THE VASOPRESSIN SYSTEM AND BEHAVIOR

EDITED BY: Heather K. Caldwell and Aras Petrulis

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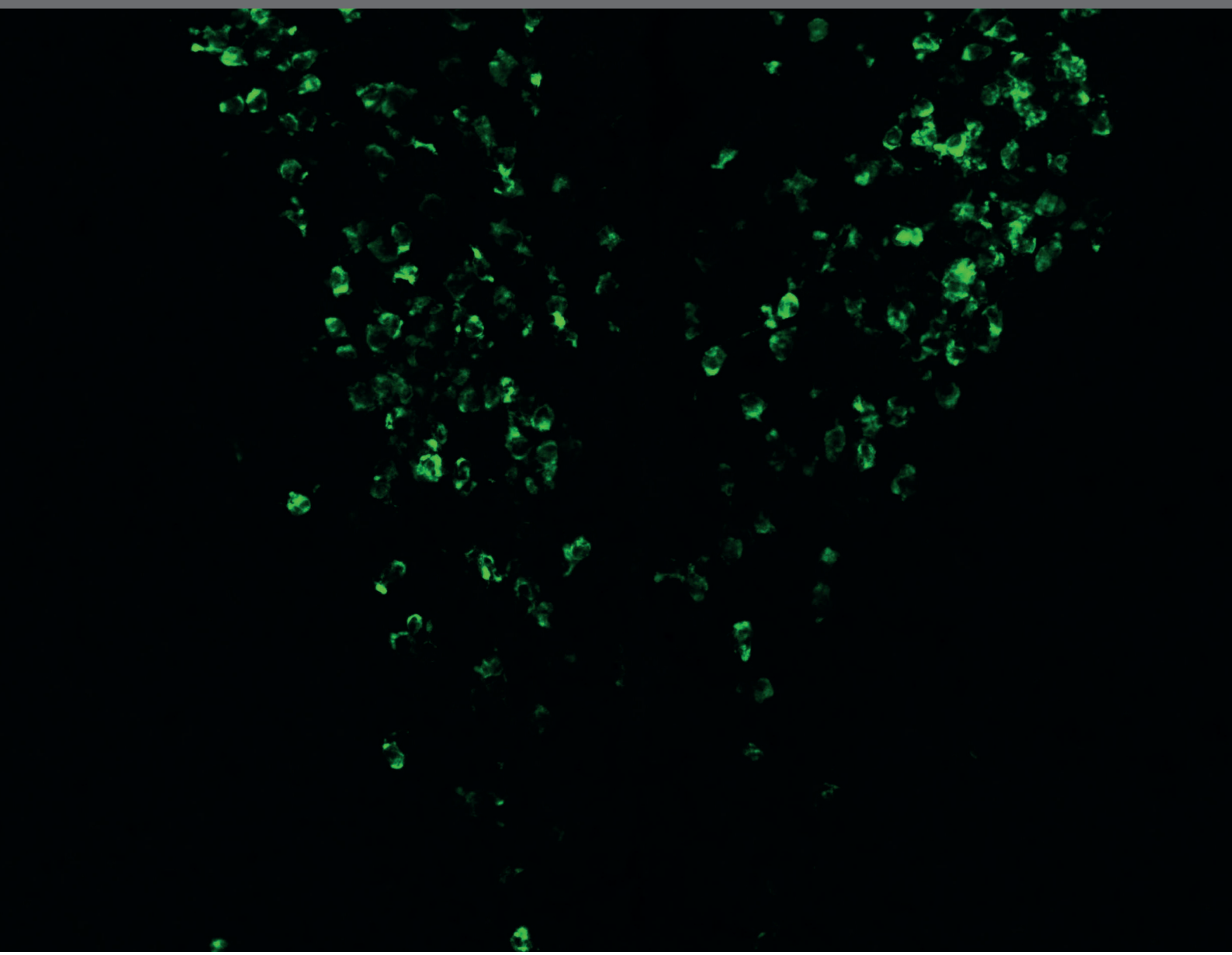

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THE VASOPRESSIN SYSTEM AND BEHAVIOR

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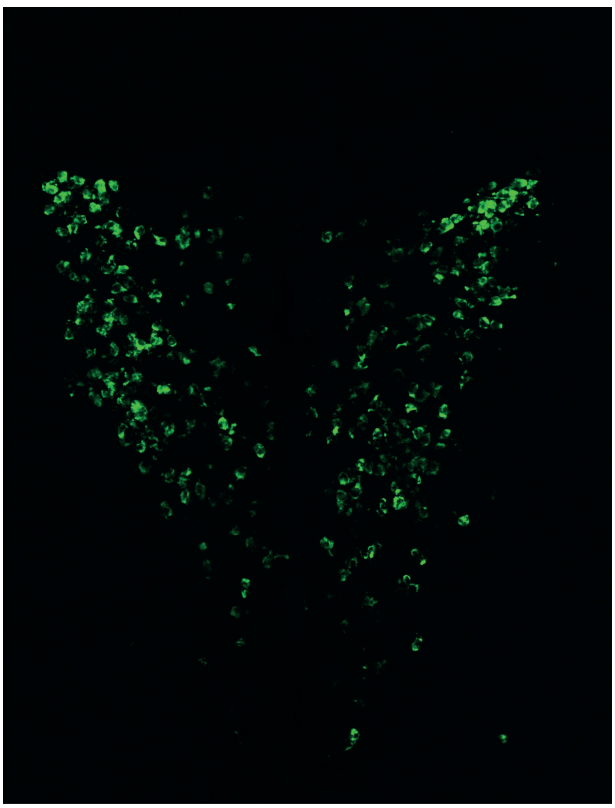

Fluorescent in situ hybridization of vasopressin-neurophysin mRNA in the mouse paraventricular nucleus of the hypothalamus. 20X magnification.
Image: Jack Whylings, M.S.

Vasopressin and its homologues are evolutionarily ancient neuropeptides that are important to the neural modulation of behavior in many species. Over the last several decades there has been an emergence of cross-species consensus with regard to the broad behavioral domains that the vasopressin system influences. However, there are nuanced species- and sex-differences in the functions of this system, as well as evidence for cross-talk between this system and the oxytocin system.

For this Research Topic, reviews and research articles from investigators across the field were solicited, with the goal to highlight some of the complexity and diversity within this system. This collection challenges researchers to broaden their

understanding of this system as well as identifies areas in which additional research is needed. Topic areas featured include:

- System complexity
- Sex and species differences
- Developmental effects
- Human and non-human primates

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HUMAN AND NON-HUMAN PRIMATES

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EDITORIAL
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Editorial: The Vasopressin System and Behavior

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Keywords: vasopressin, oxytocin, vasotocin, behavior, brain

The Editorial on the Research Topic

The Vasopressin System and Behavior

The awarding of the 1955 Nobel Prize in Chemistry to Vincent du Vigneaud, in part for his isolation and synthesis of the neuropeptide arginine vasopressin (Avp), ushered in a new era of research focused on the ways in which Avp (and its evolutionary precursor vasotocin (Avt)) regulate physiology and behavior (1, 2). While early studies focused primarily on the peripheral effects of these neuropeptides, it soon emerged that Avp, and its homologs, are important neuromodulators of behavior; a PubMed search indicates a steady rise in the number of published papers on this topic each year since the mid-1950s, with over 120 papers published per year since 2012. No doubt this continued enthusiasm is due, in part, to the evolutionarily ancient and highly-conserved nature of Avp-like molecules across vertebrate taxa (3). As a result, there is now a tremendous richness in this literature, both in species breadth and mechanistic depth. It was with this in mind that we organized this research topic on "The Vasopressin System and Behavior," in which we hoped to bring together a diversity of perspectives outlining areas of consensus and divergence, as well as to facilitate discussion.

Within this collection, numerous reviews and empirical papers explore the complexity of the Avp and Avt systems in the context of different behavioral systems and taxa. From these papers, several broad themes and some "calls to action" have emerged. First, although it is clear that Avp/Avt are key regulators of social and emotional behavior [e.g., (4–7)], there is a lack of consensus regarding the contexts under which they modulate behavior. Thus, this remains an area requiring even deeper scrutiny. For example, Carter reviews how complex interactions between the Avp and oxytocin systems can affect an animal's responses within differing emotional and social contexts. Second, a broader look at how Avp/Avt influences brain systems, rather than just individual brain regions, is required for further progress. This is underscored by papers from Ophir and Phelps et al. that outline the potential for Avp and oxytocin to alter cortical and hippocampal dynamics underlying complex social space use. Third, even though sex-differences in response to Avp have been frequently noted in the literature [e.g., (8–11)], there are still behavioral domains and taxa in which more critical evaluation of sex differences in the Avp/Avt systems are needed Taylor et al.; Wilczynski et al.; Tickerhoof and Smith; Terranova et al. Fourth, as Simmons et al., Murgatroyd et al., and Baran demonstrate, there is an important role for Avp/Avt throughout behavioral development that is currently under-examined. Fifth, as is explored in Caldwell et al. and Terranova et al., continued consideration of receptor dynamics and subtypes that mediate Avp effects will be needed to more fully understand the relationship between Avp release and its locus of action. Sixth, given the ancient origin of Avp/Avt, a continued commitment to explore their roles across species will aid in finding areas of convergence and divergence in behavioral function. This diversity of Avp/Avt behavioral action across different species is nicely illustrated

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by contributions from Baran, Rodriguez-Santiago et al., Wilczynski et al., and Latzman et al. Lastly, the Avp system is clearly important in regulating human and non-human primate behavior, as described by Latzman et al., Murgatroyd et al., Patisaul, Rilling et al., Price et al., Taylor et al. However, compared to the avalanche of data on oxytocin effects in humans, much less is known and understood regarding the how, the where, and the when of Avp effects on primate social behavior. Thus, there is a need for further detailed examinations of Avp's influence on behavior and cognition, especially within the context of human health.

We are hopeful that these diverse and thoughtful papers will be utilized by both new and more seasoned investigators to guide their work and will spark new discussions about the role of the Avp/Avt systems in behavioral regulation. Given the complexity of these systems, the diversity of species studied, and the numerous behaviors they regulate, it appears that even 60 years after the structural definition of Avp, much work still remains to be done.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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The Oxytocin–Vasopressin Pathway in the Context of Love and Fear

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Vasopressin (VP) and oxytocin (OT) are distinct molecules; these peptides and their receptors [OT receptor (OTR) and V1a receptor (V1aR)] also are evolved components of an integrated and adaptive system, here described as the OT–VP pathway. The more ancient peptide, VP, and the V1aRs support individual survival and play a role in defensive behaviors, including mobilization and aggression. OT and OTRs have been associated with positive social behaviors and may function as a biological metaphor for social attachment or "love." However, complex behavioral functions, including selective sexual behaviors, social bonds, and parenting require combined activities of OT and VP. The behavioral effects of OT and VP vary depending on perceived emotional context and the history of the individual. Paradoxical or contextual actions of OT also may reflect differential interactions with the OTR and V1aR. Adding to the complexity of this pathway is the fact that OT and VP receptors are variable, across species, individuals, and brain region, and these receptors are capable of being epigenetically tuned. This variation may help to explain experience-related individual and sex differences in behaviors that are regulated by these peptides, including the capacity to form social attachments and the emotional consequences of these attachments.

Keywords: oxytocin, vasopressin, oxytocin receptor, vasopressin receptor subtype 1a, love, attachment, prairie voles, aggression

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There is no fear in love: but perfect love casteth out fear. (1 John 4: 18)

INTRODUCTION

Oxytocin (OT) and vasopressin (VP) are ancient peptide molecules with many behavioral and physiological functions. These pleotropic peptides evolved from a single genetic source (1). OT and VP, with their receptors, function as an integrated, adaptive system, allowing the mammalian body to survive, maintain homeostasis, and reproduce in an ever-changing world. However, OT- and VP-like molecules were co-opted for other functions many times over the course of evolution (2).

Vasopressin is considered the more ancient molecule, with a central role in defense. OT, especially in a context of safety, may override the defensive functions of VP helping to facilitate the evolution of the complex cognition and selective sociality associated with human behavior, including social attachment and love (3, 4) (**Figure 1**).

Sources of individual differences in OT and VP and the sensitivity of their receptors include gender and basic genetic differences (6, 7). For example, some species, including humans and other socially monogamous mammals, such as prairie voles and dogs, have high levels of OT (8, 9) and

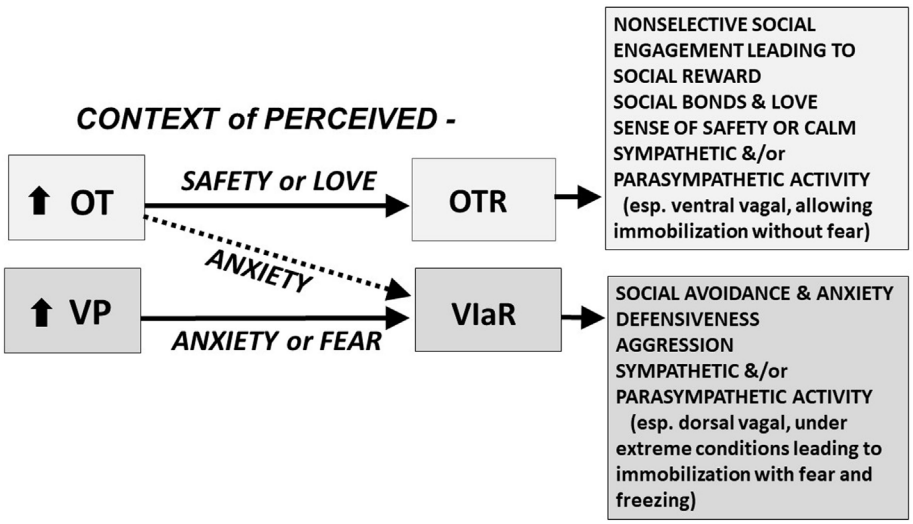

FIGURE 1 | The oxytocin (OT) and vasopressin (VP) pathway includes the OT receptor (OTR) and the V1a receptor (V1aR). We hypothesize that in a context of perceived safety, OT predominately acts on the OTR, facilitating "immobility without fear," including high levels of social engagement, social bonds, and social reward; these behaviors are at the heart of mammalian reproduction and "love." VP and the V1aR are more ancient and probably become dominant under conditions of anxiety or trauma. In a context of anxiety or fear, OT may function primarily through effects on the V1aR; under these conditions both OT and VP may act, *via* the V1aR, to induce additional anxiety, social avoidance, defensiveness, aggression, and fear. We hypothesize that under extreme conditions, fear and the V1aR may dominate leaving the individual vulnerable to "immobility with fear," which may lead to freezing and cognitive and emotional dissociation. These responses are mediated in part by interactive effects of OT and VP on the sympathetic nervous system and the parasympathetic nervous system, including the ventral vagal complex (necessary for social engagement) and the dorsal vagal complex (functioning to conserve energy and protect against shutting down in the face of trauma) (5). Other components of this adaptive system including the V1bR, and many other molecules or receptors, including those regulated by CRH, dopamine, opioids, GABA, and serotonin, play a role in the expression of social and defensive behaviors. The differential actions of OT and VP are dose, time, and brain-region dependent. The OT and V1a receptors are affected by genetics and epigenetic tuning, especially in early life.

an apparent dependence on OT to allow the expression of high sociality and attention to positive social cues. The OT receptor (OTR) and V1a receptor (V1aR) also can be epigenetically tuned by experience (10–14), increasing the capacity of OT and VP to have complex adaptive functions.

Behavioral work in this field has focused on the neurobiology of OT in social behavior and the management of stressful experiences (3, 4, 15, 16). The systems necessary for actions of OT involve extensive neural networks through the brain and autonomic nervous system. Many recent reviews describe the neural and behavioral roles of these peptides (4, 17–25). Furthermore, these networks are capable of dynamically changing (20, 26, 27), especially in early life (26, 27). Those reviews will not be duplicated here, but in conjunction with primary sources are used as background for a discussion of functional interactions between OT and VP and their receptors in the context of evolution and mammalian social behavior.

The OT and VP Pathway

Current knowledge concerning OT and VP and their receptors indicate that these are interactive components of an evolved and integrated system—here termed the OT–VP pathway (**Figure 2**). It has long been known that both peptides can bind to both the OT and VP receptors *in vitro* (28–32). Accumulating evidence dealing with diverse outcomes and from various species supports the hypothesis that when looking at the whole organism OT and VP tend to affect more than one receptor and several types of behavioral functions (7, 20, 33–35). In general in the behavioral literature, OT has received more attention than VP.

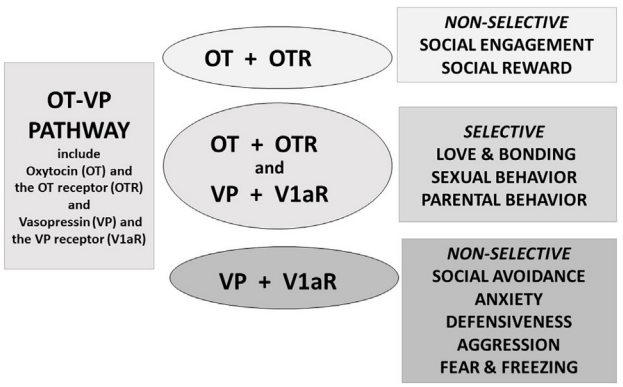

FIGURE 2 | Oxytocin (OT) and vasopressin (VP) are components of an integrated pathway. OT and VP interact dynamically with receptors [including the OT receptor (OTR) or V1a receptor (V1aR)] to influence social engagement and defensive behaviors. In many cases, OT acts in conjunction with VP, *via* the V1aR or through effects on both the OTR and V1aR, thus regulating the capacity to form selective social behaviors. OT rarely acts alone but, especially under nonthreatening or "safe" conditions, may facilitate features of "love," including social engagement, and social reward, and "immobility without fear" (36).

The OT–VP pathway allows the body to adapt to highly emotional situations and develop selective attachments. Such experiences require the presence of both peptides (37), as well as molecules associated with reinforcement, such as dopamine (38–40). Conditions under which *both* OT and VP are necessary for normal behavior include selective social behaviors and emotionally intense experiences, such as sexual behavior, parental behavior, and pair bond formation, as well as regulation of the autonomic nervous system (18, 41, 42).

Until recently OT and VP, and their receptors, were typically treated as independent systems. This is especially true in human studies of the effects of exogenous hormones (43–45). For a notable exception see studies by Rilling and associates, in which both peptides are being studied (46, 47).

Properties of OT and VP

Oxytocin and VP are small peptides that are similar in structure. Both consist of nine amino acids in a six amino acid ring, formed by cysteine bonds, and a three amino acid tail with a terminal amine group. The precursors for OT and VP consist of 12 amino acids and are synthesized and released in conjunction with carrier proteins (neurophysin 1 and 2, respectively). The precursors are later cleaved into the "mature" forms of these peptides. It is also possible that precursors and fragments of OT and VP have unidentified functions (29, 48); although not well studied, it is likely that these forms and the binding of OT and VP in blood and other tissues play a role in the functional interactions of OT and VP (49).

Oxytocin and AVP are primarily synthesized in brain regions that are critical to behavioral and physiological homeostasis. Different cells in specific brain regions produce these two peptides, including the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus (20). Anatomical studies in rodents indicate that OT and VP are synthesized in discrete areas and in separate cells within the PVN and SON; these cells also produce a network of neural projections reaching throughout the brain and spinal cord (50). For additional details of specific neural targets for OT and VP, see reviews such as those from Wang and his associates (39, 51).

Research using brain slices (25) indicates that in other brain regions, including the amygdala and the bed nucleus of the stria terminalis, both OT and VP containing cells and projections lie adjacent to each other. These OT–VP associations form local functional units, capable of rapid and often opposite interactions—for example, in brain regions associated with fear versus fear reduction. Fear responses are mediated by V1aRs in the amygdala, while OT may act to inhibit fear, depending on context (**Figure 3**) and gender (52).

Oxytocin and VP are synthesized and stored in the pituitary gland, where these peptides are thought to remain in vesicles until released as the nine amino acid forms. However, these molecules also may be released from axons within the CNS (20), as well as from the neuronal soma and dendrites or by diffusion within the brain (50). In addition, OT and VP are made throughout the body with local effects on diverse tissues, including the uterus, testes, digestive system, kidney, and thymus (53, 54). The dynamic nature of the OT–VP pathway not only makes this system exciting but also limits research in this field.

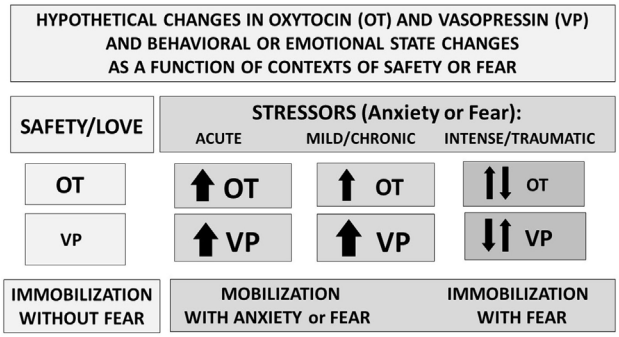

FIGURE 3 | Perceived context and the intensity of challenge can regulate the release or effects of oxytocin (OT) and vasopressin (VP). Under conditions of safety, the actions of OT may dominate, supporting high levels of sociality. In response to an acute stressor, both OT and VP increase, supporting mobilization and escape, followed in some cases by increases in social behavior especially toward "safe" conspecifics. Following intense or traumatic stressors, initial responses would include mobilization and anxiety. However, following a traumatic experience, individuals may vacillate between mobilization and immobilization with fear or revert to the more primitive response of shutting down. These patterns differ between males and females and as a function of individual life histories.

Sex differences are adaptive and commonly seen in studies of actions of OT, and especially VP. Sex differences are not always explored, but when they are, males and females frequently differ (52); this is especially true with reference to reactions to treatments involving stressors (3). Most of these studies suggest that males have either more VP (55) or are more sensitive to the effects of VP (56). For example, in a quantitative study of 22 subregions in the forebrain "social behavioral neural network" in rats, VP immunoreactivity show marked regional variation between males and females and as a function of age (57). These differences were particularly apparent in the medial amygdala, bed nucleus of the stria terminalis, and lateral septum—brain regions previously implicated in androgen-dependent sex differences and in defensive aggression. OT immunoreactivity did not show this pattern of variation in rats.

Receptors for OT and VP

The gene (*OXTR*) for the OTR is found on human chromosome 3. The same OTR located in breast, uterus, and neural tissue also is present in many other bodily tissues. Three VP receptor subtypes are expressed in different tissues, and their genes are located on separate chromosomes. The V1aR is found in nervous system and throughout the cardiovascular system with a broad set of behavioral functions. The VP V1b receptor is not only found in the pituitary but also in brain areas with a role in the management of stress and aggression (58, 59). The VP V2 receptor is localized primarily to the kidney with a classical role in fluid balance.

The V1aR evolved from and is homologous with the vasotocin receptor (2). There is a high level of homology among the OTR and the three VP receptors, especially in the extracellular binding

domain which allows OT and VP to bind to each other's receptors (30, 32, 60). The pharmacological tools available for identifying, stimulating, or blocking receptors for OT and VP often have not been sufficiently selective to allow easy identification or manipulations of these receptors (61).

