specific ways. *Horm Behav*. 2012; 61(1):50–56. [PubMed: 22033278]
- Wacker DW, Ludwig M. Vasopressin, oxytocin, and social odor recognition. *Horm Behav*. 2012; 61(3):259–265. [PubMed: 21920364]
- Wang Z, Liu Y, et al. Developmental changes in forebrain vasopressin receptor binding in prairie voles (*Microtus ochrogaster*) and montane voles (*Microtus montanus*). *Ann N Y Acad Sci*. 1997; 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(1-2):191–195.
- Wang Z, Young LJ, et al. Species differences in vasopressin receptor binding are evident early in development: comparative anatomic studies in prairie and montane voles. *J Comp Neurol*. 1997; 378(4):535–546. [PubMed: 9034909]
- Wassink TH, Piven J, et al. Examination of AVPR1a as an autism susceptibility gene. *Mol Psychiatry*. 2004; 9(10):968–972. [PubMed: 15098001]

- Wersinger SR, Caldwell HK, et al. Vasopressin 1a receptor knockout mice have a subtle olfactory deficit but normal aggression. *Genes Brain Behav.* 2007; 6(6):540–551. [PubMed: 17083331]
- Winslow JT, Insel TR. Effects of central vasopressin administration to infant rats. *Eur J Pharmacol.* 1993; 233(1):101–107. [PubMed: 8472738]
- Yang SY, Cho SC, et al. Association study between single nucleotide polymorphisms in promoter region of AVPR1A and Korean autism spectrum disorders. *Neurosci Lett.* 2010; 479(3):197–200. [PubMed: 20546835]
- Yang SY, Cho SC, et al. Family-based association study of microsatellites in the 5' flanking region of AVPR1A with autism spectrum disorder in the Korean population. *Psychiatry Res.* 2010; 178(1): 199–201. [PubMed: 20452058]
- Yirmiya N, Rosenberg C, et al. Association between the arginine vasopressin 1a receptor (AVPR1a) gene and autism in a family-based study: mediation by socialization skills. *Mol Psychiatry.* 2006; 11(5):488–494. [PubMed: 16520824]
- Young LJ, Flanagan-Cato LM. Editorial comment: oxytocin, vasopressin and social behavior. *Horm Behav.* 2012; 61(3):227–229. [PubMed: 22443808]
- Zielinski WJ, Vomsaal FS, et al. The Effect of Intrauterine Position on the Survival, Reproduction and Home Range Size of Female House Mice (*Mus-Musculus*). *Behavioral Ecology and Sociobiology.* 1992; 30(3-4):185–191.

Figure 1.

V1aR ligand binding in post-natal day 8 wild-type C57BL/6J mouse forebrain. A) Representative autoradiographs showing the rostro-caudal (1-8) ligand binding of ^{125}I – labeled linear vasopressin antagonist (NEX310, Perkin Elmer, Waltham, MA, USA), a potent and selective ligand for V1aR. B) Quantitation of relative ligand binding at P8 in males (diamonds) and females (circles) demonstrating regional differences in ligand binding density. All data are mean $\pm$ S.E.M.

Figure 2.

Odor bias behavior at P8. A) Training schematic showing training paradigms. Each block represents 1 hour. B) Each pup was tested individually for 3 minutes in a test chamber with freshly prepared odorants. C) Wild-type females, but not males, show evidence of familiarity bias in the contingent condition, but not in the not contingent condition. D) Knock-out females, but not males, show significantly enhanced familiarity preference in both conditioning paradigms. Graphed data are mean $\pm$ S.E.M. Asterisks denote $\alpha < 0.05$ significance for one-tailed student's t-tests.

Figure 3.

V1aR activation by injection with vasopressin (AVP) eliminated orienting bias in females. A) AVP injection (20ng) into the lateral ventricle of wild-type females just before testing eliminated the display of familiar odor bias. In contrast, injection of AVP into the lateral ventricles of knock-out females did not eliminate the strong familiarity bias, demonstrating that the effect of AVP was through its action on V1aR. B) AVP injection in males did not alter odor preference scores. Graphed data are mean $\pm$ S.E.M. Pharmacological data were evaluated with a priori t-tests (see text) and an omnibus ANOVA followed by Tukey's least significant difference. Asterisks indicate post-hoc significance at $p < 0.05$.

Figure 4.

Summary working model of early odor bias, including potential neurobiological mechanisms (A, B) and ethological relevance (C). A) Females tended to show an orienting bias which may be driven by female factors, with insignificant inhibition by baseline levels of AVP. B) In contrast, exogenous AVP application blocked this female-specific bias related activity. Males (not schematized) tended to show no familiar odor bias, and the absence of this presumptive circuit activity under our experimental conditions could not be further modulated by AVP signaling. C) Because life history traits in mammals depend on a protracted period of developmental maturation, changing probabilities of orienting toward familiar stimuli may lead to altered frequency of reinforced experiences in the natal area or with conspecifics more generally. This, in turn, may influence the probability of a weanling orienting toward its natal home or escaping to a new territory, or enhance social or other expertise.