In mammals, receptors for OT and/or VP are typically abundant in areas of the nervous system that regulate social, emotional, and adaptive behaviors and reward (17). Among the regions with high levels of OTR or V1aR are various parts of the amygdala, the bed nucleus of the stria terminalis, the nucleus accumbens, brainstem source nuclei for the autonomic nervous system (25, 39, 62), and systems that regulate the hypothalamic–pituitary–adrenal (HPA) axis. OTRs also are found in cortex and hippocampus; these are highly variable among species and individuals, with possible consequences for neural and behavioral plasticity (63–66). In the cortex and in the spinal cord, both the V1aR (67) and OTR are present, allowing the possibility for interactions in processes such as social cognition and pain (68).

Expressions of the OTR and V1aR in the nervous system are highly variable even within species; for example, brain regions and individual differences are related to functional and adaptive patterns of sociality and aggression (6, 12, 24, 62, 69, 70). VP and OT and in some cases their receptors may differ between males and females, and across the lifespan (7, 39, 51, 52, 71, 72). The receptor variation characteristic of this system, and especially of VP has been associated with species and individual variation in behavior and brain function. Although sometimes overlooked in behavioral research, both peptides can have regulatory effects throughout the entire body, including the autonomic nervous system (42) and the immune system (53, 73–75) with effects on inflammation and healing.

Initially, it was assumed that only one primary receptor existed for OT (76). Genes for the *OXTR* and the three VP receptors code for separate G-protein coupled receptors, each with a seven transmembrane domain. Peptides binding to these receptors trigger subcellular cascades. The subcellular signaling pathways are not identical for these different receptors. In addition, the capacity of OT or VP to activate a given receptor subcellular signaling pathway may differ according to the concentrations of the peptides and the regional location of receptors in the nervous system (20, 25, 61). These subcellular differences may help to explain the capacity of OT and VP to have different functions in various processes, such as birth (77), social behavior (17, 34), and reactivity to stressors (13, 16, 25). Further adding to the complexity of this system is the possibility that receptors for OT and VP can form heterodimers with unknown consequences for peptide binding (20).

Evolution and Sociality

Oxytocin and VP are genetic and biochemical siblings. Both originated from a single ancestral gene that produced vasotocin (1, 2). Vasotocin is found in reptiles and other vertebrates and can be measured in the mammalian fetus. OT and VP differ from vasotocin by one amino acid and from each other by two amino acids. It is estimated that the ancestral peptides arose over 500 million years ago, that VP evolved approximately 200 million years ago and OT approximately 100 million years ago, originally through gene duplication (1). The genes for OT and VP reside near each other on human chromosome 20, lying in opposite transcriptional orientations (48).

Compared with OT, VP is the more primitive molecule and closer in function to vasotocin (1, 2, 23). However, other OT-like peptides, including mesotocin and isotocin, have functions that resemble those of OT. Animals that evolved from reptiles, including mammals and birds may be particularly dependent on selective social behaviors and OT-like peptides for reproduction and survival.

Vasopressin-like molecules are critical to adaptation and water balance under difficult environmental conditions. VP can support sympathetic arousal, mobilization (flight–flight responses) or in more extreme cases a metabolically conservative, shutdown response (5). Thus, under conditions of extreme stress or trauma, VP may take precedent over OT and over survival strategies that are more prosocial or mobilized (**Figure 3**). However, the benefits of either sociality or OT also may be most easily detected in the presence of a stressor or of VP (3, 37).

Combined Actions of OT and VP

At the core of positive social behaviors are neurobiological systems that regulate fear and threats versus safety (**Figures 1**–**3**). OT typically supports immobilization without fear, necessary in interactions with family and friendly associates (36). VP supports mobilization, and in some cases defensive aggression and protection of social boundaries. By contrast, VP, in conjunction with CRH, dopamine and many other molecules, may support active and mobilized coping strategies (3, 71, 78). However, as with many features of the OT–VP pathway, exceptions exist—possibly because of the capacity of OT and VP to interact with each other's receptors (**Figure 1**).

Increasing evidence suggests that the actions of OT on the V1aR, versus the OTR, vary depending on the behavior and context being examined (7, 17) (**Figure 1**). In hamsters, fear-based or aggressive effects of OT rely on the V1aR and social reward on the OTR (34, 79, 80). The capacity of OT and VP to bind to each other's receptors adds complexity to attempts to understand both peptides. However, OT–VP interactions also are adaptive, increasing the capacity for a small number of peptides and receptors to regulate various processes across different tissues.

Both OT and VP are responsive to environmental and social demands, although in somewhat different ways (3, 16). These peptides—presumably *via* interactions with their receptors—may have diverse physiological and behavioral properties. Regional effects of OT and VP are expected and need to be investigated to fully understand the functional consequences for these peptides. Dynamic interactions either on specific receptors, due to brain region-specific actions, or due to relative availability of the peptides to a receptor (20) could help to explain the behavioral properties of these two molecules. In addition, refined behavioral studies are necessary, since the effects of OT and VP on various behaviors change across time and as behavioral context changes.

As detailed below, a number of studies have attempted to separate the effects of OT versus VP on reproduction, social behavior, and aggression (7, 17). In general, it appears that OT plus VP may be especially critical to allow selective social experiences that involve awareness the individual identity of a partner and

the experience of a social reward (18, 37). These behaviors also may require alternating between behavioral mobilization and immobilization, which is seen after trauma (36) (**Figure 3**).

Methodological Limitations and "Cross Talk" Between OT and VP

The evolved properties of OT and VP permit "cross talk" between these peptides and their receptors (7, 17, 20, 31, 33, 77) (**Figure 1**). Unexpected outcomes are sometimes reported when exogenous OT or VP is given or when a peptide or specific receptor is inactivated. This work initially depended on pharmacological agonists or antagonists, often using drugs that were relatively non-specific. More recently research, primarily in rodents, has used genetic manipulations including rodents with mutations (81) or optogenetic methods for silencing or activating genes for peptides or receptors (40, 82).

Early evidence for cross talk between VP and the OTR came from research in OT knockout (OTKO) mice (31). For example, single-unit recording from tissue slices from the ventromedial hypothalamus from OTKO mice revealed that VP was capable of stimulating this brain region. Moreover, whether findings from mutant mice can be generalized to wild-type animals remains unclear. For example, OTKO mice showed increased sensitivity to VP. This important finding suggested that the absence of OT across the lifespan could sensitize animals to later VP exposure. The molecular basis of this process remains to be discovered. Components of some functions, such as birth and maternal behavior, continue to be observed in OTKO mice (83, 84). However, upon careful examination, these behaviors often lack the full range of behavioral expression typical of wild-type animals (81).

Similar methodology also has been used to study the OTR. Reductions in social behavior and cognitive flexibility and increases in aggression and seizure susceptibility are seen in OTR-null mice (85). These behaviors in OTR-deficient mice can be rescued not only by OT but also by VP treatments. This may be another expression of the capacity of the nervous system to adapt to changes in the peptidergic systems. However, studies of animals that are missing only one allele for the gene regulating the OTR show selective deficiencies in social behaviors, but not aggression (85). This study further supports the hypothesis that positive social behaviors may be especially sensitive to the reductions in OTR activity, while more defensive and perhaps more primitive processes are preserved.

Interactions among OT and VP and their receptors allow adaptive functions in time frames that are both short term and long term. Studies comparing the short-term versus long-term interactions between OT and VP are rare. However, those studies that do exist suggest that acute versus chronic actions of OT and VP can be very different (**Figure 3**), and sometimes opposite in function (86). Based on the behavioral patterns that are seen following acute versus chronic exposure to exogenous OT, we can hypothesize that the long-term effects of OT, and possibly the effects of very high levels of OT may involve stimulation of the V1aR (**Figure 1**).

Behavioral studies dealing with OT's capacity to affect VP receptors have focused on OT's effects on the V1aR or combined effects of OT and VP on the OTR and/or the V1aR (**Figure 2**). In general, the combined effects of OT plus VP are associated with highly rewarding experiences including some components of sexual behavior, parental behavior, and pair bond formation. At present, only a very limited number of studies seem to support the notion that OT functions primarily at the OTR without the participation of the V1aR (7, 82). Among the functions that seem especially dependent on OT are comparatively "modern" mammalian functions including lactation, reversal learning, and behavioral plasticity (33, 34, 61). Whether VP can stimulate the OTR *in vivo* has received less attention (80).

INTERACTIVE FUNCTIONS OF OT AND VP

Caveats

Examples of specific studies of functional interactions within the OT–VP pathway are described below. In some cases, only a portion of the possible interactions only a portion has been tested. In most, but not all cases, OT has been shown to have the capacity to affect the V1aR. In cases deliberately involving a stressor, effects of OT or VP that were not otherwise detected may emerge. A possible role for the VP V1bR is beyond the scope of this review, but effects of stress and OT on the VP V1bR also are possible (81). Among the many other molecules of importance to the regulation of OT and VP are CRH (87), GABA (88), dopamine (38), and serotonin (89, 90); these molecules also play roles in the modulation of stress and coping. Brain region- and cell type-specific changes are another source of variation that is relevant to understanding how OT and VP interact. New technologies, such as optogenetics, are allowing more specificity in neural circuitry but are currently limited to comparatively simple behaviors or components of behavioral patterns. There is increasing evidence that the OT–VP receptor pathway is epigenetically tuned by experience, including gonadal hormones, stressors, and probably peptides as well (10, 11, 13, 14, 75). Although not reviewed here, processes such as methylation may be of particular relevance to explaining the role of context and experience in the regulation of social behavior.

Lactation

Lactation is a defining feature of mammals, and contraction of breast tissue and milk ejection requires stimulation of the OTR. Lactation arose in conjunction with the evolution of mammals and is one of the comparatively few reproductive functions that do not continue in the absence of OT or the OTR (81, 83, 84). Immature mammalian offspring depend for varying periods of time after birth on their mother's milk. Conservation of fluids is necessary for lactation and effects of VP on the kidney and blood pressure probably support normal milk production, but this is presumably under separate control from milk ejection.

Birth and Uterine Contractions

Observations at the beginning of the twentieth century offered early evidence that OT and VP interactions are components of the normal functions of these peptides. Research conducted by Sir Henry Dale in 1906 showed that an extract from the human

posterior pituitary gland was capable of producing contractions in the uterus of a pregnant cat. The pituitary gland contains both OT and VP and the effects of pituitary extracts probably reflected the effects of both peptides and possibly other hormones (91).

In his Nobel Lecture, describing the functions of the first "polypeptide," Vincent du Vigneaud mentions two assays used to test the biological activity of OT. In that research, du Vigneaud (Nobel Lectures, 1955, p. 461) used rat uterine strips, but also noted that he used the "chicken vasopressor method of Coon, which utilizes the property of OT to lower the blood pressure of the fowl and has been adopted by the United States Pharmacopeia as the method for assay for OT." The use of a vasopressor response to assay OT, indirectly acknowledged the capacity of OT to stimulate the VP system. This was one of the first of what would eventually be many lines of research documenting interactions between OT and VP.

In large mammals, OT adopts a central role in reproduction by helping, in some cases, to expel the big-brained baby from the uterus (4, 18). However, in mice and presumably other mammals, birth can occur without OT (81, 83, 84). Egg laying, which is the precursor to birth, appeared long before the evolution of mammals, and thus may rely on more ancient hormones, including VP or vasotocin.

Although OT has been assumed to play a fundamental role in birth, current evidence suggests that OT alone acting on the OTR is NOT capable of inducing normal labor and blocking only the OTR does NOT prevent premature birth. Rather both OT and VP and both the OTR and V1aR regulate uterine contractions (60, 92). Thus, it is not surprising that female mice made mutant for OT or the OTR remain capable of giving birth (83, 84). In fact, especially under conditions of stress, VP is likely to have a much greater role in birth than has been acknowledged. VP's effects on the uterus, although functionally different from OT, may help to explain premature labor and preeclampsia, which are associated with adversity or stress across the life span (77, 91).

Parental Behavior

Early research on OT revealed consequences for maternal behavior (93) and filial bonding (94). Although, a role for OT in maternal behavior is now widely accepted, this work was initially controversial (95). Apparent discrepancies regarding the necessity of OT to maternal behavior may have been due to experimental differences related to the role of stress in mothering. Effects of acute OT seem to be most apparent in the face of novelty, acute stressors or against a background of elevated HPA axis activity (96, 97). In the presence of OT, avoidance or fear of the infant may be replaced by approach and positive emotional states (3). Whether this is due to competitive inhibition of VP or more specific actions of OT on the HPA axis deserves additional study.

A functional role for VP in maternal behavior cannot be excluded. Pedersen and colleagues found that centrally administered VP increased maternal behavior in rats, although the effects of VP took longer to appear than those seen after OT. OTKO mice remain maternal to some extent, but their behavioral patterns are not identical to those in wild-type mice (81). The role of OT in maternal behavior may depend in part on the capacity of OT to directly or indirectly override the defensive effects of VP and reduce fear in the presence of young animals. VP, in conjunction with OT, also supports the capacity to protect offspring, in the form of postpartum maternal or paternal aggression in rodents (98, 99).

Threatening Environments and Aggression

Vasopressin and the V1aR may be of critical importance in the capacity for physical and emotional adaption in the presence of stressful experiences (3, 16, 24, 25). VP is involved, synergizing with CRH (78), in hypothalamic regulation of the pituitary, supporting the release of glucocorticoids and mobilized defense strategies against various physical and emotional stressors or threats (25). OT also can be released during stressful experiences and is sometimes considered a "stress-coping" molecule.

Vasopressin also plays a protective role in the behavioral defense of self and the family (3, 100). Various forms of aggression and territoriality have been related to stimulation of the V1aR in both males and females (7, 101, 102). However, at least in golden hamsters the mediation of dominance and aggression was associated with increases in hypothalamic VP in males (but not in females). By contrast, serotonin, acting in the dorsal raphe, was associated with increased aggression in females, and decreased aggression in males (90).

Avoidance of Danger and Anxiety

A growing literature associates increased central VP in the development of memory necessary for the avoidance of danger or survival (24). Psychological processes associated with anxiety and obsessions also may rely on VP (7, 103, 104). VP, in the context of other centrally active molecules, such as CRH, dopamine, and serotonin, regulates emotional states, including anxiety (**Figure 3**). Anxiety in turn can reduce the capacity to use cognitive or "top down" strategies to manage stressful experiences. VP and CRH can amplify the effects of each other on aggression and anxiety, especially during circumstances involving intense challenges (101, 103).

Increased activity in the central VP system may lower thresholds to impulsive forms of aggression, possibly by reducing cortical inhibition (105). The actions of VP also help to explain the association of anxiety and ruminations with cardiovascular risk (106). VP plays a central role in circadian rhythms and is likely to be important in sleep disturbances or elevations in blood pressure, which are also common following stress and considered defining features of posttraumatic stress (PTS) disorders. In human males, high blood levels of VP have been correlated with emotional dysregulation and aggression (107).

Vasopressin is associated with physical and emotional mobilization and helps support vigilance and behaviors needed for guarding a partner or territory (3), as well as other forms of adaptive self-defense (103). Prairie voles have provided a useful model for examining the importance of peptides in selective aggression (108). In this species, immediately after mating males became lethally aggressive toward strangers, but not familiar partners or family members; this response was blocked by antagonists for the V1aR (109). The formation of partner preferences and pair bonds requires access not only to the V1aR but also the OTR (37). Mate

guarding and parental aggression offer examples, among several, suggesting the importance of both OT and VP and their receptors in behaviors that are socially selective (**Figure 2**).

Non-Selective versus Selective Social Behaviors

Oxytocin's role in social behaviors has been documented in many species, including humans (4, 18, 110, 111). Based primarily on work in nonhuman animals, in many, but not all cases, the effects of OT are mediated *via* VP receptors. This seems to be the case in behaviors that are non-selective, such as a general tendency toward sociality or gregarious behavior (**Figure 2**). This may include behavioral patterns involving social recognition (56, 112). Among other examples, in which both OT and VP receptors were examined, are social contact, including lying adjacent to another member of the same species (113) and huddling with conspecifics in the presence of the odor of a predator (114); these were facilitated by OT but only when the V1aR was accessible. In another example, in golden hamsters the effects of OT on social reward required access to the OTR (80). By contrast, in hamsters for OT to affect aggression, activation of the V1aR was necessary (79).

Research, initially conducted in prairie voles, demonstrated the capacity of OT to increase social contact between adults (115). This work led to studies showing a role for OT and the OTR in the formation of selective social bonds (116). However, in studies in which either the OTR or VP V1a were blocked, both OT and VP receptors were necessary for pair bond formation (37). When both OTR and the V1aR were blocked animals showed very low levels of contact behavior. In pair bond formation, OT and VP interact with motivational and reward systems and may enhance or otherwise amplify the effects of other molecules including dopamine and opioids in specific brain regions, including those that have been implicated in both maternal behavior and social bonding (38, 81, 117).

Social Learning and Conditioning

Research on the behavioral effects of the OT–AVP system began with studies of memory, including avoidance learning (118) and social recognition (56, 112). These continue to be major topics in studies of the functions of the OT–VP pathway (24, 33). Learning of context and cues, as well social salience, may be affected by access to the OTR. There is an increasing tendency to direct attention to specific brain regions. In rodents, brain systems involved in reinforcement and reward, including the nucleus accumbens and ventral tegmental area, have high levels of both OTR and dopamine. OT-related sociality, probably in conjunction with the actions of dopamine, is reinforcing (40, 82). Only a few studies have suggested functions in which OT acts solely *via* the OTR, without access to the V1aR. For example, in mice, exogenous OT is capable of modulating fear conditioning following treatments directed at the lateral septum (119). OT in the lateral septum reduced fear following a positive social encounter but facilitated fear conditioning after a prior negative social encounter (120). In rats, fear conditioning also was enhanced by OT administered in the bed nucleus of the stria terminalis; blocking access to the OTR eliminated conditioned fear responses, while non-conditioned fear responses were not affected (121). In addition, in rats, effects of peripherally administered OT on neural activation in the central amygdala (indexed by cFos expression) continued to be present even following treatment with a V1aR antagonist. OT may act to increase sociality in the face of fear or challenge, including effects of exogenous OT measured by regional change in cFos in other brain areas. As one example, neural activation by OT in the hypothalamus and brain stem did require V1aR stimulation; among the other brain regions in which cFos was increased by OT and blocked by a V1aR antagonist were the SON, PVN, locus coeruleus, and nucleus tractus solitarius (35). The latter brain areas have many functions, including regulation of the autonomic nervous system and HPA axis, which are necessary for the optimal expression of social behavior (5).

Dose-Dependent Effects of OT: More Is Rarely Better

When infant prairie voles received a low dose of exogenous OT immediately following birth, they showed as adults increased OT in the CNS and an increased tendency to form a pair bond. However, when higher doses of OT were administered, a single exposure to OT in early life disrupted the later capacity to pair bond. Females exposed neonatally to a high dose of OT later preferred a stranger. Stranger preference in prairie voles is very atypical (122, 123) and, especially in males, is most commonly associated with stressful experiences or stress hormones including CRH and cortisol (87, 124).

These and many other experiments suggest that the effects of OT are dose dependent. Low doses may appear to be beneficial, while higher doses of OT can have detrimental behavioral consequences and in some cases may stimulate the VP receptor. Low to moderate doses of OT, especially as acute treatments may reduce anxiety in the face of a challenge or stressor. By contrast, larger amounts of OT, especially if given as a chronical treatment may no longer be anxiolytic, and can have the opposite effect. Chronic or very high levels of OT can reduce the capacity to respond to OT possibly by reducing OTR or binding to the OTR, while also allowing OT to activate VP receptors (86). In another example, when male mice were tested in a social stress paradigm, chronic and high levels of OT (given centrally) were associated with an increase in anxiety-like behaviors; in that study OTR binding was also reduced in the amygdala and septum (125). Perhaps in individuals primed by negative experience, small amounts of OT are capable of activating VP receptors, further supporting mobilization and potentially defensive emotional or behavioral responses. Based on data from OTKO mice, in which the VP system was sensitized (31), we also can hypothesize that individuals (including humans) with low levels of endogenous OT might be more likely to experience increased VP-like activities even when given OT.

Studies of OT, and less commonly VP, using intranasal infusions have generated an increased interest in the behavioral effects of these peptides. The intent is to non-invasively deliver peptides to the brain and there is increasing evidence that this is possible (126, 127). However, it is useful to note, based on imaging studies in

rodents, that the tissues activated by exogenous peripheral versus central applications of OT are not identical (128). Furthermore, the concentrations chosen for most human studies are generally arbitrary and based on doses of OT medically available as an intranasal "lactational aid." Studies using different amounts of OT are needed to examine possible threshold differences among individuals as a function of gender, experience, and emotional lability (129). Different doses of a given peptide can produce different effects, and dose–response curves are only just appearing in this literature (130).

Are There Unique Functions for OT?

In mammals, we have argued that under optimal conditions OT appears to serve as a physiological metaphor for "safety" (18). OT is of special relevance to physical and mental protective adaptations that involve high levels of sociality, a sense of psychological safety within a family or familiar social group, as well as emotional regulation that is necessary for mental health and higher levels of rational cognition (4). At least in rodents, OT seems to play an important role in cortical functions necessary for social cognition (24) and social reward (79).

Oxytocin also promotes autonomic flexibility in the face of threats (42). Parental care and social support in a safe context are particularly important in species of mammals adapted to live in extended families or groups, including humans and prairie voles (131). Social contact between adults or adults and offspring is a defining feature of most families. However, social contact, necessary for mating, parental behavior and nursing, can be dangerous and requires a physiological and autonomic state that permits "immobilization without fear" (36). This behavioral response may be especially adaptive in females but also may leave females more sensitive than males to the consequences of traumatic experiences and symptoms of PTS.

Mammals, with their comparatively large brains, are particularly vulnerable to the need for oxygen, and under extreme conditions the functions of OT may shift from social behavior to survival and protection of the cortex, including dissociation or even loss of consciousness (4) (**Figure 3**). In mice, exogenous OT elicited a transient activation of cortical regions and a sustained activation of hippocampal and forebrain regions. It is interesting to note that in mice intranasal VP produced a sustain deactivation of pathways associated with cortical function. Many effects of VP still existed when OTRs were genetically deleted, presumably reflecting the capacity of VP to activate cortico-parietal, thalamic, and mesolimbic regions *via* VP V1aRs (105). Whether V1aRs, possibly responding to OT, can assume such roles in primates needs additional study (62).

Does OT Act Alone?

Many important functions including birth and selective social behaviors, another form of learned behavior, appear to rely on *both* OT and VP and their receptors. It is uncommon to find evidence that OT functions solely *via* the OTR. Lactation is one comparatively "modern" function of OT (81). Social reward may be another OT–OTR based function (79, 80), perhaps requiring a co-activation of localized dopaminergic systems (17).

Under circumstances of acute stress or prolonged isolation OT (in females) can be released (132) (**Figure 3**). If acting on the OTR or the V1aR this OT could allow stress coping (16). However, especially after early-life adversity, epigenetic sensitization or upregulation of VP (133) and V1aR (11) can occur. Under these conditions, OT may no longer be sufficient to be protective. Furthermore, OT may stimulate VP receptors. Thus, although OT is normally protective against stress, if it acts on the VP receptor system the effects may be seen as exacerbation of stress reactivity or anxiety. This may be a particular problem in individuals with a history of trauma and neglect, for whom the effects of exogenous OT have been reported as socially negative or "antisocial" (110, 134, 135).

Mechanisms for OT–VP Interactions

The mechanisms underlying OT–VP interactions *in vivo* remain largely to be understood. In the face of a challenge, the interactive effects of OT and VP appear to be hierarchical. The more modern peptide, OT, may act *via* the presumably older V1aR either as an agonist or perhaps as a competitive antagonist (**Figure 1**). Furthermore, the functions of OT and VP may be regulated by various other processes, including differential availability of OT or VP (or their receptors) which may be regulated locally in the nervous system (20).

The Paradox—Why Are the Social Effects of OT Unpredictable?

As data have accumulated, apparent inconsistencies or "paradoxical" effects of OT have emerged (44, 45). For example, a tendency toward parochial behavior and "outgroup" rejection was described in some human studies after intranasal OT (45, 134, 135). Treatment with OT also has been implicated in increased aggressive tendencies in certain kinds of computer games, an effect that was attributed to an OT-induced increase in social salience (136). These responses may be adaptive but also could reflect the kind of receptor "cross talk" described in studies in nonhuman animals.

In mice (86, 137) and voles (138), chronic OT exposure has been either relatively ineffective or even had negative effects on social behavior. When OT levels are high or chronically elevated their effects may be primarily due to stimulation of VP receptors with a concomitant downregulation of the OTR. This pattern of exposure to either exogenous or endogenous OT might support mobilization and potentially defensive responses, rather than positive sociality and a reduction in anxiety (**Figure 3**).

The history of the individual, including prior exposure to early-life stress, also can influence the response to OT. Early maltreatment also has been associated with an increase in endogenous OT (27, 139). In another example, individuals who described themselves are relatively lonely were less likely to show an OT-associated increase in parasympathetic activity (140). Perceived loneliness, isolation in early life or maltreatment might alter thresholds for physiological consequences of exogenous OT, also possibly by upregulating VP receptors and/or downregulating the OTR.

In the presence of a challenge or a negative environment (141), OT of either endogenous or exogenous origins, perhaps acting on VP receptors, could support arousal, including activation of CRH, the sympathetic nervous system and other components of the HPA and autonomic nervous system (5, 140). The interactive effects of OT and VP, including actions on the V1aR may help to explain the observation that treatment with OT has frequently been associated with antisocial behaviors, especially in a context of fear or danger.

SUMMARY

Across the lifespan, the effects of OT and VP dynamically interact to adjust to and influence the perception of fear and safety. VP is the evolutionarily older molecule with presumably the older receptors. VP is implicated in mobilized behaviors including defense of self and the family. Among the patterns of behavior for which both OT and VP may be necessary are sexual behavior (142), paternal behavior, and pair bonding (18). OT is of special relevance to adaptations that involve high levels of sociality, a sense of psychological safety within a family or familiar social group, as well as emotional regulation and higher levels of rational cognition (4). Furthermore, working together OT and VP, and their receptors, create a biological and genetic pathway that regulates attachment and bonding, which in turn may be protective against threats or other forms of challenge.

The nature of interactions of OT and VP at their receptors needs further study, especially *in vivo* and the epigenetic context of development (26). There is considerable interest in using OT-like molecules as therapeutics. However, the evolved and dynamic features of the OT–VP pathway create difficulties for attempts to study OT and VP independently. These also pose challenges for the usefulness of drugs based on this system, including those commonly used around the time of birth, such as synthetic forms of OT, which may affect both the OT and VP receptors.

AUTHOR CONTRIBUTIONS

CSC conceived and wrote this review.

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Navigating Monogamy: Nonapeptide Sensitivity in a Memory Neural Circuit May Shape Social Behavior and Mating Decisions

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The role of memory in mating systems is often neglected despite the fact that most mating systems are defined in part by how animals use space. Monogamy, for example, is usually characterized by affiliative (e.g., pairbonding) and defensive (e.g., mate guarding) behaviors, but a high degree of spatial overlap in home range use is the easiest defining feature of monogamous animals in the wild. The nonapeptides vasopressin and oxytocin have been the focus of much attention for their importance in modulating social behavior, however this work has largely overshadowed their roles in learning and memory. To date, the understanding of memory systems and mechanisms governing social behavior have progressed relatively independently. Bridging these two areas will provide a deeper appreciation for understanding behavior, and in particular the mechanisms that mediate reproductive decision-making. Here, I argue that the ability to mate effectively as monogamous individuals is linked to the ability to track conspecifics in space. I discuss the connectivity across some well-known social and spatial memory nuclei, and propose that the nonapeptide receptors within these structures form a putative "socio-spatial memory neural circuit." This purported circuit may function to integrate social and spatial information to shape mating decisions in a context-dependent fashion. The lateral septum and/or the nucleus accumbens, and neuromodulation therein, may act as an intermediary to relate socio-spatial information with social behavior. Identifying mechanisms responsible for relating information about the social world with mechanisms mediating mating tactics is crucial to fully appreciate the suite of factors driving reproductive decisions and social decision-making.

Keywords: hippocampus, lateral septum, mating system, memory, nucleus accumbens, prairie voles (*Microtus ochrogaster*), social behavior, retrosplenial cortex

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INTRODUCTION

The nonapeptides vasopressin (VP) and oxytocin (OT), and their non-mammalian homologs, are crucial regulators of social behavior across taxa (Goodson and Bass, 2001; Goodson, 2008; Goodson and Thompson, 2010). Indeed they are well known for their roles in social behavior, pairbonding, and mating systems (Young and Wang, 2004; Carter et al., 2008; Insel, 2010), including in humans (Heinrichs et al., 2009). It is less recognized that they were first studied in behavioral neuroscience for their central effects on memory (de Wied, 1971; Bohus et al., 1978b; Pedersen and Prange, 1979; McEwen, 2004). Somewhat ironically, mating systems are inherently dependent on social and spatial memory. For example, most theories of the evolution of mating systems emphasize the importance of space use.

Unfortunately, how animals use information about the spatial distribution of conspecifics and the social context of interactions to inform mating decisions has been drastically underappreciated. Individual mating tactics should reflect the social landscape (i.e., context) in which animals find themselves. For example, most males must consider the defendable resources located within a given territory, the number of mating partners they are capable of monopolizing, and the activity of their mate(s) and neighbors (Emlen and Oring, 1977; Shuster and Wade, 2003). Based on their best estimate of the context and the status of their own body condition, individuals should adopt a mating tactic that will most likely produce the greatest reproductive success. The assessment of these factors will largely depend on integrating social and spatial information (i.e., the identity and location of potential mates or competitors). This process requires behavioral coordination by multiple neural mechanisms, including a major role for the action of VP and OT in the forebrain.

Here, I begin by discussing theory for mating systems emphasizing space use as a driving force. I then introduce and briefly review the nonapeptide neuromodulatory system (vasopressin and oxytocin) and its role in pairbonding and memory. I next outline the connectivity between several neural structures in which nonapeptides assert an influence and may serve as the foundation for a putative "socio-spatial memory neural circuit". Finally, I speculate on how this putative circuit may function and interact with other known circuits to influence reproductive decisions and mating tactics using prairie voles as a case study example. Although, some attention has been dedicated to understanding female prairie vole reproductive decision-making and the mechanisms therein (Zheng et al., 2013b) in this review I primarily consider males, unless otherwise stated. While many of the theoretical and presumably neuroanatomical details are likely to generalize between the sexes, sexual selection and sex-dependant selection have also produced important differences that are likely to alter the proximate and ultimate processes that shape reproductive decision-making in males and females. It is crucial to consider both sexes if we are to ever achieve a full understanding of the mechanisms that contribute to such important decisions, and the dynamic interactions that follow. Nevertheless, deepening our concept of mating systems by incorporating memory represents significant progress toward understanding how neural mechanisms govern complex behavior.

HOW MIGHT SOCIAL OR SPATIAL MEMORY INFLUENCE MATING SYSTEMS?

Ultimately, mating systems are social systems that represent the outcomes of several individual reproductive choices. Indeed, mating systems are emergent properties of populations, whereby each individual assesses the ecological and social landscape in which they find themself and adopts a tactic based on that information (Oliveira et al., 2008). In this review I use terms like "assess", "adopt", and "evaluate" in the behavioral ecology sense of the words. This is meant specifically to reflect information gathering (however reliable or unreliable) and the outcome of a computation in which that information was used to inform the probability that the world is in a particular state, and weighed against the expected gains of performing various behaviors given that state. From this perspective, "cognition" is deeply rooted in the behavioral ecology of all species (Sherry, 2006; Dukas and Ratcliffe, 2009). Identifying the social and cognitive factors that sculpt mating decisions is necessary to predict reproductive success, and to understand how individual decisions contribute to and shape social organization at large.

At their core, mating systems represent the most common social arrangements of individuals of a given population or species. They are often synonymously conflated with breeding arrangements and portrayed as a collection of mating decisions for the purpose of reproduction. It should be stated that how animals mate and how they live may not perfectly map on to each other, and breeding arrangements and social arrangements often differ (Kleiman, 1977). Indeed, alternative tactics commonly evolve in mating systems, and frequently take the form of territorial vs. "sneaker" tactics, although other forms of alternative reproductive tactics also exist (Oliveira et al., 2008).

In simple terms, mating systems can be characterized by the average number of mates (or at least social partners) that males and females of a given population most commonly have (Shuster and Wade, 2003). This way of categorizing reproductive behavior creates four general categories of mating systems: polygyny (one-male—multi-female units), polyandry (one-female—multi-male units), promiscuity (a.k.a., polygamy, polygynandry; multi-male—multi-female units), and monogamy (one-male—one-female units). Just how common each mating tactic is varies by taxa. For example, although monogamy is common in birds (Owens and Bennett, 1997), it is very rare among mammals (Kleiman, 1977). Nevertheless, monogamy is particularly interesting considering that humans represent one mammalian species that readily engages in this mating system. For reviews of mating systems see Apollonio et al. (2000); Clutton-Brock (1989); Greenwood (1980); Orians (1969); Shuster and Wade (2003).

Although, mating systems are conceptualized in terms of reproductive behaviors, they are frequently defined in terms of social and spatial behavior. Emlen and Oring (1977) argue that the potential for polygyny is contingent on a male's ability to monopolize resources that attract females by establishing large territories (an entity inherently rooted in space). In fact, the size and exclusivity of territories are measures commonly used to define mating systems. For example, mammalian monogamy is most likely to evolve when females occupy small but exclusive home ranges, thereby increasing the difficulty for males to monopolize several females (Komers and Brotherton, 1997).

Abbreviations: AC, Anterior Commissure; AT, Anterior Thalamus; BST, Bed Nucleus of the Stria Terminalis; CeA, Central Amygdala; CC, Corpus Callosum; DG Dentate Gyrus; EC, Entorhinal Cortex; f, Fornix; HPC, Hippocampus; LS, Lateral Septum; LDTh, Laterodorsal Thalamus, MB, Mammillary Bodies; MeA, Medial Amygdala; MS, Medial Septum; NAcc, Nucleus Accumbens; PAR, Parietal Cortex; PAG, Periaqueductal Grey; PFC, Prefrontal Cortex; POA, Preoptic Area; PC, Posterior Commissure; RSC, Retrosplenial Cortex; SHi, Septohippocampal Nucleus; Sub, Subiculum; VPall, Ventral Pallidum; VTA, Ventral Tegmental Area; 3V and 4V, Ventricles, third and fourth.

In practice, a male must be proficient at defending resources while monitoring the activity of his mate or mates to successfully mate-guard or exclude competitors. Presumably, polygynous (and promiscuous) males must track the temporal progression of the estrous cycle of nearby females, establish territories, and remember neighbors (Brotherton and Komers, 2003). Males that are in the right place at the right time are likely to experience a reproductive advantage over others. Alternatively, a monogamous male must monitor the activity of his neighbors, both in terms of which individuals directly threaten his fitness through cuckoldry or infanticide, and indirectly through challenging his resource holding potential (Brotherton and Komers, 2003). He may also benefit greatly by tracking the identity and location of neighbors that may boost his fitness through pairing (or extra pair) opportunities (Brotherton and Komers, 2003). Monogamy should therefore require males to associate the spatial distribution of conspecifics with their social identity: are they competitors, pairbonded mating partners or potential extra-pair mates? Ultimately, a male's decision to adopt a particular mating tactic should be informed by associations with space use (*spatial memory*), distinguishing between neighbors (*social memory*), and accounting for the spatial distribution of those neighbors (*socio-spatial memory*).

THE SOCIALLY MONOGAMOUS PRAIRIE VOLE

Perhaps no species is better studied with resect to both natural behavior comprising their mating system and the neurobiological mechanisms that govern important aspects of the mating system than prairie voles (*Microtus ochrogaster*). Prairie voles overwhelmingly demonstrate behaviors consistent with a monogamous mating system (e.g., Thomas and Birney, 1979; Gavish et al., 1981; Getz et al., 1981; Carter and Getz, 1993). In the lab, males and females form long-lasting social preferences for a mating partner (i.e., a pairbond), demonstrate aggressive behavior toward strangers, and provide care for offspring (Witt et al., 1988; Williams et al., 1992; Winslow et al., 1993; Carter et al., 1995). In the field, prairie voles tend to form breeding units in which one male and female breeder attend to offspring until they disperse (McGuire et al., 2013). Animals adopting this mating tactic are often referred to as "residents", largely because males and females occupy overlapping home ranges and appear to exclude other conspecifics (Getz et al., 1993; Solomon and Jacquot, 2002; Ophir et al., 2008a).

The majority of males adopt residency (∼60–75%), one reason why prairie voles are considered monogamous (Getz et al., 1993). But being a monogamous species need not exclude males or females from attempting to engage in extra-pair mating. In fact, resident males face a trade-off between mate guarding to increase "faithful" in-pair reproduction and "unfaithful" extra-pair mate seeking (Ophir et al., 2008a; Phelps and Ophir, 2009; Okhovat et al., 2015). Here, I refer to the former as "*true residents*" and the latter as "*roving residents*" or simply "*rovers*". The proportion of these two types of residents has not been formally investigated, but some studies hint that they are roughly equally common (Getz et al., 1993; Ophir et al., 2008a; McGuire and Getz, 2010; Okhovat et al., 2015). Home range size of true residents and rovers are similar, but the way they use the same amount of space might differ. For instance, how rovers and true residents navigate and move within the same area and interact with conspecifics is almost surely different and would reflect their chosen tactics. This would most likely express itself in the degree to which they remain at the nest and mate guard and the degree to which they visit the nests of other females.

Beyond true residents and rovers, another important and less common (∼25–30% of the population) tactic referred to as "*wandering*" exists. In this case, male and female wanderers occupy much larger home ranges compared to residents, they do not appear to be territorial, and they presumably attempt to mate multiply (Getz et al., 1993; Solomon and Jacquot, 2002; Ophir et al., 2008a; McGuire and Getz, 2010; McGuire et al., 2013). It is unclear if adopting a resident or wandering tactic produces greater reproductive payoffs, but our data suggest that males prefer to form bonds (Blocker and Ophir, 2016) and that residents may have greater reproductive success while wanderers are making the best of a bad job (Ophir et al., 2008a; Phelps and Ophir, 2009). Taken together, it is most accurate to characterize the overall mating system of prairie voles as socially monogamous, in which animals engage in alternative reproductive tactics.

NONAPEPTIDES, MONOGAMY, AND MEMORY

Oxytocin and vasopressin are integral to many forms of mammalian social behavior (Goodson, 2008; Goodson and Thompson, 2010). Although knowledge of the function of nonapeptides in social behavior across an array of species has progressed at a remarkable pace, a foundation of knowledge has been built around prairie vole social behavior, affiliation, and pairbonding. Indeed, nonapeptides are necessary and sufficient for the production of prairie vole pairbonds (Young and Wang, 2004; Johnson and Young, 2015)—a hallmark of their socially monogamous mating system. For example, manipulation of VP and its receptor (V1aR) in the ventral pallidum (VPall) or lateral septum (LS), and OT or its receptor (OTR) in the nucleus accumbens (NAcc) can either facilitate or diminish the pairbond (Winslow et al., 1993; Williams et al., 1994; Cho et al., 1999; Liu et al., 2001; Liu and Wang, 2003; Lim et al., 2004; Ross et al., 2009; Keebaugh et al., 2015). These and other limbic structures, sometimes referred to as a "*pairbonding neural circuit*" (Young and Wang, 2004), have formed the basis for understanding the neurobiology of social affiliation and monogamy (for reviews see Carter and Keverne, 2002; Young and Wang, 2004; Carter et al., 2008; Donaldson and Young, 2008; Insel, 2010; McGraw and Young, 2010; Carter, 2014; Lieberwirth and Wang, 2014; Johnson and Young, 2015).

The expression of nonapeptide receptors across the brain can reveal how evolution has shaped the mechanisms that impact mating decisions (Ketterson and Nolan, 1992). For example, studies famously comparing monogamous and

non-monogamous vole species indicate that nonapeptide receptor profiles (particularly within the aforementioned areas) are good predictors of mating system (Insel and Shapiro, 1992; Insel et al., 1994). Similar characterizations have since been performed in many other species with different mating systems or social organization (c.f., Kelly and Ophir, 2015). How broadly the relationship between nonapeptides and mating system extends beyond voles is unclear, but some evidence suggests parallel results may exist for humans and chimpanzees (Hammock and Young, 2006; Donaldson et al., 2008; Walum et al., 2008; Hopkins et al., 2012). Strangely, the extraordinary individual variation in prairie vole V1aR or OTR density does not differ between residents and wanderers (Ophir et al., 2008b, 2012; Zheng et al., 2013b). Evidence demonstrating that residents produce more fertilized embryos (Ophir et al., 2008a) suggests that natural selection has eliminated the standing variation in the pairbonding neural circuit to predispose prairie voles to adopt a socially monogamous lifestyle. It should be noted that this conclusion is built on the assumption that unborn embryos are a rough proxy of fitness. However, this measure does not account for variation in parental care these offspring would have received, the lifetime reproductive success of the breeding unit, or ultimately survival and subsequent reproduction of the offspring (see Ophir et al., 2008a; Blocker and Ophir, 2016) which might have altered the "fitness advantage" of bonded males in either direction. Nevertheless, if there is indeed a reproductive advantage to being paired, then the mechanisms that promote pairing should be advantageous to all males. Therefore, any individual variation in the neural phenotype that is known to facilitate (or gate) bonding, should be low and all males have the same "bonding" neural phenotype, more or less. Our neural data appear to support this interpretation.

Considerable evidence from the mid-twentieth century demonstrated that OT and VP affect the process of learning and memory, either directly or indirectly by altering arousal (c.f., McEwen, 2004). The original work in this area focused on the impact of VP and OT in passive or active avoidance learning (Bohus et al., 1972, 1978b; de Wied, 1991), but has also expanded to understanding navigation (i.e., hippocampal-dependent cognition; e.g., Engelmann et al., 1992; Everts and Koolhaas, 1999), retrieval and relearning in visual discrimination (Alescio-Lautier et al., 1987), and social recognition and social memory (e.g., Ferguson et al., 2002; Albers, 2012; Stevenson and Caldwell, 2012). The main neural targets on which VP and OT assert effects on memory include the hippocampus, the cingulate and retrosplenial cortices, septum, several subunits of the thalamus, hypothalamus, and other limbic structures such as the amygdala and medial preoptic area (e.g., Popik and Van Ree, 1998; Ferguson et al., 2001; McEwen, 2004; Ophir et al., 2008b). More recently, increasing attention has been dedicated to understanding the roles of nonapeptides in the hippocampus and hippocampal-dependent memory. For instance, Egashira et al. (2004) showed that vasopressin is necessary to perform a hippocampal-dependent spatial memory task, and Tomizawa et al. (2003) showed that hippocampal oxytocin may be necessary for long-lasting spatial memory. Interestingly, OT appears to enhance hippocampus spike transmission by modulating fast-spiking interneurons, effectively improving the signal-to noise ratio (Owen et al., 2013).

Much of the evidence has led to the idea that VP and OT appear to have opposite effects on learning and memory, with VP facilitating memory consolidation and retrieval, and OT potentially serving as an amnestic (Bohus et al., 1978a; Kovacs and Telegdy, 1982; Argiolas and Gessa, 1991; de Wied, 1991; McEwen, 2004). For example, nonapeptides are functionally important for social recognition (Gabor et al., 2012). Blockade of endogenous VP in the septum and the hippocampus (dorsal and ventral portions) disrupts social recognition, whereas OT blockade only impacts social recognition when administered to the ventral hippocampus and not the septum or dorsal hippocampus (van Wimersma Greidanus and Maigret, 1996). More specifically, central and peripheral injections of VP facilitate social recognition, whereas OT injections have no effect or attenuate it (Bohus et al., 1978a,b; Koob et al., 1981; Dantzer et al., 1987; Popik and Vetulani, 1991; Benelli et al., 1995). The role of OT on memory is much less clear than that of VP. In fact OT appears to have a dose-dependent effect on memory. High doses of OT produce amnestic effects but low doses facilitate recognition (Popik et al., 1992a,b). OT's dose-dependent influence on social recognition is probably explained by the types of OT metabolites that bind to OTR (Burbach et al., 1983; Popik et al., 1996; Popik and Van Ree, 1998), but could also be explained by OT-V1aR cross reactivity (de Wied, 1991; Manning et al., 2008; Song et al., 2014, 2016). Moreover, differences in how and where VP and OT impact social recognition appear to vary by sex (Gabor et al., 2012). Nevertheless, V1aR antagonists clearly block social recognition (Engelmann and Landgraf, 1994; Landgraf et al., 1995), while OTR antagonists block the facilitating effects of OT on social recognition at low doses and the attenuating effects of social recognition at high doses (Benelli et al., 1995).

As a cautionary warning, these results, particularly those regarding OT just discussed, highlight the importance of considering the route of administration, dose, timing, and the behavioral tests that are used to assess learning and memory. Administration and dose matter because both can potentially have confounding effects of arousal (Baldi and Bucherelli, 2005). Furthermore, nonapeptides do not readily cross the blood-brain barrier and therefore peripheral (e.g., intranasal or intraperitoneal injections) and central (e.g., targeted or intracerebroventricular) administration can have different results (Neumann et al., 2013). It is also unclear if peripheral administration has direct or indirect effects. Moreover, the administration of exogenous nonapeptides may have important and different dose-responses, as conveyed in the example given above. The timing of administration of pharmacological agents, or the like, may also impact memory in different and important ways because they may impact acquisition, consolidation, and/or expression of memory, which each follow different timelines. Finally, the behavioral test matters because the behavior of interest will change in line with the expected procedure for most behavioral tests of memory, however "memory" is an interpretation of the observed behavior (e.g., olfactory inspection, or visits to a particular area in space)

rather than an observable behavior itself. Factors such as these are important to be mindful of when interpreting the studies that have investigated learning and memory and in particular the influences of nonapeptides on these processes, and may help explain why results may appear contradictory (e.g., why OT might appear to both facilitate and attenuate memory, see above).

A FUNCTIONAL MEMORY NEURAL CIRCUIT

A tremendous effort has been dedicated to describing and understanding the processes of learning and memory and the neural mechanisms that govern these processes. It is not my intent to review this entire literature here. However, I do aim to provide a brief and somewhat simplified synopsis of the neural circuit and structures therein that are closely associated with memory. For more exhaustive reviews of this topic see Aggleton and Brown (1999); Brown and Aggleton (2001); Eichenbaum et al. (2007); Fanselow and Dong (2010); Strange et al. (2014), and Zola-Morgan and Squire (1993).

The Hippocampus

The hippocampus (HPC) is probably the best-known neural structure associated with learning and memory. It is necessary for many forms of higher-level memory including episodic memory (i.e., recalling experienced events), contextual memory, and spatial memory (Hirsh, 1974; Nadel et al., 1985; Zola-Morgan and Squire, 1993; Rolls, 1996; Mizumori, 2007; Smith and Bulkin, 2014; Strange et al., 2014; Bulkin et al., 2016). Indeed some have characterized the HPC as a structure that generates representations of multi-dimensional spatial maps, while others have argued it is a center for assessing context (Hirsh, 1974; Nadel et al., 1985; Zola-Morgan and Squire, 1993; Rolls, 1996; Mizumori, 2007; Smith and Bulkin, 2014; Strange et al., 2014; Bulkin et al., 2016). The hippocampus is a highly conserved forebrain structure that takes its name from the curved sea horse-like shape it takes in the human brain. In rodents the HPC is shaped more like a cashew that curves at an angle along the anterior-posterior and dorsal-ventral axes. Although the orientation and location of the HPC has been rotated and drifted over mammalian evolution, this structure still maintains its basic configuration: the dorsal HPC (sometimes referred to as the septal pole, and represented by the anterior component of the HPC in rodents), and the ventral HPC (a.k.a., the temporal pole, representing the posterior component of the rodent HPC) (Strange et al., 2014). Although the cellular anatomy and connectivity within the HPC is fairly consistent throughout the length of the HPC, these two components appear to be functionally distinct, with the dorsal HPC accounting for the episodic memory, spatial map and navigational functions, and the ventral HPC relating to emotional memory, affect, and stress (Moser and Moser, 1998; Fanselow and Dong, 2010). Homologs of this structure take many forms in other taxa, like the HPC-equivalent found in the dorsal pallium in birds, or the aptly named mushroom bodies of some insects (Strausfeld et al., 1998; Kempermann, 2012). In mammals, the neuroanatomy and connectivity within the HPC is captured by the so-called "tri-synaptic loop", a one-way loop of axonal connections from the entorhinal cortex (EC), penetrating through the subiculum, and through the sub-structures of the HPC (the dentate gyrus, CA1, and CA3) (Amaral and Witter, 1995; Brewer et al., 2013). To complete this loop, the axons of the cells in CA1 project to the neurons of the EC and subiculum.

Despite its central role in learning and memory, the HPC is functionally and anatomically connected with several other structures that work in concert to enable many important aspects of learning and memory. This extended memory circuitry has been described in detail elsewhere (Gabriel, 1993; Aggleton and Brown, 1999; Mizumori et al., 2000; Smith et al., 2012). Briefly, the so-called hippocampus-anterior thalamic axis (**Figure 1**) forms the basis of this circuit and incorporates the HPC, the fornix, mammillary bodies, retrosplenial cortex (RSC), and thalamic nuclei. The HPC sends and receives projections to/from the anterior thalamus (AT) via the fornix, and projects to the mammillary bodies via the fornix. The HPC, however, is also bidirectionally connected to the EC and RSC, and sends projections to the prefrontal cortex. Signals entering the HPC from the EC initiate the tri-synaptic loop. In addition to its bidirectional connection with the HPC, the RSC is bidirectionally connected with the parietal cortex and the AT.

The Retrosplenial Cortex

The RSC is a key component of the brain's memory and navigation systems (Vann et al., 2009; Miller et al., 2014) and is

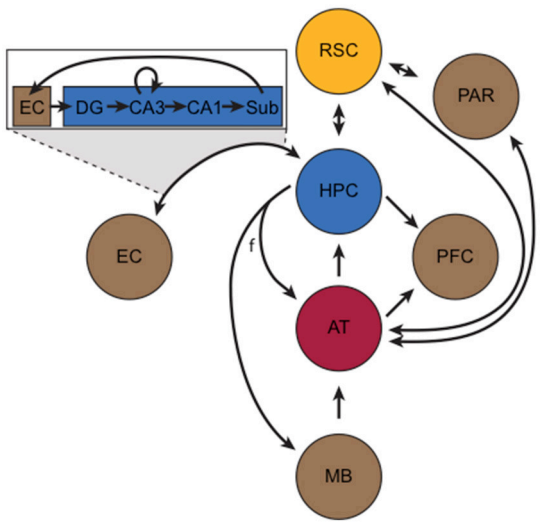

FIGURE 1 | The hippocampus-anterior thalamic axis (adapted from Aggleton and Brown, 1999). At its core, the circuit incorporates the hippocampus (HPC, blue), retrosplenial cortex (RSC, yellow), and the anterior thalamus (AT, red). Other important structures (in brown), include the mammillary bodies (MB), entorhinal cortex (EC), parietal cortex (PAR), and the prefrontal cortex (PFC). The fornix (f) and other axonal projections are represented by black arrows. The box portrays the "tri-synaptic loop" between the EC and the subunits of the HPC, including the subiculum (Sub), dentate gyrus (DG), and CA1 and CA3 subfields.

located along the posterior portion of the cingulate cortex (Jones et al., 2005). Lesions to the RSC are remarkably similar to the effects of hippocampal lesions, including impairments in episodic memory (Valenstein et al., 1987; Bowers et al., 1988), contextual memory (Keene and Bucci, 2008a,b), and spatial navigation (Sutherland et al., 1988; Harker and Whishaw, 2002; Vann and Aggleton, 2002). Not only is the RSC bi-directionally connected with other components of itself (van Groen and Wyss, 1992a,b), but the HPC and RSC are reciprocally interconnected (Wyss and Van Groen, 1992; Jones and Witter, 2007), and this bidirectional communication is likely critical for memory. Inactivation of the RSC disrupts hippocampal representations (Cooper and Mizumori, 2001), and it appears to be an important consolidation target for hippocampal-dependent memories (Katche et al., 2013), especially contextual memories (Keene and Bucci, 2008c; Cowansage et al., 2014; Czajkowski et al., 2014).

The Anterior Thalamus

The AT (consisting of the anterior dorsal, anterior ventral, anterior medial, and lateral dorsal nuclei) is bidirectionally connected with the HPC and RSC (synapsing in the granule layers of the RSC) (van Groen et al., 1993). The AT is a major subcortical target for HPC output (Swanson and Cowan, 1977; Aggleton et al., 1986), and like the RSC, it is involved in many of the same memory and navigation functions as the HPC. For instance, the AT is a critical site of damage in diencephalic amnesia (Aggleton et al., 2011). In rodents, AT neurons exhibit directional firing (i.e., head direction cells; Taube, 1995) and AT lesions reliably disrupt spatial navigation (Aggleton and Nelson, 2015), sequence memory (Wolff et al., 2006), and contextual memory (Law and Smith, 2012). Furthermore, AT lesions cause large-scale disruption of HPC and RSC functioning (Jenkins et al., 2002, 2004; Savage et al., 2011). In addition to the aforementioned connectivity, the AT also sends afferents to the prefrontal cortex and to the parietal cortex. In turn, the AT receives efferents from the HPC (via the fornix), mammillary bodies, and RSC. Similarly, the parietal cortex receives input from several structures, but most notably the dorsal RSC and laterodorsal (LDTh) subdivision of the AT. Moreover, the LDTh acts as a transitional nucleus projecting to both limbic and neocortical areas, and the presence of head direction cells in this structure is an interesting point of convergence with other areas central to spatial cognition (Mizumori and Williams, 1993; Taber et al., 2004). Anatomically, the LDTh provides extensive afferent input to the subicular complex of the hippocampal formation and sends dense projections to the RSC (van Groen and Wyss, 1992b). Indeed, the RSC is bi-directionally connected with the LDTh (van Groen and Wyss, 1992a,b). Taken together, the HPC, RSC and AT (including the LDTh) are central components of an extended limbic memory circuit that is vastly important for mediating spatial, episodic, and context dependent memory (**Figure 1**).

Other Structures: The Septum and Septohippocampal Nucleus

It is important to make clear that the classic functional memory neural circuit just discussed is a relatively simplified model. Extending this model, the HPC, septohippocampal nucleus (SHi), and septum form a reciprocal circuit among themselves (Rye et al., 1984; Gaykema et al., 1990), which is directly involved in memory (Khakpai et al., 2013). Both the dorsal and ventral portions of the HPC project to the LS (Fanselow and Dong, 2010; Strange et al., 2014). Although the LS receives massive glutamatergic fiber input from the hippocampus via the fornix, the medial septum (MS) sends significant cholinergic and GABAergic projections to the hippocampus (Jakab and Leranth, 1995; Swanson and Risold, 2000). Meanwhile, the SHi, which is centrally involved but not necessary for learning and memory (Parent and Baxter, 2004), provides feedback between the HPC and (primarily) the MS (Giovannini et al., 1994; Marighetto et al., 1994). Interestingly, lesions of the medial septum disrupt hippocampal theta oscillations (Lawson and Bland, 1993) and impair spatial memory (Winson, 1978; Leurgeb and Mizumori, 1999). Thus, the septal connections back to the HPC and SHi primarily travel through the MS. However, the medial and lateral septa are themselves tightly and reciprocally connected to each other, and the LS can inhibit HPC function via the MS-SHi (Giovannini et al., 1994; Marighetto et al., 1994; Jaffard et al., 1996; Desmedt et al., 1999). Feedback through indirect LS regulation of the HPC via the SHi is accomplished through the glutamatergic receptors in the LS that exert an inhibitory effect of cholinergic cells in the MS, which in turn influences HPC function (Giovannini et al., 1994; Marighetto et al., 1994; Jaffard et al., 1996; Desmedt et al., 1999). Thus, there are cytoarchitectural and functional connections among the septum and hippocampus enabling direct communication from the HPC to LS and indirect LS feedback regulation of the HPC via the SHi.

THE SOCIAL DECISION-MAKING NETWORK AND THE PAIRBONDING NEURAL CIRCUIT

Central to the study of social behavior is a core set of interconnected limbic structures, collectively recognized as the social behavior network (SBN). These include the LS, preoptic area (POA), central and medial amygdala (CeA and MeA), bed nucleus of the stria terminalis (BST), anterior hypothalamus (AH), ventromedial hypothalamus (VMH), and midbrain (i.e., periaqueductal gray, PAG) (Newman, 1999; Goodson, 2005). By definition, these core nodes of the SBN are involved in the regulation of many forms of social behavior, are reciprocally connected, and are influenced by sex steroid hormones (Newman, 1999). For example, various combinations and permutations of the activation of these structures are necessary or important for the expression of sexual behavior, aggression, parental care, and social grouping within and across species (c.f., Numan, 2015). Somewhat recently, the SBN was extended to integrate reward circuitry into a larger network, called the social decision-making network (SDMN), comprised of the SBN structures and the NAcc, VPall, striatum, basolateral amygdala, ventral tegmental area (VTA), and notably the HPC (O'Connell and Hofmann, 2011a,b, 2012). These latter structures are tightly networked key nodes in or key accessories to the mesolimbic reward system (for review see Ikemoto, 2010). The

mesolimbic reward system has become commonly regarded as the neural network where salience and valence of stimuli is processed (Alcaro et al., 2007; Wickens et al., 2007; Ikemoto, 2010). Dopamine, particularly in projections from the VTA to the NAcc, is a major factor in this function, but of course it is not the only important signaling molecule (Spanagel and Weiss, 1999). Much of the work on the mesolimbic reward system has been done under the premise of understanding mood disorders, addictive behavior, or reinforcement learning (Berridge and Robinson, 1998; Alcaro et al., 2007; Koob and Volkow, 2010; Dichter et al., 2012). But clearly natural, and in particular social, behavior heavily relies on reward (Schultz, 2000, 2006), which is probably one of the reasons this network appears to be ubiquitously shared across taxa. For an extensive review on the structure, function, and connectivity of nodes of the SDMN across four major vertebrate taxa, see (O'Connell and Hofmann, 2011b).

Although, no mention of nonapeptides was made in the original characterization of the SBN (Newman, 1999), these structures are largely sensitive to VP and OT action (Albers, 2015). For example, all of the SBN/SDMN structures, with the exception of the POA and VTA (but see Hammock and Young, 2005), express V1aR, OTR, or both in prairie voles (Zheng et al., 2013b). Not surprisingly, all nodes of the pairbonding neural circuit described above (see Young and Wang, 2004) are contained within the SDMN (with the one exception of the prefrontal cortex, which might also be important to include). Arguably, the decision to form a pairbond falls safely within social decision-making, and from this point of view it is reasonable to consider the pairbonding neural circuit as a sub-unit of the SDMN.

A CASE FOR SOCIO-SPATIAL MEMORY AS A FACTOR FOR MATING SYSTEM

The relationship between social and spatial memory, mating decisions, and the role of nonapeptides therein is likely to be much more than coincidental. Social decision-making necessarily relies on an individual's ability to assess the social and spatial landscape in which it finds itself and then act on that information. Such decision-making should be context dependent and plastic, yet open to the stabilizing or canonizing forces of natural selection. The action of nonapeptides as modulators of social behavior provides a plausible mechanism by which such plasticity can be maintained by natural selection. Indeed, individual differences in nonapeptide receptor expression may contribute to differences in socio-spatial memory and to differences in mating tactics, possibly as a consequence of its impact on memory. For instance, non-monogamous male meadow voles (*M. pennsylvanicus*) perform better than monogamous prairie voles in several mazes testing spatial memory (Gaulin and FitzGerald, 1989). Interpretations of these and other related results indicate that spatial memory (i) may facilitate navigating larger home ranges, (ii) differs systematically between mating systems, and (iii) potentially helps shape mating systems (Gaulin and FitzGerald, 1989; Jones et al., 2003), supporting the idea that memory is important for mating decisions. How social and spatial memory might operate within species to shape, and possibly promote, particular mating decisions is an open question and may vary based on the species under investigation.

Of particular importance here is the function of VP and OT in the RSC and HPC, respectively. Some polygamous rodents have larger HPC or RSC than monogamous congeners (Gaulin and FitzGerald, 1989; Gaulin, 1992; Clint et al., 2012; Jasarevic et al., 2012; Kingsbury et al., 2012), implicating these brain areas as being important for mating systems. Although interspecific comparisons of nonapeptide receptors in the HPC or RSC are limited, Insel et al. (1991) demonstrated that promiscuous mice (*Peromyscus maniculatus*) express more oxytocin receptor in the hippocampus (CA1 sub-region) than a monogamous congener (*P. californicus*), providing some of the first evidence that variation in nonapeptide receptor expression might relate to mating system.

In contrast, we have found no evidence suggesting that RSC or HPC *volume* predicts mating tactics within prairie voles (Kingsbury et al., 2012; Rice et al., in review). However, although size and volume of brain structures are commonly linked with information processing and its behavioral consequences (e.g., Sherry et al., 1992; Maguire et al., 2000), sheer size of structures is only one aspect of neural function. The neural mechanisms that operate within structures can also have a profound influence on neural processing and behavior (Roth et al., 2010). To this end, expression patterns of nonapeptide receptors within these structures predict successful adoption of monogamous or non-monogamous tactics (Ophir et al., 2008b, 2012; Okhovat et al., 2015, see below). This suggests that the most successful residents are more sensitive to VP and OT binding in these brain areas than the most successful wanderers. Overall, variation of VP and OT receptor expression within regions associated with memory processing appears to reflect the variance in the sensitivity to these neuromodulators, and hence their ability to impact memory, particularly for socially relevant contexts. Nonapeptides are, therefore, highly likely to play an important and nuanced role in modulating reproductive success and mating tactics via structures associated with memory.

A NONAPEPTIDERGIC SOCIO-SPATIAL MEMORY CIRCUIT

Based on the material discussed above, I propose that the influence of VP and OT in a putative "socio-spatial memory neural circuit" shapes reproductive decisions. In the remainder of this article, I attempt to outline this neural circuit in which the brain areas that contribute to social decision-making (and pairbonding in particular) interface with social and spatial memory processing to enable animals to successfully navigate and operate within a social context. Considering that successful mating tactics necessarily rely on an individual's ability to locate mates and competitors in space and are often related to (if not defined by) space use, it is probable that social and spatial memory have coevolved to—at least in part—serve the purpose of facilitating social behavior and mating success.

The composition of the proposed network is based largely on neuroanatomical studies of connectivity between structures subserving social behavior and/or memory. Specifically, I refer to the extensive connectivity among the components of the limbic memory circuit, and their axonal connections with core areas within the pairbond neural circuit described throughout this review. In abstract terms, it is plausible that nonapeptide action in this memory circuit functions to integrate socio-spatial information to shape mating decisions in a context-dependent fashion. This context dependency is a notion supported by work demonstrating that social recognition varies based on the social environment in which it is tested (Zheng et al., 2013a).

This hypothesis predicts that neuromodulation by VP and OT in the memory circuit functions to evaluate the social landscape for potential mating and bonding opportunities. The degree to which these areas enable an animal to accurately account for the identity and location of conspecifics (mates and competitors) would be fed into the SDMN and specifically the pairbonding neural circuit. These behavioral networks could use that information to weight the probabilities that reproductive success can be maximized based on engaging in certain reproductive behaviors. Thus, communication between these nonapeptide sensitive circuits could shape reproductive tactics by biasing decision-making for remaining single or forming (faithful or unfaithful) bonds. The functional evidence discussed below is based on observations in prairie voles, which I use here as an example of how this might work.

At the center of this putative nonapeptide-governed socio-spatial memory circuit is the HPC, RSC, LDTh, SHi, and the LS (see **Figure 2**). With the exception of the LS, each of these areas demonstrates profound individual variation in either V1aR or OTR across individuals, indicating that variable sensitivity to the neuromodulatory influences of nonapeptides in these structures can account for individual variation in behavioral outcomes. Individuals also demonstrate the same clearly stereotyped patterns of nonapeptide receptors in the RSC, LDTh, HPC, and SHi, and these patterns predict reproductive success of those individuals based on their chosen mating tactic. Specifically, successfully breeding residents express the greatest densities of RSC and LDTh V1aR and HPC and SHi OTR, while successfully breeding wanderers express the least (Ophir et al., 2008b, 2012). Although neither V1aR nor OTR density shows this pattern in the LS, V1aR expression in the LS does show a non-significant trend that is consistent with the patterns seen in these four other structures (Ophir et al., 2008b). Moreover, OTR density in the HPC, SHi, and LS is significantly and positively correlated across these structures (Ophir et al., 2012), further supporting the idea that nonapeptide action coordinates the modulation of this network of memory processing brain structures.

Taken together, there is a strong precedent for the HPC, RSC, LDTh, SHi, and septum to either directly contribute to, or indirectly aid in, the processing of social and spatial memory. The connectivity and coordinated VP/OT sensitivity among these structures suggests an integrated network of memory-related structures. Functionally, this network could contribute to solving the cognitive demands of mating tactics within a social system. The main memory-processing components of this circuit (HPC, RSC, LDTh, and SHi) may make it possible to also process socially contextual information defined, in part, by the density and distribution of conspecifics in the surrounding environment. This premise is tentatively supported by the data discussed above.

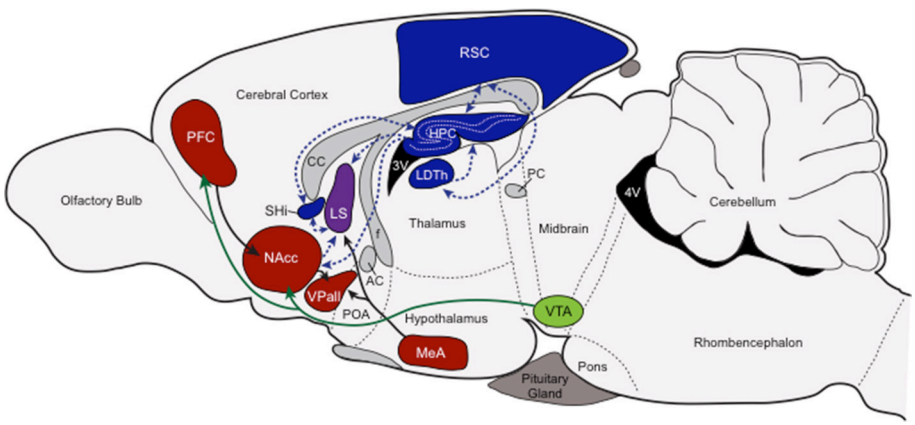

FIGURE 2 | Putative socio-spatial memory neural circuit. This figure provides a cartoon schematic of the connections among the nonapeptide expressing memory areas, the connections among the pairbonding neural circuit, and the connections between these two circuits to showcase how socio-spatial memory might influence reproductive decision-making. Neural structures involved in processing socio-spatial memory (blue), and their projections (blue dashed arrows), include the retrosplenial cortex (RSC, V1aR) the laterodorsal thalamic nucleus (LDTh, V1aR), the hippocampus (HPC, OTR), and the septohippocampal nucleus (SHi, OTR). Neural structures involved in the pairbonding neural circuit (adapted from Young and Wang, 2004), and their projections, are also presented. Structures of this circuit that contain either V1aR or OTR (red) include the prefrontal cortex (PFC, OTR), nucleus accumbens (NAcc, OTR), ventral pallidum (VPall, V1aR), and medial amygdala (MeA, V1aR); the dopaminergic ventral tegmental area (VTA; green) is also included. The lateral septum (LS), pictured in purple, contains both OTR and V1aR and can be considered both a "memory" and "social behavior/pairbonding" node. Other abbreviations: CC, corpus callosum; AC, anterior commissure; POA, preoptic area; f, fornix; PC, posterior commissure; 3V and 4V, third and fourth ventricles.

The functional relationships and connectivity among these structures suggest that male prairie voles may rely on socio-spatial memory processing to shape the behavioral phenotype demonstrated by monogamous residents (including true residents and rovers) and non-monogamous wanderers. Within this framework, the LDTh and RSC influenced by VP, and the HPC and SHi influenced by OT may function to process context dependent learning and memory. But for this information to be useful in shaping reproductive behavioral outcomes, these "socio-spatial memory" structures would need to interface with the SDMN, and in this case specifically with the pairbonding neural circuit (**Figure 2**).

WHERE MEMORY AND SOCIAL BEHAVIOR MEET: CONNECTIONS TO THE PAIRBOND NEURAL CIRCUIT

I have suggested that the purported circuit detailed above may function to assess the socio-spatial context, enabling males to evaluate the probable reproductive value of forming bonds with females. The mechanisms for establishing and maintaining a pairbond have been relatively well characterized and are briefly summarized above (i.e., the pairbonding neural circuit, Young and Wang, 2004). In some instances the probability of forming bonds should be high. For example, males should be predisposed to form mating-induced bonds with females when such opportunities arise. This should be particularly true when pairing opportunities are promising because pairing appears to boost reproductive success (Ophir et al., 2008b; Okhovat et al., 2015; Blocker and Ophir, 2016). On the other hand, males should never forgo the opportunity to mate, even if it is unlikely to lead to a pairbond. In practice, if a male finds itself in a social context where several females are present but none is available for pairing, it would still greatly benefit from mating, but not pairing with those females. In fact, forming bonds with unavailable females would pose a great cost to males. In each of these cases, it would be important for the socio-spatial neural circuit to communicate with the pairbonding neural circuit and have the capacity to adjust the probability that a bond will form when mating occurs.

Where might the socio-spatial memory circuit interface with the pairbonding circuit? The lateral septum is one place where the two circuits converge. The LS is potentially unique in its role in relating learning and memory with social decision-making for several reasons. First and foremost, the LS can be considered a "memory" structure, a "social behavior" structure, or a "pairbonding" structure. Indeed, the LS, which is sensitive to both OT and VP, is important for many forms of social behavior (Goodson and Thompson, 2010), including social recognition (Ferguson et al., 2002; Gabor et al., 2012), and for establishing pairbonds (Liu et al., 2001). Interestingly, the action of VP in the LS, appears to be specific to learning and memory of social but not non-social information (Everts and Koolhaas, 1997). The LS's necessary and sufficient role in pairbond formation could be interpreted as enabling animals to make associations between the highly rewarding experiences from social affiliation and mating with the identity of a particular individual (Young et al., 2005). Therefore, in this and many other ways, the LS is most likely functioning as a general "association maker." In the context of the proposed neural circuitry, the LS could aid in identifying the relative roles each conspecific might play in that individual's life (i.e., same-sex competitor, pair-mate, or non-mate female). Alternatively, the LS could promote or inhibit social grouping preferences, coloring the valences associated with learning the identities of neighbors and their relationships in space (Goodson and Wang, 2006; Goodson et al., 2009a,b; Kelly et al., 2011).

The nucleus accumbens is another particularly promising candidate area for integration of memory and social behavior. In particular, accumbal OTR densities might modulate hedonic interactions, biasing males to either form pairbonds or remain single. The NAcc is an integral component of the pairbonding neural circuit and has a well-established functional role in reward (Berridge and Robinson, 1998; Salgado and Kaplitt, 2015). Manipulations of oxytocin, dopamine, or mu-opioid receptors in this structure can alter the propensity to form bonds (Johnson and Young, 2015), and OTR density in the NAcc may modulate bonding by altering the intensity of reward (Liu and Wang, 2003; Aragona et al., 2006; Ross et al., 2009). Monogamous species of voles have higher densities of OTR in the NAcc than non-monogamous species (Insel and Shapiro, 1992; Insel et al., 1994), and OTR density in the NAcc is greater in paired resident prairie voles than the un-paired wandering males (Ophir et al., 2012). The NAcc is also the only pairbonding neural structure that differs between paired residents and single wanderers (Ophir et al., 2012). Furthermore, NAcc OTR is positively associated with OTR expression in several other important neural structures central to social decision-making including the prefrontal cortex and the amygdala (Ophir et al., 2012). Importantly, the NAcc receives strong projections from the HPC and LS, and it sends afferents to the LS and other limbic structures (Powell and Leman, 1976; Swanson and Cowan, 1977; Kelly and Domesick, 1982; Groenewegen and Russchen, 1984), suggesting that it is well positioned to serve as a relay center between the memory processing circuit outlined above and the social decision-making and pairbonding circuits (**Figure 2**).

Might the NAcc serve as a "tuning knob" (Young and Hammock, 2007) to bias males to bond or remain single? As just stated, the difference between adopting monogamous or non-monogamous tactics is related to OTR differences observed in the brain. In other words, OT may govern the behavioral differences in mating tactics via an OTR density-dependent neuromodulatory influence. With greater OTR density in the NAcc, resident males should be more sensitive to OT-modulated reward associated with mates. But, these data do not make it clear if the NAcc OTR phenotype preceded bonding in the field (i.e., a fixed phenotype that predicted the probability of bonding) or if it is dynamic and responsive to the social environment. Dynamic OTR in the NAcc could make it possible for animals to adjust their affiliative responses based on the context and, indeed, perception of accumbens-mediated reward can change based on the social context (Thiel et al., 2008). As it turns out, OTR in the NAcc is dynamic and epigenetically regulated, and this flexibility alters the likelihood that male and female prairie voles will form bonds (Wang et al., 2013; Duclot et al., 2016). Thus, accumbal

OTR density could dynamically change based on the socio-spatial context, thereby altering the valence of the reward associated with mating. As a result, the reward associated with mating with a particular individual may only be sufficient to induce pairbonds when the social context is judged to be optimal or appropriate for forming bonds. Such changes in NAcc OTR could therefore impact normal functioning of the pairbonding neural circuit, enhancing or curtailing the probability of pairbond formation. If this is true, OTR in the NAcc could play a pivotal role as a bridge between socio-spatial neural structures that predict monogamous mating tactics, and neural structures that enable monogamous bonds to form. Further, OT action in the NAcc may broadly impact the SDMN, which could have a cascading effect on other aspects of sociality, thereby contributing to much larger behavioral consequences beyond bonding.

DO RESIDENT AND WANDERER BRAINS SHOW DISTINCT NONAPEPTIDE PATTERNS?

It is clear that monogamous resident and non-monogamous wandering male prairie voles demonstrate distinct behavioral phenotypes, and that aspects of their brains differ (see above). To explore just how different these neural phenotypes are, I conducted hierarchical clustering analysis (JMP 12.0; SAS) of previously published nonapeptide receptor expression in pairbonding [VPall, NAcc, LS, MeA, and prefrontal cortex (PFC)] and memory (HPC, SHi, LDTh, RSC) areas (see **Figure 2**) taken from monogamous residents and non-monogamous wanderers living freely in outdoor semi-natural enclosures (for details see Ophir et al., 2008a,b, 2012). Hierarchical clustering groups data using an association matrix of pairwise *r*-values (for example, see Ophir et al., 2009). Thus, the correlations within each matrix provide a description of how well the relationships among variables relate to each other. It should be noted that cluster analyses like these make no *a priori* assumptions about grouping order or strength. Several interesting patterns are notable from this analysis, however I will focus on just two.

The most striking pattern that these analyses revealed is that resident male brains show two branches of tight clustering; one comprised of most of the pairbonding-associated structures (VPall, MeA, NAcc), and the other containing all of the memory-associated structures (HPC, SHi, LDTh, RSC) (**Figure 3A**). The LS, which expresses both OTR and V1aR was split between these two clusters (LS V1aR in the "bonding branch", and LS OTR in the "memory branch"), potentially reflecting its multifaceted role in bonding and memory. Although, OT action in the PFC, which clustered with memory structures, has been implicated in pairbonding (Young and Wang, 2004; Smeltzer et al., 2006), it is important for many forms of memory and primarily implicated in goal-directed behavior (Miller and Cohen, 2001).

In contrast to the clear pattern seen in residents, wanderers show a much greater degree of intermingling of OTR and V1aR expressing memory and bonding neural structures (**Figure 3B**). One interpretation of this pattern is that structures that contribute to these two different behaviors are non-distinct and show little cohesion, suggesting that the wanderer brain has little structure distinguishing between nonapeptide sensitive memory and bonding areas. Another interpretation of these results is that the nonapeptide-regulated structures that subserve bonding and memory are highly integrated. The latter interpretation is supported by the fact that, compared to the resident cluster, more of the correlations that were used to construct the wandering cluster were significant (following the false discovery rate correction for multiple comparisons). But how such integration across the two circuits operates, whether they work to improve or interfere with memory, and/or how that information is ultimately related to mating decisions remain interesting and unanswered questions.

A second noteworthy feature of these cluster analyses is the placement of the NAcc in the two clusters (**Figure 3**). In residents, it is closely associated with other structures that, like itself, are

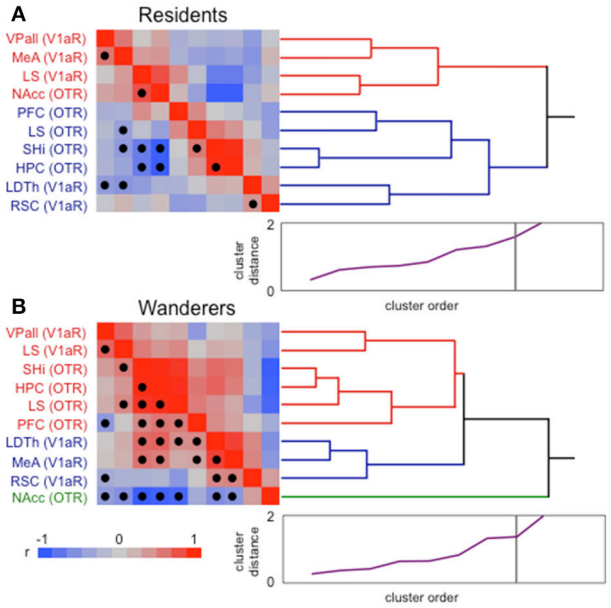

FIGURE 3 | Hierarchical clustering analysis of resident and wandering male prairie voles. The clusters are composed of vasopressin receptor 1a (V1aR) and oxytocin receptor (OTR) expression in "memory" and "pairbonding" neural structures of paired residents **(A)** and single wanderers **(B)** living freely in semi-natural enclosures. Ward linkages were used in these analyses. Correlation matrices (on left) present strength (hue) and direction of correlations (red positive and blue negative; see legend at bottom) used to create the dendrograms (on right). False discovery rate-adjusted significant relationships are marked with a solid circle. For residents **(A)**, adjusted alpha = 0.0144, and significant p's ranged from 0.0067 to < 0.0001. For wanderers **(B)**, adjusted alpha = 0.0311, and significant p's ranged from 0.0302 to < 0.0001. Scree plots (bottom right of each panel, purple line) have a point for each cluster join. The ordinate (0–2) is the distance that was bridged to join the clusters at each step. Often, there is a natural break where the distance jumps suddenly. These breaks suggest natural cutting points to determine the number of clusters. The length of the branches in the dendrogram tree diagram is on a distance scale and shows the actual joining distance between each join-point. Thus, the longer the branch lines are, the larger the difference between samples having a common link. HPC, hippocampus; LDTh, laterodorsal thalamus; LS, lateral septum; MeA, medial amygdala; NAcc, nucleus accumbens; PFC, prefrontal cortex; RSC, retrosplenial cortex; SHi, septo-hippocampal nucleus; VPall, ventral pallidum.

necessary and sufficient to induce pairbonds in prairie voles (see above). In wanderers, however, it was excluded from the branch containing all the other structures, effectively creating a single branch on its own. This is despite several significant (and negative) correlations with almost all the other structures that were fed into the analysis. These results are even more interesting considering that OTR density in the NAcc was significantly greater in residents than wanderers (Ophir et al., 2012). Perhaps the "isolation" of NAcc OTR from the other structures in the wanderer brain is another reflection of its potentially pivotal role as a node enabling/preventing communication between structures associated with reproductive decision-making and socio-spatial memory.

Cluster analyses such as these are useful to get a general sense of potential relationships across the brain. In this case, a main point is that resident and wandering brains demonstrate very distinct patterns of V1aR and OTR expression within these two circuits. Unfortunately, it is difficult to make specific functional conclusions from descriptive analyses like these. Nevertheless, these data clearly demonstrate that individuals that have adopted two distinct alternative reproductive tactics also demonstrate different broad-scale neural phenotypes. The different patterns of nonapeptide receptor associations have the potential to shape memory processing and pairbonding in very different ways by acting on the coordination of networks of nuclei that are potentially important for evaluating the social landscape and shaping mating tactics.

CONCLUDING REMARKS

The ability to navigate space and relate that ability to social interactions is something that has been relatively unappreciated in discussions of mating system. I have attempted to make the case that these behaviors are integral to mating systems and in particular for successful monogamy. I have provided evidence supporting the hypothesis that neural mechanisms involved in socio-spatial memory shape the mating decisions resulting in differential mating tactics, and that these processes are functionally modulated by nonapeptides (VP and OT). Such data have led to the hypothesis that a putative "socio-spatial memory neural circuit" informs reproductive decisions. Presumably, this putative network enables prairie voles to assess the social landscape and bias their decision-making for remaining single or forming (faithful or unfaithful) bonds to maximize their probability of reproductive success in nature. Such a decision-making process largely accounts for the form of mating system prairie voles demonstrate (i.e., social monogamy with multiple alternative reproductive tactics). Importantly, despite species differences that are sure to exist, the larger function of this circuit—assessing the social and spatial landscape to inform reproductive decision-making—is likely to be a general feature of brains in many species. Therefore, this putative circuit need not be limited to explaining the interface between memory and reproductive decisions in prairie voles. Indeed it is likely to extend beyond addressing reproductive decisions related specifically to monogamy.

The extensive connectivity among the memory-related brain structures, and their axonal connections with core areas within the pairbonding neural circuit appears to form a larger network of structures, distinct in their functions but bound by their shared sensitivity to nonapeptides. This provides a foundation on which this network has the potential to subserve the larger (and emergent) behavioral function of integrating socio-spatial information to shape mating decisions in a context-dependent fashion. I have argued that these "memory" structures are likely to work with the SDMN via the LS and NAcc to enable the evaluation of the social landscape to weight reproductive decisions that determine individual mating tactics and ultimately mating systems. It is clear that this hypothesis will require sufficient testing, but I have aimed to provide a framework from which novel hypothesis and new predictions can be generated. Ultimately, I hope that this article broadens the discussion of social and spatial memory, mating systems and social behavior, and inspires crosstalk between these fascinating and inextricably linked areas of research.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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REVIEW
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Individual Differences in Social Behavior and Cortical Vasopressin Receptor: Genetics, Epigenetics, and Evolution

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Social behavior is among the most complex and variable of traits. Despite its diversity, we know little about how genetic and developmental factors interact to shape natural variation in social behavior. This review surveys recent work on individual differences in the expression of the vasopressin 1a receptor (V1aR), a major regulator of social behavior, in the neocortex of the socially monogamous prairie vole. V1aR exhibits profound variation in the retrosplenial cortex (RSC), a region critical to spatial and contextual memory. RSC-V1aR abundance is associated with patterns of male space-use and sexual fidelity in the field: males with high RSC-V1aR show high spatial and sexual fidelity to partners, while low RSC-V1aR males are significantly more likely to mate outside the pair-bond. Individual differences in RSC-V1aR are predicted by a set of linked single nucleotide polymorphisms within the *avpr1a* locus. These alternative alleles have been actively maintained by selection, suggesting that the brain differences represent a balanced polymorphism. Lastly, the alleles occur within regulatory sequences, and result in differential sensitivity to environmental perturbation. Together the data provide insight into how genetic, epigenetic and evolutionary forces interact to shape the social brain.

Keywords: cognitive ecology, balancing selection, enhancer elements, single nucleotide polymorphism, *Microtus ochrogaster*, neuroendocrinology, monogamy

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Individual differences in social behavior are remarkably common. Male lizards vary dramatically in their display colors and aggressive behaviors (Sinervo and Lively, 1996). Male sunfish may differ profoundly in their parental care (Gross, 1991), while bluehead wrasses can shift body color, behavior, and even sex in response to social environments (Semsar and Godwin, 2004). Indeed, evolutionary theory has long known that the fitness value of specific behavioral traits may depend on the frequency of such traits in the population, or on the population density of conspecifics (Maynard-Smith and Price, 1973). Similar forces have been hypothesized to shape individual differences in human personality (Keller and Miller, 2006; Penke et al., 2007), resilience to developmental trauma (Boyce and Ellis, 2005), and even the variety of human faces (Sheehan and Nachman, 2014). Understanding the genetic and epigenetic factors that shape individual differences in social behavior is thus of fundamental importance to both our basic understanding of behavior, and to our understanding of natural variation related to health and disease.

Behavioral neuroscience is often focused on model species in which genetic diversity has been intentionally purged. This has the advantage of minimizing variation that could confound

the study of species-specific traits, and this strategy has enabled substantial insights into the role of developmental factors in shaping adult behavior. Intrauterine environments (Ryan and Vandenbergh, 2002), parental care (Weaver et al., 2004), and environmental complexity (van Praag et al., 2000), for example, all have profound influences on the development of brain and behavior. The decision to study genetically similar individuals, however, precludes studying genetic variation or how it interacts with developmental environments to shape natural behavior.

Non-traditional model species offer a variety of strengths that complement traditional foci of behavioral neuroscience (Phelps, 2010; Taborsky et al., 2015). For example, by studying species in which genetic diversity has been actively retained by derivation from wild stock, it is possible to examine how genetic variation contributes to brain and behavior. In addition, species may be chosen that exhibit interesting social phenotypes not exhibited by traditional model systems. Among mammals, recent examples include the study of pair-bonds (Young and Wang, 2004; Ophir et al., 2007), non-sexual bonds (Beery and Zucker, 2010), elaborate vocalizations (Blondel and Phelps, 2009; Crino et al., 2010), and the elaboration of paternal care (Bendesky et al., 2017). Work on non-traditional rodents and primates, moreover, can employ many of the technologies developed for common mammalian models (e.g., Lim et al., 2004). These attributes make them powerful supplements to common approaches in social neuroscience.

In the current paper, we offer a detailed review of our work on individual differences in the vasopressin system of prairie voles, a socially monogamous rodent that has become a powerful model for the study of attachment. We focus more specifically on cortical differences in the abundance in the vasopressin 1a receptor, the predominant form in the brain. Our focus, the retrosplenial cortex (RSC), is a brain region critical to spatial and contextual memory, and an increasing focus of research in both humans and rodents (Harker and Whishaw, 2002; Vann et al., 2009; Kingsbury et al., 2012; Ranganath and Ritchey, 2012; Cowansage et al., 2014; Todd and Bucci, 2015). The expression of V1aR in the RSC is profoundly variable among individual prairie voles, and has been linked to both spatial behavior and sexual fidelity in the wild (**Figure 1**). We begin by introducing prairie voles as models in social neuroscience and neuroendocrinology.

PRAIRIE VOLES AS MODELS OF MONOGAMY

The prairie vole, *Microtus ochrogaster*, is a small North American rodent that lives in grasslands. It is known both for its wild fluctuations in population density and for its ability to form enduring pair-bonds (Thomas and Birney, 1979; Carter et al., 1986, 1995; Getz et al., 1993, 2001; Pizzuto and Getz, 1998). Males and females live in pairs and share care of offspring. Roughly 25% of these young are sired outside the pair (Ophir et al., 2008b). Male pair-bonding is accompanied by a dramatic increase in aggression and a reduction in homerange. While paired males live as aggressive, territorial "residents," up to 45% of males may

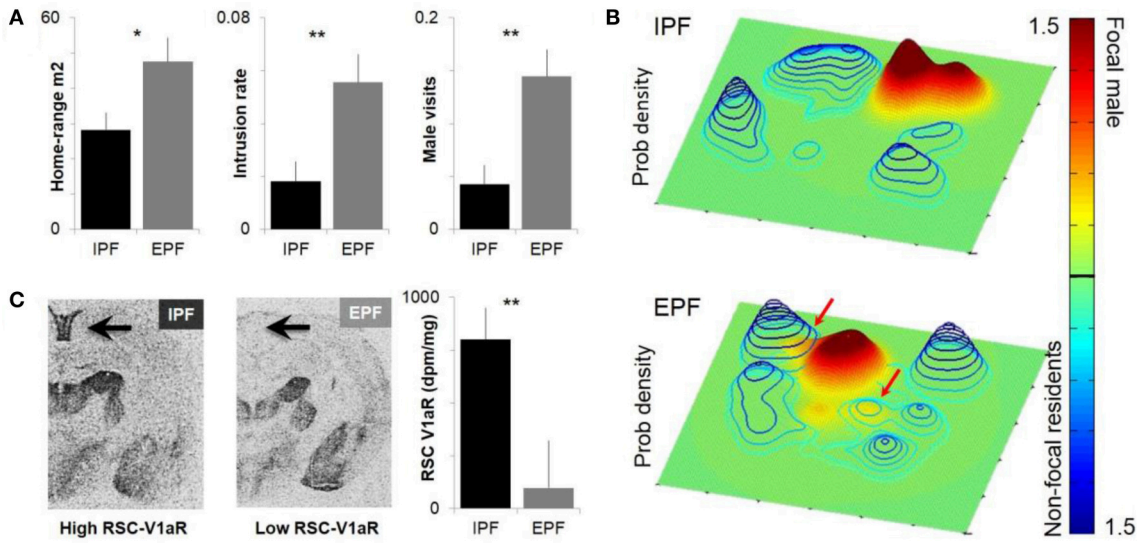

FIGURE 1 | Individual differences in male space-use and sexual fidelity are predicted by RSC-V1aR abundance. **(A)** Males who sired young only within a pair (intrapair fertilization, IPF) and males who sired at least one embryo with a non-partner (extrapair fertilization, EPF) differ in homerange size, the rate of intrusions onto resident male territories, and the rate at which their own core homeranges were visits by other males. **(B)** Sample probability density estimates of paired males within a common enclosure. Focal males are shown on a green-red scale and a solid surface, non-focal residents in blue wired surface. X and Y axes correspond to dimensions of the outdoor enclosure. Top panel shows an IPF male that does not intrude on the territories of other bonded males; bottom panel depicts an EPF male who intrudes on two other male territories. Colorbar depicts probability density (0–1.5 × 10e-3) that a focal male or non-focal resident will be at a given point in space. **(C)** IPF and EPF males differ in the abundance of V1aR in the RSC, which predicts individual differences in space use. $^{*}P < 0.05$, $^{**}P < 0.01$. Modified with permission from Okhovat et al. (2015).

live as unpaired, non-territorial "wanderers" (Getz et al., 1993; Solomon and Jacquot, 2002).

Space use is a critical component of variation both within and between these alternative male tactics. Residents with small, exclusive homeranges have high mating success with their respective partners (Ophir et al., 2008c; Phelps and Ophir, 2009). Residents with larger homeranges gain extra-pair fertilizations (EPFs) but are more often cuckolded. Wanderers have larger, less exclusive homeranges than residents, but only those with the largest homeranges obtain EPFs (Ophir et al., 2008c; Phelps and Ophir, 2009). Thus, for both residents and wanderers, larger and less exclusive homeranges translate into increases in extrapair paternity; only residents, however, face trade-offs between EPFs and IPFs. Space use differs between residents and wanderers, but it also predicts patterns of paternity within tactics.

VASOPRESSIN AND MATING SYTEM

Arginine-vasopressin (AVP) is a nine-amino acid peptide implicated in a wide variety of social behaviors. Among vertebrates, AVP and its homologs are commonly linked to aggression, courtship and other social behaviors (Goodson and Bass, 2001; Caldwell et al., 2008). Among mammals, neurons of the bed nucleus of the stria terminalis and the medial amygdala express AVP at higher levels in males than females (De Vries et al., 1994), a finding thought to contribute to the importance of the peptide to male social behavior (Cho et al., 1999; but see Bosch, 2013; Dumais and Veenema, 2016, for examples of vasopressin functions in female social behaviors). Although this neuropeptide is consistently implicated in social behavior, its effects can be highly species-specific. This specificity seems to emerge from species differences in the neural distribution of the vasopressin 1a receptor (V1aR). Prairie voles, for example, exhibit high V1aR in a reward region, the ventral pallidum, that influences pairbond formation (Winslow et al., 1993; Insel et al., 1994). Injection of a vasopressin antagonist into the ventral pallidum blocks pair-bonding in male prairie voles (Lim and Young, 2004). Remarkably, viral overexpression of pallidal V1aR enables normally promiscuous male meadow voles to form attachments (Lim et al., 2004).

Although the ventral pallidum causes species differences in pair-bond formation among voles, this mechanism does not seem to be general. We recently measured pallidal V1aR in seven species of *Peromyscus*, for example, and found it did not reliably predict mating system across deer mice (Turner et al., 2010). The consensus seems to be that the ability of vasopressin and its homologs to modulate social behavior is an ancient innovation common among vertebrates (Goodson, 2005; Ho et al., 2010; O'Connell and Hofmann, 2011, 2012). The effects of the hormone on a particular social behavior, however, can emerge in a variety of ways, presumably by acting anywhere in a series of connected brain regions important for social behavior (e.g., Goodson, 2005; O'Connell and Hofmann, 2011, 2012).

Although differences between monogamous and promiscuous vole species are shaped by pallidal V1aR, residents and wanderers have *identical* patterns of neural V1aR (Ophir et al., 2008c). The abundance of V1aR in the ventral pallidum is remarkably consistent across individual prairie voles, with the high levels needed for pair-bonding apparently fixed within the population (Phelps and Young, 2003). Given that bonded males have higher fitness, it seems likely that selection has cleared heritable variation in pallidal V1aR abundance (Phelps and Ophir, 2009). Differences between resident and wandering males seem to represent differences in opportunity rather than neural V1aR abundance (Ophir et al., 2008c). Somewhat surprisingly, although there are no differences in V1aR between residents and wanderers, more subtle behavioral variation within each tactic is associated with the abundance of V1aR in the RSC (Ophir et al., 2008c; **Figure 1**).

To examine this relationship, we collared and radiotracked animals in the field, using the locations determined over the course of a few weeks to estimate the probability a given animal would be at a particular point in space (Ophir et al., 2008c; Okhovat et al., 2015; **Figure 1**). From these probability landscapes, we can estimate the core of an animal's homerange, and the extent to which the animal intrudes into the core homeranges of its neighbors. The data reveal that having low RSC-V1aR is associated with more territorial intrusion, increased rates of being intruded upon, and increased extra-pair paternity (Phelps and Ophir, 2009; Okhovat et al., 2015; **Figure 1**). [Interestingly, RSC-V1aR was not associated with female behavior (Zheng et al., 2013)]. Together these data suggest that vasopressin function shapes individual differences in memory, space-use and sexual fidelity in the field. Given the prominent role of the RSC in spatial memory, we hypothesize that males with low RSC-V1aR are less adept at remembering the spatial location of social interactions. In this scenario, low RSC-V1aR males intrude more because they are less able to recall the details of a punitive encounter with a resident male; males with high RSC-V1aR, in contrast, seem to avoid intruding on male territories, and so are better equipped to guard their mates. An alternative (but not mutually exclusive) hypothesis is that RSC-V1aR influences space use and sexual fidelity by shaping the strength of a bond, or by promoting a male's ability to keep track of his mate. These alternatives have yet to be tested.

GENETIC VARIATION AT THE AVPR1A LOCUS PREDICTS RSC-V1AR EXPRESSION

Having identified profound individual differences in cortical V1aR (Phelps and Young, 2003), and linked them to individual differences in behavior (Ophir et al., 2008c; Phelps and Ophir, 2009), we next asked whether individual differences in RSC-V1aR abundance were genetic, epigenetic, or both. From our field data, there were two plausible explanations: that differences in behavioral experiences somehow drove the individual differences in V1aR, or that V1aR in the RSC preceded and perhaps caused the behavioral differences in space-use and fidelity. If RSC-V1aR

variation caused behavioral differences, what was the origin of the cortical variation?

A variety of findings suggested that RSC-V1aR was not caused by the experience of intra-pair or extra-pair paternity, but was some complex combination of genetic and developmental regulation of the *avpr1a* locus. First, there are no sex differences in RSC-V1aR abundance, nor are there any differences between paired and single animals (Phelps and Young, 2003; Ophir et al., 2008c), suggesting that neither sex steroids nor mating experience influenced expression. Moreover, work by Hammock and Young (2005) bred lines of prairie voles with long or short microsatellite lengths in the *avpr1a* promoter, and found that they differed substantially in RSC-V1aR abundance. This demonstrates that *cis*-acting sequence variation contributes to RSC-V1aR. The story became more complicated, however, because neither Hammock et al. (2005), nor our own lab (Ophir et al., 2008a) found microsatellite length to predict RSC-V1aR in outbred animals. Our hypothesis was that the *avpr1a* microsatellite is not causal, but that it was imperfectly linked to neighboring single-nucleotide polymorphisms (SNPs) that are responsible for individual differences. Studies that bred for long or short microsatellites would also select for different frequencies of any linked SNPs.

To test the hypothesis that SNPs were shaping RSC-V1aR abundance, we looked at natural variation in RSC-V1aR and sequence variants from a large population of lab-reared and wild-caught prairie voles across ∼8 kb of the *avpr1a* locus (**Figure 2**). We found 151 SNPs overall (Okhovat et al., 2015). None of these SNPs predicted V1aR in brain regions implicated in bonding and aggression (ventral pallidum or lateral septum). However, we found four tightly linked SNPs predicted RSC-V1aR. These four SNPs were found upstream of the first exon (SNP -1392), within the intron (SNP 2170 and 2676) and in the second exon (SNP 3506; all SNPs are numbered with

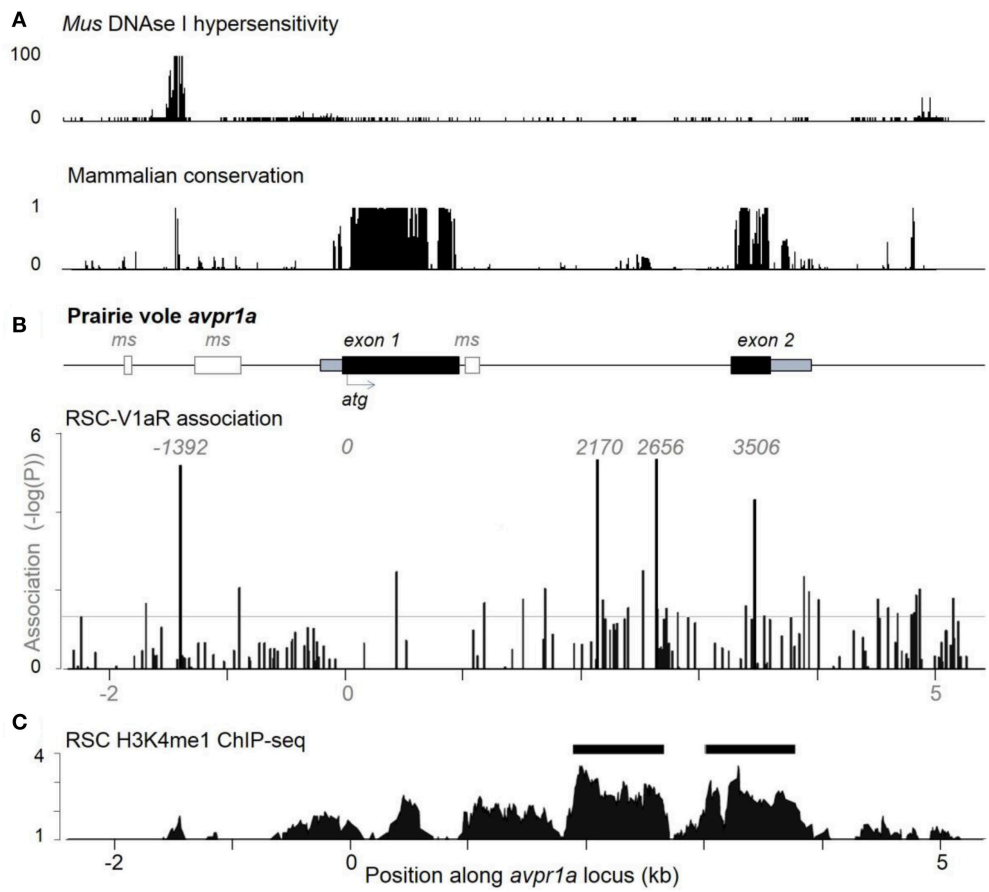

FIGURE 2 | Individual differences in RSC-V1aR abundance are well predicted by 4 linked single nucleotide polymorphisms at the *avpr1a* locus. **(A)** ENCODE data on DNAse hypersensitivity (top) from the cortex of adult *Mus musculus* (top), and conservation of corresponding sequences across mammals (below). **(B)** The structure of the prairie vole avpr1a locus includes two exons (UTRs in gray, CDS in black), and three microsatellite sequences (ms, white). The microsatellite upstream of the first exon has been the subject of numerous studies. Below, vertical bars represent the strength of association (-logP) between each identified SNP and RSC-V1aR abundance. Four strongly linked SNPs (positions -1392, 2170, 2656, 3506) were highly associated with RSC-V1aR and survived multiple comparison corrections. Horizontal bar corresponds to uncorrected α ($P = 0.05$). **(C)** Chromatin immunoprecipitation sequencing (ChIP-seq) targeting the enhancer marker H3K4me1 reveals significant enrichment (compared to input DNA controls) within the intron, as well as within the second exon. Horizontal scale depicts position in kilobases (kb) of *avpr1a* locus, aligned to all panels in figure. Data used in association analyses included both males and females. Modified with permission from Okhovat et al. (2015).

respect to translation start site; **Figure 2B**). We refer to the set of SNPs that correspond to high RSC-V1aR as the HI allele, and the opposite set of SNPs as the LO allele. We replicated this association on a third population of wild-derived animals, crossing parents heterozygous for the alleles to produce HI/HI and LO/LO homozygotes in the same litter (Okhovat et al., 2015). We found that the HI and LO alleles were strong, replicable, and robust predictors of not only RSC-V1aR, but also *avpr1a* transcript abundance, suggesting that these predictive SNPs affect *avpr1a cis*-regulation.

When located within regulatory regions, SNPs can alter gene expression by changing the epigenetic properties of the locus. Remarkably, all four RSC-associated *avpr1a* SNPs co-localized with markers of gene regulation. SNP -1392 was within an deoxyribonuclease I (DNAse I) hypersensitive site, a marker of open chromatin; moreover, this open chromatin was centered on a highly conserved binding site for the transcription factor CTCF, a factor known to shape gene regulation through its contributions to chromatin looping (Phillips and Corces, 2009, **Figure 2A**). Distal regulatory sequences that interact directly with promoters to regulate transcription are known as enhancers, and can be identified through their characteristic histone modifications (Heintzman et al., 2009). One such mark is the monomethylation of lysine 4 in histone 3 (H3K4me1), which marks both active and poised enhancers (Heintzman et al., 2009). We performed ChIP-seq on the RSC of prairie voles, and found that one such enhancer site was located in the *avpr1a* intron and overlapped with both SNP 2170 and SNP 2676 (**Figure 2C**). There was also a second putative enhancer that overlapped with the second *avpr1a* exon and SNP 3506 in the HI/LO alleles.

Interestingly, SNP 2170 is a T/G polymorphism that alters the presence/absence of a CpG site located within a putative intron enhancer. This site is also weakly linked to additional polymorphic CpG sites (polyCpG) within the same enhancer, leading to significant HI and LO allelic differences in CpG availability; the LO allele, which is associated with lower RSC-V1aR, has significantly more CpG sites in the putative intron enhancer compared to the HI allele (Okhovat et al., 2015, 2017b). CpG sites are the main targets for DNA methylation—a well-known epigenetic modification that can regulate gene expression—therefore, we hypothesized that *avpr1a* genotype differences in enhancer CpG could lead to differences in enhancer methylation and *avpr1a* expression in the RSC.

We found that both lab-reared and wild-caught showed significantly different levels of DNA methylation in the intron enhancer (Okhovat et al., 2015, 2017b). There was also a negative correlation between overall enhancer methylation and *avpr1a* transcription (Okhovat et al., 2015, 2017a), suggesting that enhancer methylation lowers RSC-V1aR by reducing *avpr1a* transcription, consistent with commonly reported silencing effects of DNA methylation (Nan et al., 1998). While enhancer methylation predicted individual differences in RSC-V1aR, methylation of the *avpr1a* promoter did not (Okhovat et al., 2017a). Although promoter methylation is generally silencing (Bird and Wolffe, 1999), our data indicate that the *avpr1a* promoter is generally un-methylated, whether the locus is active or not. This finding is in line with recent studies that suggest promoters are often unmethylated, even in cell types in which they are not expressed—thus a lack of methylation is necessary but not sufficient for gene expression (Rollins et al., 2006; Lister et al., 2013). Methylation and sequence variation in regulatory elements outside of the promoter area—especially within enhancer sequences—may be better predictors of expression.

A detailed analysis of HI and LO allele sequences suggested at least two mechanisms by which sequence variation and epigenetic mechanisms might interact at the *avpr1a* enhancer. First, allelic differences in CpG abundance and overall enhancer methylation could account for differences in expression via allele-biased recruitment of repressive methyl-binding proteins—such as MeCP2 (Bird, 2002). Alternatively, binding of transcription factors may be influenced by sequence changes generated by SNP 2170. Based on published position weight matrices, some transcription factors, including GATA2—which is expressed in the mouse RSC—bind preferably to the LO allele (Okhovat et al., 2017b). Therefore, differential binding of transcription factors due to both genetic and epigenetic variation at the intron enhancer may drive allele-biased changes in RSC-V1aR abundance. While further research is required to elucidate the exact molecular consequences of sequence variation in the intron enhancer, these findings provide promising explanations for the variation observed in RSC-V1aR.

SELECTION MAINTAINS ALLELIC VARIATION RELATED TO RSC-V1AR ABUNDANCE

Although individuals can vary tremendously in social behavior, as well as in gene expression and brain function, we know relatively little about how DNA sequence variation contributes to meaningful differences in brain and behavior. We have reviewed data showing that individual differences in RSC-V1aR predict behavior of male prairie voles in the field, and that these brain differences are due at least in part to genetic variation at the *avpr1a* locus. Here, we examine whether there is evidence that natural selection has actively maintained variation in brain in behavior.

Our first analysis was to revisit data on paternity and fitness obtained from animals in the field (**Figure 1**). We asked whether there was a difference between paired and single males in their overall fitness, as measured by the number of pups that they sired in the field. We found that paired males sired more young (Ophir et al., 2008b), demonstrating that selection favors the capacity to form pair-bonds. However, we did not find a difference in the fitness of males who mated exclusively with a partner (IPF), and those who mated at least once outside a pairbond (EPF), suggesting that both faithful and unfaithful males do comparably well in the conditions we examined (Ophir et al., 2008c). We examined the relative fitness of HI and LO RSC alleles in our field study and found that they did not differ significantly in fitness (**Figure 3A**; Okhovat et al., 2015). However, when we examined how this fitness was obtained, we found that HI alleles were more fit than LO alleles in the

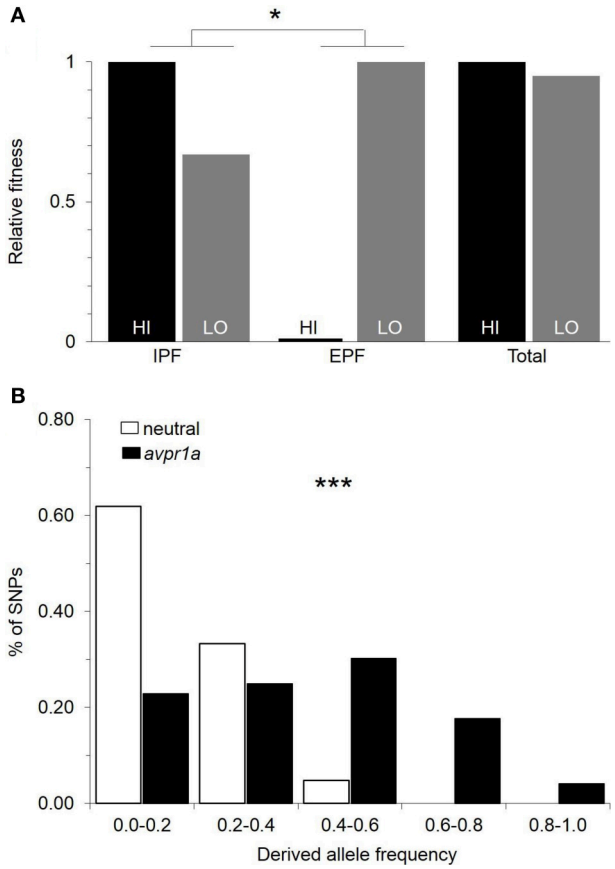

FIGURE 3 | Natural selection maintains variation in RSC-V1aR abundance. **(A)** Relative fitness of HI and LO alleles measured in the context of intrapair (IPF) and extrapair (EPF) fertilization rates obtained by male prairie voles. Selection is measured by the difference in fitness of the two alleles. The differences in the direction and strength of selection in IPF and EPF contexts were tested with a permutation test. **(B)** Comparison of the frequency spectra of polymorphisms for *avpr1a* (black) and neutral loci (white) reveals a significant excess of intermediate frequency alleles in *avpr1a*. $^{*}P < 0.05$, $^{***}P < 0.001$. Modified with permission from Okhovat et al. (2015).

context of intra-pair fertilizations, while LO alleles were more fit in the context of extra-pair fertilizations (**Figure 3A**; Okhovat et al., 2015). This is consistent with the view that the diversity of V1aR in the RSC represents a "balanced polymorphism" of the social brain, in which faithful and unfaithful male mating behaviors provide alternate but equivalent sources of evolutionary fitness.

While these results were encouraging, our field studies were a snapshot in time, tested under a single set of population densities and over a narrow range of conditions. We used evolutionary genetic approaches to test whether there was a history of selection actively maintaining variation at the *avpr1a* locus. A new mutation is, by definition, at low frequency within a population, and in the absence of selection, it is more likely to be lost than to drift to high frequency; thus most variable sites in the genome are at low frequencies (Hudson et al., 1987; Tajima, 1989). If selection is actively maintaining alternative forms of an allele, however, both forms tend to be at intermediate frequencies, and neighboring sites are also at higher frequencies than is characteristic of the genome as a whole (Hudson et al., 1987; Tajima, 1989). We compared the frequencies of mutations at the *avpr1a* locus to those in three putatively neutral genes (Okhovat et al., 2015), or across the entire genome (Berrío Escobar, 2017). We found that indeed, the *avpr1a* locus had higher frequencies of SNPs than was characteristic of the rest of the genome, suggesting that selection actively maintained this diversity (**Figure 3B**). Moreover, this signal was concentrated in the vicinity of the SNPs that defined the HI and LO alleles—a region of the *avpr1a* locus that did not predict expression in other brain regions (Okhovat et al., 2015; Berrío Escobar, 2017). Together these data suggest that RSC-V1aR diversity represents adaptive variation in brain, behavior, and cognition.

The high degree of linkage between the SNPs that defined HI and LO alleles seems unusual, because many intervening polymorphisms are unlinked to HI and LO alleles. We used permutation tests to ask whether these SNPs were significantly more linked than we would expect by chance (Berrío Escobar, 2017). We found that the SNPs were significantly more linked than predicted based on the distance between them—a pattern suggesting that the selection had favored specific combination of nucleotides across sites. Such epistasis across regulatory regions is poorly studied, but not without precedent. For example, recent data suggest that SNP-by-SNP interactions among non-coding elements play an important role in human disease (Dinu et al., 2012; Jamshidi et al., 2015). Such epistasis may reflect interactions among transcription factors that bind at different sites, contributions to chromatin looping and conformation, or any of the many other molecular changes needed to effectively coordinate transcription at a locus (e.g., Grubert et al., 2015). Whether the HI and LO SNPs interact remains to be determined, but our evidence of non-random linkage further suggests a causal role for these polymorphisms.

DEVELOPMENTAL VARIATION AT THE *AVPR1A* LOCUS

Although SNPs in *avpr1a* regulatory sequences seem to have a major role in regulating RSC-V1aR abundance, a variety of data suggested that environmental factors may also be at play. For example, lab-reared voles had a stronger association between HI and LO alleles and RSC-V1aR abundance than did wild-caught prairie voles (Okhovat et al., 2015). This observation suggested that RSC-V1aR variation might also be shaped by the environmental variation that voles are naturally exposed to in the wild (e.g., population and resource fluctuations, Getz et al., 2001). In fact, previous work on prairie voles (Bales et al., 2007; Prounis et al., 2015) and rats (Francis et al., 2002) demonstrated that developmental manipulations can alter V1aR regulation in the RSC and other brain regions. While the exact molecular mechanisms for these neuronal changes are not known, environmentally induced changes in neuronal gene expression are often mediated by molecular epigenetic modifications, such as DNA methylation (Szyf and Bick, 2013).

Given that HI and LO alleles differ in the abundance of CpG sites within the putative intron enhancer, and that the methylation of this enhancer is negatively associated with RSC-V1aR abundance (Okhovat et al., 2015, **Figure 4A**), we hypothesized that LO

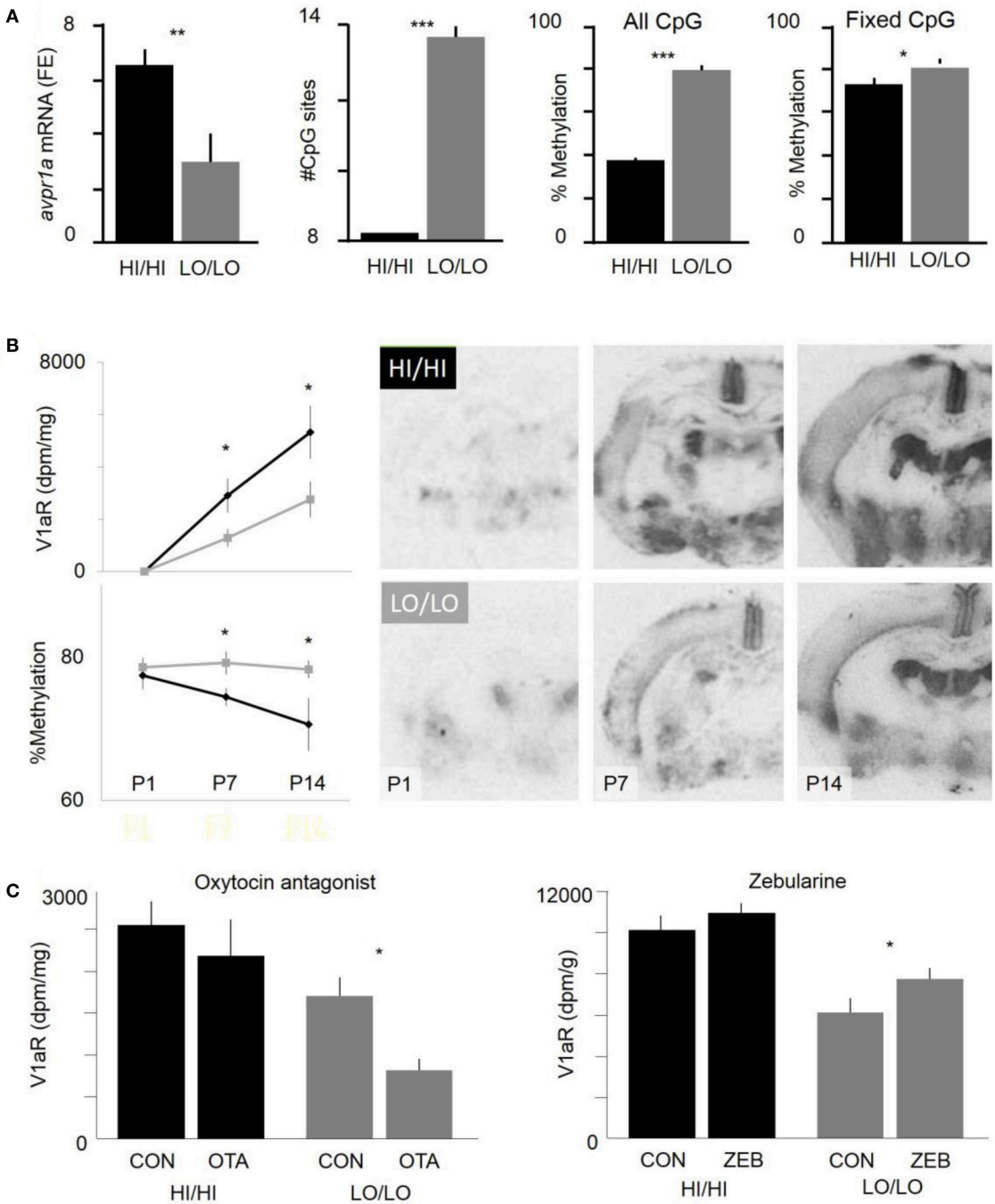

FIGURE 4 | Interaction of genetic and epigenetic differences in cortical V1aR abundance. **(A)** Effects of HI/LO genotype on RSC *avpr1a* mRNA abundance (fold enrichment, far left), the number of CpG sites within the intron enhancer, the methylation status of the intron enhancer, and the methylation status of enhancer CpGs that lack sequence variation ("fixed CpGs", far right). Graphs represent animals at weaning age (21d). **(B)** Ontogeny of RSC-V1aR abundance (top left) and methylation of fixed CpGs within the intron enhancer (bottom left). Images represent autoradiograms of brains with median RSC-V1aR abundance for each genotype and time point (postnatal days 1, 7, and 14). **(C)** Genotype-specific effects of neonatal (P1) manipulations of oxytocin antagonist (left) and a methylation inhibitor (right) on RSC-V1aR abundance. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data included both male and female offspring. Modified with permission from Okhovat et al. (2015, 2017a).

alleles may be more sensitive to developmental perturbations that influence CpG methylation.

Typically, rodent brains undergo periods of dramatic developmental change in gene expression and methylation; such critical periods are often highly responsive to environmental variation in parental care, diet, or stress (Roth and Sweatt, 2011). However, based on genetic makeup, individuals can vary in their sensitivity and response to these early developmental perturbations, a phenomenon known as gene-by-environment interactions (GxE). In prairie voles, neuronal V1aR abundance undergoes drastic changes postnatally (Wang et al., 1997). To begin to understand how genotype interacts with development, we examined the ontogeny of RSC-V1aR in HI/HI and LO/LO genotypes. We found that one-day-old HI/HI and LO/LO voles lacked RSC-V1aR (Okhovat et al., 2017a). However, significant genotype differences in RSC-V1aR quickly emerge during the first postnatal week (**Figure 4B**). Interestingly, genotype differences in *avpr1a* enhancer methylation also appear during this period, indicating that enhancer methylation may be involved in early-life regulation of RSC-V1aR (Okhovat et al., 2017a, **Figure 4B**).

To assess HI and LO differences in susceptibility to early-life perturbation, newborn pups were exposed to oxytocin receptor antagonist, a manipulation that is sometimes considered analogous to poor parenting, and that has been shown to alter adult RSC-V1aR of voles (Bales et al., 2007). This postnatal treatment reduced RSC-V1aR later at weaning age (21 days), demonstrating that *avpr1a* regulation is sensitive to early developmental and environmental perturbations (Okhovat et al., 2017a, **Figure 4C**). This sensitivity, however, was only detected in LO/LO pups, and not their HI/HI siblings. Similarly, we used a global inhibitor of methylation, zebularine (Cheng et al., 2003) to manipulation methylation in newborn pups. We found that zebularine treatment increased RSC-V1aR in LO/LO 21d animals but not in their HI/HI siblings (Okhovat et al., 2017a, **Figure 4C**). Overall, these data present a remarkably coherent picture in which the high CpG density of LO alleles made them both more sensitive to the silencing effects of the oxytocin receptor antagonist, and to the demethylating effects of zebularine. LO alleles seem to be more developmentally sensitive, while HI alleles seem to be constitutively highly expressing.

While HI and LO alleles differ in their sensitivity to developmental perturbation, examination of the methylation of the intron enhancer suggests a more complex story than we hypothesized. Enhancer methylation *was not* influenced by these developmental manipulations (Okhovat et al., 2017a). While HI and LO genotypes exhibited GxE interactions, this effect does not seem to be due to CpG density differences in the putative intron enhancer alone. It is likely that genetic differences in the intron enhancer are inherited along with genetic variation at additional unexamined enhancers. Indeed, methyl-DNA immunoprecipitation (meDIP) identifies additional differentially methylated near *avpr1a*, but outside of our original focus (Okhovat et al., 2017a, **Figure 5**). Examining whether any of these sites also contain sequence differences between HI and LO alleles may clarify how genetic variation in *avpr1a* regulatory mechanisms contributes to sensitivity to developmental perturbation, and how these interact with regulatory regions we have already identified.

CONCLUSIONS AND FUTURE DIRECTIONS

Our work began with the observation that the distribution of vasopressin 1a receptor in the RSC was surprisingly variable across individuals (Insel et al., 1994; Phelps and Young, 2003). We found that this variation predicted patterns of space-use and sexual fidelity in the field, with high levels of RSC-V1aR associated with sexual fidelity, and low levels associated with infidelity—even among paired males (Ophir et al., 2008c; Okhovat et al., 2015). Field paternity data and patterns of standing variation within the genome both suggest that variation at the *avpr1a* locus has been actively maintained by selection. Lastly, the alleles that drive differences in RSC-V1aR influence not only the mean level of vasopressin receptor, but also its sensitivity to developmental perturbation. Although this work spans diverse levels of analysis, from the function of chromatin to tests of selection in natural environments, there are a number of interesting questions that remain unanswered.

From a molecular perspective, while HI and LO alleles cause differences in RSC-V1aR abundance, we do not yet understand how nucleotide variation translates into differences in *avpr1a* function. Which of the four linked SNPs, if any, are causal? The case is strongest for the intron SNP 2170: it is a polymorphic CpG site associated with a cluster of polymorphic CpGs; it occurs within a region of chromatin that displays an enhancer-specific histone mark; it exhibits differential methylation between genotypes; its methylation status is associated with RSC-V1aR in animals from both lab and field; and it exhibits a pattern of nucleotide diversity that indicates a history of balancing selection (Okhovat et al., 2015, 2017a,b; Berrío Escobar, 2017). The 5′ SNP (-1396) has been less studied, but is also promising. It flanks a strongly conserved CTCF binding site and resides within a region of open chromatin (Okhovat et al., 2015, **Figure 1A**). The unusually tight linkage between these sites similarly suggests some coordinated function (Berrío Escobar, 2017). These data, however, fall short of demonstrating that either of these SNPs is causal. Moreover, the fact that developmental perturbations influence RSC-V1aR without altering the methylation status of the intron enhancer suggest that there are other, more distal regulators—an interpretation reinforced by the existence of differentially methylated regions outside of the immediate *avpr1a* locus (**Figure 5**). Whether such distal regulators bear sequence variation that contributes to HI and LO alleles remains to be determined. Our ChIP-seq approach allows for the exhaustive identification of distal regulatory sites, but conformation capture methods such as Hi-C will be needed to identify sites that make contact with *avpr1a* promoter, and that are thus likely to be directly shaping *avpr1a* function (Mifsud et al., 2015). Gene therapy methods using *cas9* to target deletions of putative enhancers, or using inactivated *cas9* fused to chromatin-remodeling enzymes to shape the function of specific regulatory sequences (Senís et al., 2014)

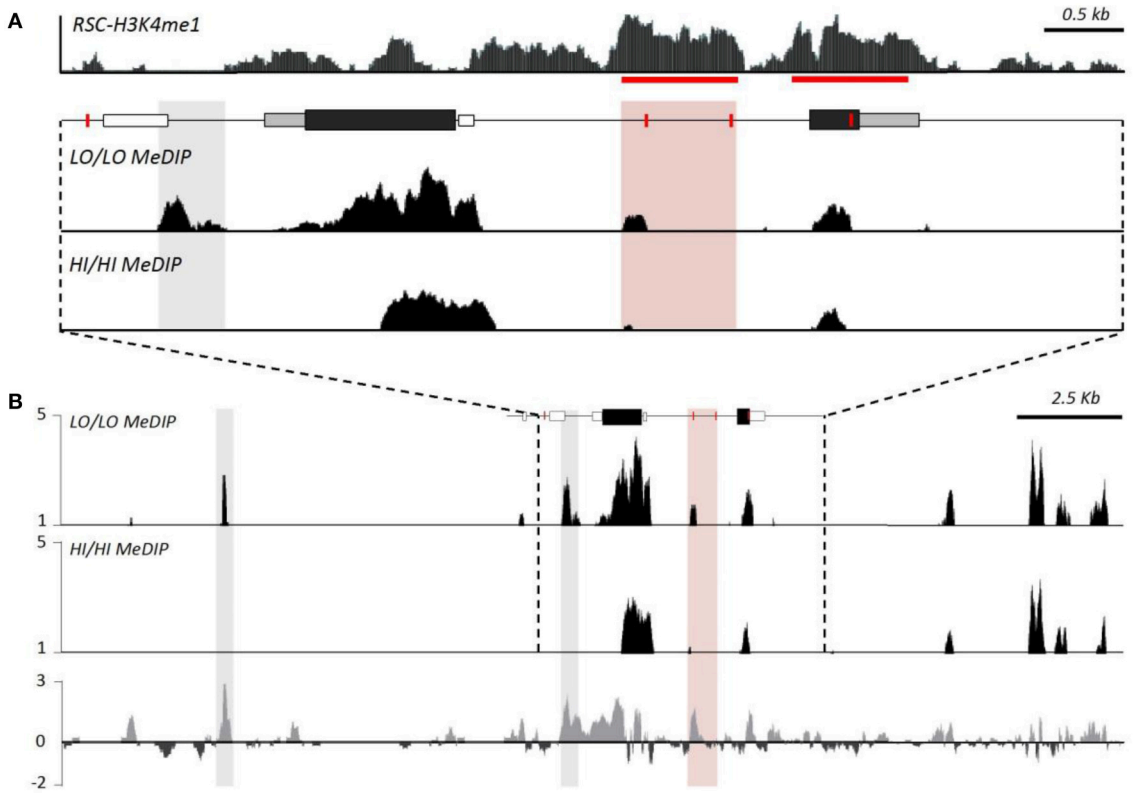

FIGURE 5 | Genotype differences in methylation suggest distal regulators of avpr1a function. **(A)** Top panel depicts relative read depth (fold enrichment) of H3K4me1 reads denoting putative enhancers at the avpr1a locus. Bottom panels depict read depths from methyl-DNA immunoprecipitation sequencing (meDIP) targeting the RSC of HI/HI and LO/LO prairie voles. The data confirm enhanced methylation near SNP 2170 (pink box) of LO/LO genotypes, as well as revealed a differentially methylated region (DMR) just upstream of the transcription start site (where there are no genetic differences between HI and LO alleles). **(B)** A more expanded view of the locus reveals a strong DMR ∼10 kb upstream of the locus; it is not known whether this or other more distal sites also differ in their underlying sequence. Modified with permission from Okhovat et al. (2017b).

provide a means for more directly determining whether specific nucleotides shape cortical expression of the *avpr1a* locus, and how such nucleotides interact with developmental experience.

While the molecular underpinnings of RSC-V1aR will offer novel insights into the nature of GxE and their substrates, a second series of unanswered questions concerns the exact nature of the relationship between RSC-V1aR and behavior. The behavioral functions of the RSC are an area of active investigation in both humans and traditional laboratory rodents. From a neuroanatomical perspective, the RSC is interconnected with the hippocampus, entorhinal cortex, anterior thalamus, and laterodorsal thalamus—a circuit central to episodic and spatial memory (Aggleton, 2014). Indeed, the RSC is active during navigation tasks, and in rats the RSC contains head-direction cells (Vann et al., 2009; Todd and Bucci, 2015). Imaging studies of humans (and rodents) at rest reveal that the RSC is one of two major nodes of the "default mode network"—a group of brain regions active when not performing a task (Spreng et al., 2008; Lu et al., 2012; Stafford et al., 2014). The second major node is the anterior cingulate cortex, a major target of the RSC (Spreng et al., 2008; Lu et al., 2012; Stafford et al., 2014). One interpretation is that the RSC connects a posterior circuit that processes memory, with a more anterior prefrontal circuit that processes decision-making; in human studies, the default mode network activity is sometimes interpreted as daydreaming, in which memory is used to simulate possible actions (Spreng et al., 2008).

Causal manipulations of RSC function confirm its role in a variety of memory-related tasks, but there is not a clear consensus on exactly how the RSC contributes to memory. In one recent study, Cowansage et al. (2014) used activity-dependent expression of channel rhodospins to tag and manipulate RSC neurons that were active during exposure to a shock-associated context. They found that activation of these neurons could elicit freezing responses in the absence of the context. One interpretation of these data is that the RSC serves to either encode or retrieve long-term memories and, through its reciprocal projections with the hippocampus, allow access to those memories during related experiences (Todd and Bucci, 2015).

The existing literature suggests a variety of alternative hypotheses for the role of RSC-V1aR in space-use and sexual

fidelity. Our core observation is that a male with high V1aR intrudes less on territories of neighboring males, more effectively guards his mate, and mates predominantly with his partner. One hypothesis is that animals with high V1aR are better able remember the locations of social interactions—this could translate into the observed patterns of space-use and fidelity by making high V1aR males better able to guard mates (Okhovat et al., 2015). Similarly, having low cortical V1aR may impair the ability to recall locations of punitive encounters, making low-V1aR males more likely to intrude on neighboring territories and gain extra-pair copulations (Ophir et al., 2008c). In addition, there may be something non-spatial about the role of the RSC in social interaction—it may shape memory for one's partner, for example, or facilitate discrimination between remembered individuals through its projections to prefrontal cortices. Whatever the pattern proves to be, a rich set of studies aimed at dissecting the cognitive aspects of bonding, navigation, choice, and fidelity remain to be done.

Aside from the specific insights the above studies offer, they also provide a general framework for thinking about variation in the nervous system and its relationship to social behavior. First, they demonstrate that genetic variation in brain function can be a source of adaptive behavioral variation within a species. Our understanding of genetic variation in the nervous system is incredibly understudied, and this work provides a novel perspective on how diverse brains can be. A second value is that the studies illustrate how modern tools for interrogating chromatin function can be used to identify specific DNA sequences likely to be important to the regulation of behavior. On a genome scale, combining these sequencing tools with evolutionary genetics will allow researchers to more quickly identify which among the many thousands of regulatory sequences (and billions of nucleotides) are likely to be playing a causal role in gene expression (e.g., Pollard et al., 2006; Boyd et al., 2015). Lastly, we show how identifying nucleotide variation within specific regulatory sequences allows one to explore the interactions between genetic and epigenetic variation. Together such approaches will be a tremendous aid not only to our understanding of natural behavior, but in our quest to identify how variation in the genome interacts with the environment to shape the diversity of social behavior related to both health and disease.

AUTHOR CONTRIBUTIONS

SP conceived and outlined the review, wrote the introduction, conclusion and future directions, assigned manuscript tasks and revised all other sections. MO wrote the sections on genetics and epigenetics. AB wrote the section on evolutionary analyses.

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frontiers in Neuroscience

REVIEW
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Social Context, Stress, Neuropsychiatric Disorders, and the Vasopressin 1b Receptor

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The arginine vasopressin 1b receptor (Avpr1b) is involved in the modulation of a variety of behaviors and is an important part of the mammalian hormonal stress axis. The Avpr1b is prominent in hippocampal CA2 pyramidal cells and in the anterior pituitary corticotrophs. Decades of research on this receptor has demonstrated its importance to the modulation of social recognition memory, social forms of aggression, and modulation of the hypothalamic-pituitary-adrenal axis, particularly under conditions of acute stress. Further, work in humans suggests that the Avpr1b may play a role in human neuropsychiatric disorders and its modulation may have therapeutic potential. This paper reviews what is known about the role of the Avpr1b in the context of social behaviors, the stress axis, and human neuropsychiatric disorders. Further, possible mechanisms for how Avpr1b activation within the hippocampus vs. Avpr1b activation within anterior pituitary may interact with one another to affect behavioral output are proposed.

Keywords: social recognition memory, aggression, neuropsychiatric disorders, hormonal stress response, animal models

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INTRODUCTION

It is well-established that the neuropeptide arginine vasopressin (Avp) is important to the neural modulation of mammalian behavior. However, the nuances of how Avp modulates behavior within specific brain regions via its two centrally expressed receptors, the Avp 1a (Avpr1a) and the Avp 1b receptor (Avpr1b), continues to be a robust and exciting area of research. While the Avpr1a has been heavily studied for several decades, the Avpr1b, which was discovered later, appears to be much more discretely localized and has a wholly different role in the modulation of behavior than the Avpr1a.

Since the initial papers describing the cloning of the Avpr1b (Lolait et al., 1995; Saito et al., 1995), its importance to the neural regulation of social behavior and the modulation of the hormonal stress response has come to light. Prominently expressed in the CA2 region of the hippocampus as well as the anterior pituitary gland (Young et al., 2006), Avp signaling through the Avpr1b can affect numerous behaviors, including social memory and aggression. Within the CA2 region of the hippocampus, the Avpr1b is hypothesized to be important for the processing of chemosensory information associated with social context, which in turn affects behavioral output (Stevenson and Caldwell, 2012; Pagani et al., 2015). Avpr1b expression in the anterior pituitary corticotrophs helps synergize the Avp signal with corticotropin releasing hormone (CRH) to facilitate the release of adrenocorticotropic hormone (ACTH). In fact, depending on the type of stressor, Avp signaling through the Avpr1b can have more of an impact on ACTH release than CRH (Ma et al., 1997, 1999).

While the aforementioned roles of the Avpr1b may seem disparate, there are important possible points of intersection. For instance, the stress response under both acute and chronic conditions can result in changes in anxiety or mood (Roper et al., 2011), ultimately shaping how an organism might interpret its social world, in turn affecting social behavior. So, while central signaling of Avp via the Avpr1b is often considered distinct from its pituitary action, it is important to consider how they may interact. Given the complexity of the central Avp system, as well as the many behaviors Avp is known to affect, this review will focus on the role of Avp signaling via the Avpr1b in the modulation of behaviors such as social memory and aggression as well as the importance of this system to the functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Lastly, the role of the Avpr1b in humans and the potential implications of this work in the context of neuropsychiatric disorders will be explored.

THE AVPR1B IS IMPORTANT FOR SOCIAL RECOGNITION MEMORY

One critical component of social context is the ability of an animal to remember conspecifics, termed social recognition memory. Social memory also contributes to social cognition, which essentially requires an animal to remember a conspecific that they have interacted with previously. The ability to remember is key to helping an animal decide whether they should engage or avoid an interaction. The broader social context is also important to social memory. For example, the presence of other conspecifics or predators may impact an animal's choice to engage in certain behaviors, or even interfere with memory formation. As mentioned previously, there is compelling evidence that the Avpr1b is important for social recognition memory, specifically, its acquisition (Smith et al., 2016). This conclusion is based on studies utilizing Avpr1b knockout ($^{-/-}$) mice, as well as excitotoxic lesions and optogenetic activation of the CA2 region of the hippocampus.

Across a variety of tests, Avpr1b$^{-/-}$ mice display deficits in social recognition memory, despite having normal olfaction (Wersinger et al., 2002, 2004). In an 11-trial habituation/dishabituation task Avpr1b$^{-/-}$ males show normal habituation and dishabituation to stimulus females—mice should decrease interaction times (as measured by proximity to the stimulus animal) across trials 1–10 when exposed to the same mouse repeatedly, i.e., habituation, and then increase their interaction time when exposed to a new mouse on trial 11, i.e., dishabituation. However, the durations of their investigation times are significantly lower in several of the trials compared to controls. These results suggest that Avpr1b$^{-/-}$ mice are able to habituate to a familiar female and are able to recognize a novel female. However, the decreases in time spent investigating the stimulus mouse compared to controls could be indicative of decreased social motivation.

Consistent with this hypothesis, Avpr1b$^{-/-}$ mice commonly demonstrate deficits in interacting with social stimuli (Wersinger et al., 2004; Yang et al., 2007; DeVito et al., 2009). Generally speaking, Avpr1b knockouts prefer a novel mouse over an inanimate object (Yang et al., 2007); although, in this particular study one cohort of null mutant and heterozygous Avpr1b mice failed to spend more time in the chamber housing the novel mouse relative to the chamber with the novel object. Additionally, Avpr1b knockouts spend less time with a familiar mouse vs. an empty compartment compared to controls (DeVito et al., 2009). Avpr1b$^{-/-}$ mice also differ from wildtype controls in an olfactory social investigation task in which mice are exposed to male, female, and clean bedding in three trials such that preference for (1) male or female, (2) female or clean, and (3) male or clean bedding are assessed (Wersinger et al., 2004). While control animals exhibit the expected preference for female over male bedding and soiled (male or female) bedding over clean, Avpr1b$^{-/-}$ mice display no preference for any type of bedding, which too suggests decreases in social motivation.

While Avpr1b knockout mice can habituate/dishabituate to social stimuli when there are short intertrial intervals, knockouts display memory deficits in more challenging tasks requiring temporal memory. When tested in the 2-trial social recognition test, which requires an animal to discriminate between a novel and a familiar animal with a 30-min intertrial interval, Avpr1b$^{-/-}$ males have impaired social recognition, as they are not able to discriminate between a novel and a familiar female (Wersinger et al., 2002; DeVito et al., 2009; **Figure 1**). Interestingly, Avpr1b$^{-/-}$ males do not seem to have any deficits in spatial memory (Wersinger et al., 2002; DeVito et al., 2009), but do exhibit impairments in two different tasks assessing temporal memory. In a "when" task that asks mice to discriminate between familiar objects presented at different time points as well as in an object-trace-odor task where mice are asked to learn associations with odors, Avpr1b$^{-/-}$ mice fail to recall or integrate the associations after a time delay (DeVito et al., 2009). Thus, it appears that genetic disruption of the Avpr1b can compromise an animal's ability to retain the memory of a conspecific beyond a short period of time.

While much of the work to date has focused on males, since females' behaviors are often not as robust on some of the aforementioned tasks, there is evidence that female Avpr1b$^{-/-}$ mice may also have deficits in social recognition memory. Specifically, female Avpr1b$^{-/-}$ mice have an abnormal Bruce effect (Wersinger et al., 2008). The Bruce effect is a pheromonally-mediated response in which a female will abort her pregnancy, i.e., pregnancy block, following the presentation of a novel male or novel male odor (Bruce and Parrott, 1960). Interestingly, unlike controls, Avpr1b$^{-/-}$ females fail to terminate their pregnancies in the presence of an unfamiliar male (Wersinger et al., 2008). Thus, Avpr1b$^{-/-}$ females are not able to identify the unfamiliar male as being "new," which is consistent with the hypothesis that the Avpr1b is important for processing olfactory cues, including accessory olfactory cues, which help the animal determine its social context and ultimately its behavioral response.

While studies that have utilized knockout mice have provided critical insight into the role of this receptor, one of the shortcomings of traditional knockout mice is that the gene is absent throughout the body from the point of fertilization, which in turn could result in some sort of developmental

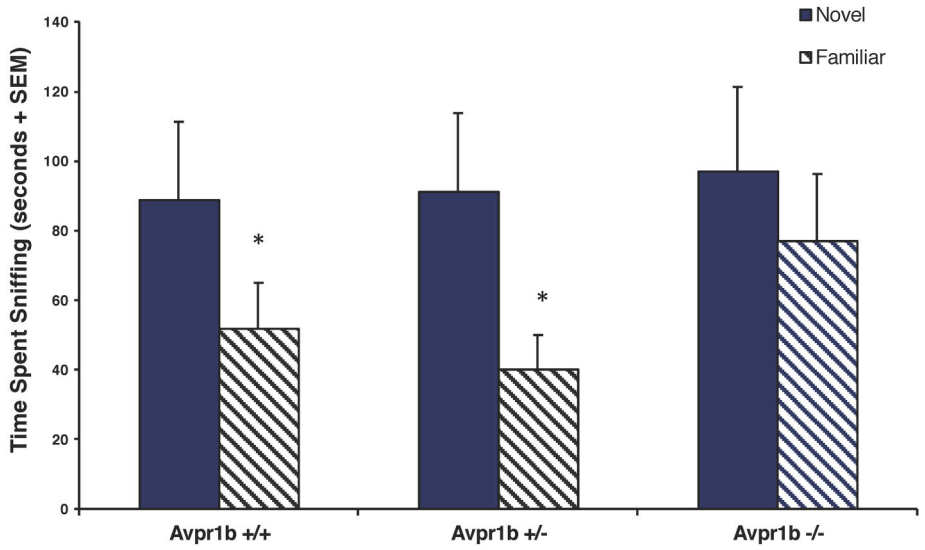

FIGURE 1 | Social recognition is impaired in Avpr1b$^{-/-}$ males as compared with their wild-type (Avpr1b$^{+/+}$) and heterozygous (Avpr1b$^{+/-}$) littermates. In a social recognition test with a 30-min interval between trials, Avpr1b$^{-/-}$ males do not appear to recognize a familiar female, compared to controls. Data are expressed as mean + SEM. *Significantly less than first exposure, $P < 0.05$. Modified and reprinted from Wersinger et al. (2002) with permission from Nature Publishing Group.

compensation. Fortunately, lesion studies and optogenetic work have confirmed much of what has been observed in Avpr1b$^{-/-}$ mice. These studies have further implicated the CA2 region of the hippocampus. More importantly, Avpr1b expression within the CA2 region was confirmed to play a role in the neural modulation of social behavior. When the CA2 region of the hippocampus is excitotoxically lesioned in males, social recognition memory is impaired in both the 2-trial social discrimination test and 11-trial habituation/dishabituation social recognition test (Stevenson and Caldwell, 2014). Further, targeted Cre-driven viral inactivation of CA2 pyramidal neurons results in a loss of social memory and a decrease in preference for social novelty. However, this inactivation does not impact sociability, as the mice still prefer a familiar littermate over an empty chamber (Hitti and Siegelbaum, 2014). During the acquisition phase of a social memory task (but not its retrieval phase), optogenetic stimulation of the Avp projection that originates in the paraventricular nucleus and extends to the CA2 region of the hippocampus, increases social recognition memory indefinitely. This effect on social memory is blocked when the Avpr1b antagonist SSR149415, also referred to as Nelivaptan, is injected into the CA2 region of the hippocampus (Smith et al., 2016). Taken together, these data provide compelling evidence that the deficits in social memory observed in Avpr1b$^{-/-}$ mice are likely due to Avp action through the Avpr1b within the CA2 region of the hippocampus.

THE AVPR1B IS IMPORTANT FOR NORMAL AGGRESSIVE BEHAVIOR

Competitive behaviors, such as aggression are important for social bonds between conspecifics. That said there are significant sex differences in the hormonal and neural regulation of aggression. Intermale aggression, for instance, is androgen-dependent in rodents. Whereas, in female rodents, aggressive behaviors are primarily observed in post-parturient females, being rarely observed in virgin females. However, whether male or female, aggressive behavior is characterized by both offensive and defensive elements and is commonly evaluated using a resident-intruder test for territorial aggression.

The Avp system, particularly its signaling through the Avpr1a, is consistently implicated in the modulation of aggressive behaviors (Ferris et al., 1997, 2006). However, even with its more limited distribution, the Avpr1b also appears to be important for normal displays of aggression in rodents. In hamsters for instance, oral administration of the Avpr1b antagonist SSR149415 at both 10 and 30 mg/kg doses significantly reduces the duration of resident male hamsters' frequency and duration of offensive sideways behaviors, olfactory investigation, chase behaviors, and flank marking compared to the vehicle and the 1 mg/kg dose groups (Blanchard et al., 2005). Similarly, in mice, oral administration of SSR149415 reduces the duration of offensive aggression in a resident-intruder test and decreases the number of defensive bites in those forced to encounter a threatening predator (Griebel et al., 2003). Conversely, in lactating Wistar rats neither intracerebroventricular nor site specific (MPOA and BNST) infusion of SS149415 10-min prior to a maternal defense test have an effect on aggressive behaviors (Bayerl et al., 2014, 2016). However, it is possible that this lack of effect is due to the use of virgin female Wistar rats as stimulus animals, though it is important to note that lactating Long Evans rats have been shown to attack female intruders more than male intruders (Haney et al., 1989). Thus, it is also plausible that this represents a species-specific effect of this antagonist. It is also important to acknowledge that SSR149415 has previously been

shown to have a high affinity for the human oxytocin receptor (OXTR) and could have affinity for the rodent Oxtr as well (Griffante et al., 2005). That said, data from Avpr1b$^{-/-}$ mice support the assertion that the Avpr1b is important for aggressive behavior within both sexes.

Compared to wildtype controls, Avpr1b$^{-/-}$ males have lower attack frequencies, longer attack latencies, and Avpr1b$^{-/-}$ males that do display aggressive behaviors display fewer agonistic behaviors (Wersinger et al., 2002, 2004). These observed reductions in aggressive behavior also extend to other mouse strains, with reduced aggressive behaviors persisting in Avpr1b$^{-/-}$ males that are crossed with the more aggressive *Mus musculus castaneus* (Caldwell and Young, 2009; **Figure 2**). Importantly, these deficits in aggressive behavior are specific to social situations as Avpr1b$^{-/-}$ males display normal predatory aggression (Wersinger et al., 2007). When Avpr1b$^{-/-}$ males are used as intruders, to see if they will defend themselves against an attack, they display defensive postures in the absence of defensive attacks, but show fewer retaliatory attacks (Wersinger et al., 2007). This observation appears to hold true for females as well, with only 20% of lactating Avpr1b$^{-/-}$ females displaying aggressive behaviors directed toward an intruder compared to 90% of lactating Avpr1b +/+ females. Further, of those lactating Avpr1b$^{-/-}$ females that do attack, the latency to attack and number of attacks remain significantly lower compared to controls (Wersinger et al., 2007).

Of course, aggression is complex with different neural networks activated depending on the type of aggression. For example, defensive contexts activate the posteroventral medial amygdala and dorsomedial ventromedial hypothalamus, whereas in offensive contexts the posterodorsal medial amygdala appears to play more of a role (Swanson, 2000). For intermale and maternal aggressive behavior, many of the nodes within the social behavioral neural network (SBNN) have been identified as being important for their regulation (for review see, Nelson and Trainor, 2007). To determine which brain areas are important for the neural regulation of aggression in Avpr1b$^{-/-}$ males and females, a couple of immediate early gene (IEG) studies have been performed. Two different IEGs, cFos and early growth response factor 1 (EGR1), have been studied in both Avpr1b$^{-/-}$ male and Avpr1b$^{-/-}$ lactating females following a single exposure to an intruder male. While no genotypic differences in cFos immunoreactivity were observed in either Avpr1b$^{-/-}$ males or Avpr1b$^{-/-}$ lactating females, a genotypic difference in EGR1 immunoreactivity was observed within the ventral bed nucleus of the stria terminalis (BNSTV) and the anterior hypothalamus (AHA), with male Avpr1b$^{-/-}$ mice having reduced EGR1 immunoreactivity in both brain regions relative to controls (Wersinger et al., 2002; Witchey et al., 2016). As both the BNSTV and AHA are implicated in the neural circuitry of aggression, we hypothesize that they may be part of the downstream circuit influenced by Avpr1b expression in the CA2 region of the hippocampus.

Like social memory, CA2 Avpr1b is also known to directly affect aggressive behavior. When the Avpr1b is overexpressed via microinjection of a lentiviral vector into the dorsal CA2 region of Avpr1b$^{-/-}$ males, their deficits in aggressive behavior are partially rescued (Pagani et al., 2015). Based on the work of Cui et al. (2013), it has been established that there is a Avp-ergic projection from the PVN to the CA2 region (this is what was driven in the aforementioned study by Smith et al., 2016). What happens downstream of the activation of Avpr1b within this region is still being determined. However, based on the connectivity of the CA2 region to other parts of the brain and what is known about the neural regulation of aggressive behavior a possible circuit can be hypothesized. The CA2 region has numerous efferent projections within the hippocampus (CA1, CA2, and CA3) as well as projections to the medial septum, the dorsal part of the LS, the triangular septal nucleus, the nuclei of the diagonal bands of broca, and the supramammillary nuclei (Chevaleyre and Siegelbaum, 2010). Of these, the projections to the septal regions are the most apparent link to the aggression circuit. Specifically, lesions or pharmacological inactivation of the LS leads to increased aggression (Slotnick et al., 1973; Potegal et al., 1981; McDonald et al., 2012) and conversely, electrical stimulation of the LS suppresses aggression (Potegal

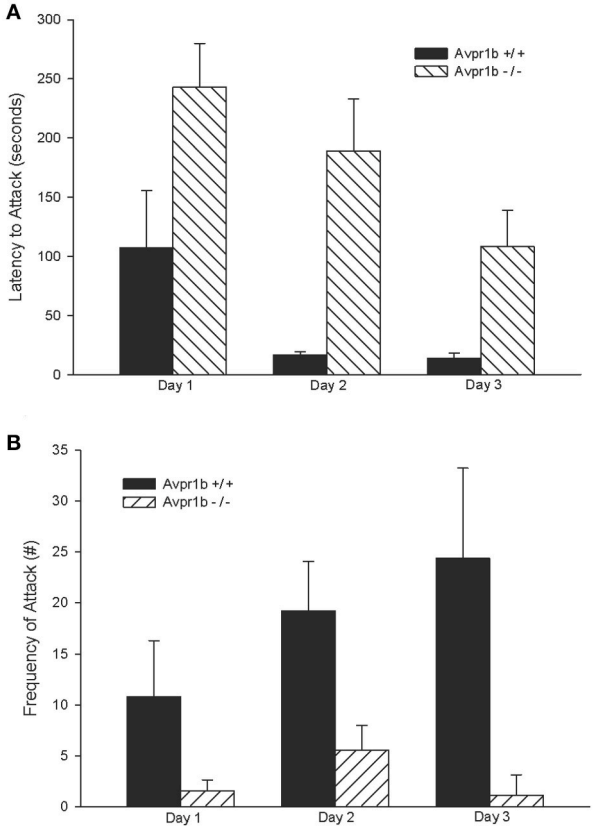

FIGURE 2 | Intermale aggression is impaired in Avpr1b$^{-/-}$ males on a more "wild" background, i.e., a 50/50 mixture of *Mus musculus* and *Mus musculus castaneus*, as compared with their wild-type (Avpr1b$^{+/+}$) littermates. In a resident-intruder test Avpr1b$^{-/-}$ males have longer attack latencies compared to Avpr1b$^{+/+}$ mice **(A).** Avpr1b$^{-/-}$ mice also display fewer attacks compared to Avpr1b$^{+/+}$ mice **(B).** Data are expressed as mean + SEM. For **(A,B)** there is a main effect of day and genotype, but no interaction, $P < 0.05$. Modified and reprinted from Caldwell and Young (2009) with permission from Elsevier.

et al., 1981). Further, increases in cFos expression are observed within the LS following intermale, interfemale, and maternal aggression (Kollack-Walker and Newman, 1995; Delville et al., 2000; Davis and Marler, 2003; Hasen and Gammie, 2005). It is also important to mention that the LS lies upstream of the BNSTV and AHA (Ferris et al., 1990; Staiger and Wouterlood, 1990); thus providing a potential circuit that might explain the EGR-1 data in Avpr1b$^{-/-}$ males (Witchey et al., 2016).

AVPR1B RECEPTORS EXPRESSED IN THE PITUITARY CORTICOTROPHS PLAY AN IMPORTANT ROLE IN THE STRESS RESPONSE

Given its well-defined role as a critical regulator of the HPA-axis, the Avpr1b has been studied extensively in the context of the hormonal stress response. Most of these studies have utilized SSR149415 and in rats there is scientific consensus that administration of SSR149415 prior to a variety of stressors decreases plasma ACTH compared to controls (Serradeil-Le Gal et al., 2003; Chen et al., 2008; Zhou et al., 2011; Jasnic et al., 2013; Ramos et al., 2016). Evidence that pharmacological disruption of Avpr1b signaling affects CORT are less consistent and seem to depend primarily upon the type of stressor as well as the route of administration of SSR149415, as its biological activity can differ depending on how it is administered (Roper et al., 2011). For example, in male Wistar rats intracerebroventricular administration of SSR149415 before air jet stress results in decreases in heart rate, blood pressure, and CORT compared to untreated controls (Stojicic et al., 2008); the effects on ACTH in this study are not known as they were not measured. However, when SSR149415 is administered intravenously or orally to male Sprague–Dawley rats prior to noise or dehydration stress, respectively, they show decreases in their ACTH response but no change in CORT compared to controls (Chen et al., 2008). Interestingly, when SSR149415 is given intraperitoneally to male Wistar or Fischer rats before heat stress or cocaine withdrawal, respectively, there are significant decreases in both ACTH (**Figure 3**) and CORT compared to controls (Zhou et al., 2011; Jasnic et al., 2013). It is also important to note that none of the studies that have utilized SSR149415 have found that this antagonist can bring ACTH or CORT concentrations back to pre-stressor levels, indicating only a partial reversal. As noted earlier, cross-talk with the Oxtr also remains a possibility (Serradeil-Le Gal et al., 2003; Griffante et al., 2005; Oost et al., 2011), which further complicates the interpretation of these studies.

Beyond measures of HPA-axis function, studies that have utilized SSR149415 have also examined the effect of this antagonist on the expression of numerous stress phenotypes, primarily those with anxiety- or depressive-like features. In both rats and mice, treatment with SSR149415 results in fewer defensive attacks in a defense battery, less time spent immobile in a forced swim test, more foot shocks in a punished drinking task, and more open arm entries in an elevated plus maze (Griebel et al., 2002; Serradeil-Le Gal et al., 2003). Taken together, these data suggest that antagonism of the Avpr1b has an overall anxiolytic effect. This is consistent with studies in which the effects of Diazepam and SSR149415 have been directly compared, with SSR149415 having more potent anxiolytic and antidepressant effects during both punished drinking tasks and elevated plus maze tests, as compared to diazepam (Serradeil-Le Gal et al., 2003). While the aforementioned studies utilized only a single dose, data from a chronic dose study found similar effects. Specifically, Breuer et al. (2009) administered chronic doses of SSR149415 intraperitoneally to Sprague-Dawley rats who were hyperactive as a result of an olfactory bulbectomy. They found that after 14 days of treatment, SSR149415 was able to bring olfactory bulbectomy-related hyperactivity back to control levels, being as effective as imipramine. Further, this effect persisted for a week after the cessation of treatment, which suggests that SSR149415 may have long-lasting effects. Chronic treatment with SSR149415 has also been shown to reduce dysphoria, as measured by intracranial self-stimulation in rat nicotine-withdrawal models (Qi et al., 2015). Interestingly, Sprague-Dawley rats chronically administered SSR149415 into the dorsal hippocampus via minipump display decreases in anxiety-like behaviors in an elevated plus maze (Engin and Treit, 2008). This latter study hints at a possible point of intersection between the peripheral effects of the Avpr1b and those within the brain with regards to anxiety and mood.

Some of the lack of consensus regarding the effects of SSR149415 on the HPA-axis are likely due to real differences in how various stressors affect the HPA-axis. Variation in the timing of the data collection post stressor (Roper et al., 2011), as well as differences between rodent species and strains (Roper

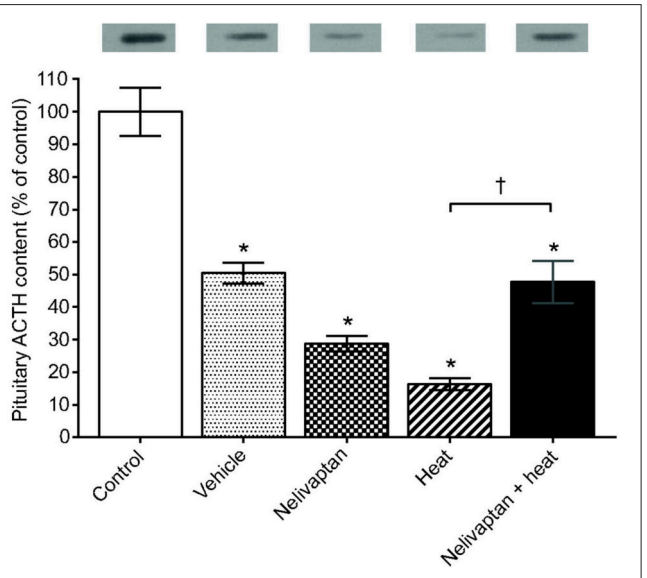

FIGURE 3 | Pretreatment with SSR149145 (i.e., Nelivaptan) prior to heat exposure results in increased pituitary adrenocorticotropic hormone (ACTH), as measured by western blot. Data are expressed as mean ± SEM. *A significant difference between the control and treated group of animals and †A significant difference between treated groups, $P < 0.05$. The results of the western blot for ACTH are presented above each bar. Reprinted from Jasnic et al. (2013) with permission from Company of Biologists LTD.

et al., 2010) are also important to consider. Another way to think about the mixed results would be to think about how SSR149415 affects the performance of the system as a whole. For example, an increase in peripheral Avp and an increase in Avpr1b protein expression within the pituitary has been observed when the Avpr1b is blocked by SSR149415, suggesting that Avpr1b expression in the pituitary is partially dependent on peripheral levels of Avp (Jasnic et al., 2013). Additionally, Ramos et al. (2016) found that the combination of SSR149415 and the CRH receptor antagonist SSR125543 was especially effective at lowering ACTH concentrations across three different types of stressors. Given that the Avpr1b and the CRH receptor type 1 are known to dimerize without impairing ligand binding and can modulate one another (Young et al., 2007; Murat et al., 2012), more research is needed to fully understand how SSR149415 is impacting signaling in this system.

Avpr1b$^{-/-}$ mice too have provided insights into the role of this receptor in the mediation of the stress response. Avpr1b$^{-/-}$ mice have normal resting ACTH levels but, as observed in the antagonist studies, following a variety of stressors, Avpr1b$^{-/-}$ males have weaker ACTH responses (Tanoue et al., 2004; Lolait et al., 2007a,b; Stewart et al., 2008b; Roper et al., 2010). Complementing SSR149415 studies, there are also stressor-dependent differences in CORT responses in Avpr1b$^{-/-}$ mice. For example, dehydration stress results in decreases in plasma CORT concentrations (Roberts et al., 2011). Male Avpr1b$^{-/-}$ mice subjected to acute restraint and shaker stress have a blunted ACTH response (Stewart et al., 2008b; Roper et al., 2010) However, while males show a blunted ACTH response after forced swim test, CORT levels appear normal (Stewart et al., 2008b). Interestingly, female Avpr1b$^{-/-}$ mice are more consistent in their ACTH and CORT response to stressors, showing reductions in both ACTH and CORT after lipopolysaccharide, ethanol, and selective serotonin reuptake inhibitor treatments (Lolait et al., 2007b; Stewart et al., 2008a).

With regards to stress phenotypes, the data are mixed in Avpr1b$^{-/-}$ mice. No genotypic differences are reported for forced swim, chronic isolation, elevated plus, and open field tests (Wersinger et al., 2002; Caldwell et al., 2006; Itoh et al., 2006). Even in Avpr1b$^{-/-}$ mice in which Avpr1b function in the CA2 region of the hippocampus is partially restored, no significant genotypic differences in anxiety-like behaviors are observed (Pagani et al., 2015). These results could be due to a compensatory mechanism, such as the upregulation of Oxtr in response to an absence of Avpr1b (Nakamura et al., 2008), but currently it is not clear why there is no obvious stress phenotype.

To better understand the role of Avpr1b in the mediation of stress behaviors, studies have focused on identifying the distribution of the Avpr1b and its local inhibition. In male Wistar rats, Avpr1b-associated immunoreactivity has been found in areas such as the amygdala, LS, nucleus accumbens, hippocampus, as well as others (Hernando et al., 2001). Of these areas, both the basolateral and medial amygdala have been implicated specifically in the mediation of anxiety by Avpr1b (Salome et al., 2006), with evidence for additional modulation via the Oxtr (Litvin et al., 2011), while the Avpr1b in the LS is suspected to be involved in depressive states (Stemmelin et al., 2005). In addition, infusions of SSR149415 into the dorsal hippocampus, amygdala, or LS of male Sprague-Dawley rats results in decreases in anxiety- and depressive-like behaviors, as measured by elevated plus or forced swim tests (Stemmelin et al., 2005; Salome et al., 2006; Engin and Treit, 2008; Zai et al., 2012). Even though none of these targeted infusion studies measured changes in ACTH or CORT levels, they do point to places in the brain where the peripheral and central effects of Avpr1b may interconnect. Further studies are required to fill the gaps in our understanding of the intersection of peripheral and central effects of Avpr1b.

DATA SUGGEST THAT THE AVPR1B MAY PLAY A ROLE IN HUMAN NEUROPSYCHIATRIC DISORDERS, EMOTIONAL EMPATHY, AND HAVE THERAPEUTIC POTENTIAL

Much of the work implicating the Avpr1b in humans has emerged from genetic studies of single nucleotide polymorphisms (SNPs). Currently, there is evidence that SNPs within the Avpr1b sequence may impact social behaviors and aggression, as well as play a role in neuropsychiatric disorders, particularly those associated with dysregulation of the HPA-axis, such as mood and anxiety disorders (van West et al., 2004; Dempster et al., 2007; Keck et al., 2008; Zai et al., 2012). As reviewed above, animal models have linked the Avpr1b to the neural regulation of social recognition memory and aggression. Similarly, studies in humans have found Avpr1 SNPs to be involved in prosociality (Wu et al., 2015). It is well-established that in humans prosociality and empathy work hand in hand with each other; the former being a voluntary behavior exhibited to benefit others and the latter the ability to respond to others' emotions. For instance, carriers of the G allele of the Avpr1b SNP rs28373064 are more prosocial and empathetic, with the effects being mediated by emotional empathy rather than cognitive empathy (Wu et al., 2015). Furthermore, the c-allele of the AVPr1b SNP rs35369693 is associated with aggressive behavior in children aged 9–15 (Zai et al., 2012). Several other haplotypes have been reported that have yet to be genotyped, thus re-sequencing of the Avpr1b gene will be required to identify other possible variants and their association to childhood-onset aggression (Zai et al., 2012).

A common phenotype among patients with affective disorders is dysregulation of the HPA-axis (Dempster et al., 2007). In humans, variations in the Avpr1b gene have been found to be associated with mood disorders. van West et al. (2004) found that separate allele distributions along the 12-kb Avpr1b receptor gene are protective against recurrent major depression in a Swedish compared to Belgian adult population diagnosed with unipolar depression. Specifically, Avpr1b-s1, s2, s3, s4, and s5 SNP without a frequent G allele is protective in the Swedish population and Avpr1b-s5 SNP with a frequent G allele is protective in the Belgian population (van West et al., 2004). Consequently, the protective SNPs found in the Van West et al. study were used to investigate their involvement in childhood-onset mood disorders. In a study of Hungarian children diagnosed with

a mood disorder prior to 15 years of age, genetic markers in the Avpr1b gene (rs28373064, rs35369693, and rs33985287) are directly associated with affective status in children. More importantly, this association is sex-specific, with these genetic markers being more common in females compared to males (Dempster et al., 2007). These findings are consistent with a study performed in twins that found that the heritability of depression is greater in females (42%) compared to males (29%) (Kendler et al., 2006).

Given that preclinical work in animal models suggests that antagonism of Avpr1b with SSR149415 can reduce anxiety-like and depressive-like behaviors (Griebel et al., 2002; Overstreet and Griebel, 2005), SSR149415 was approved for clinical trials. Unfortunately, to date, the data from the animal models does not appear to translate to humans. In a Phase II clinical trial in patients with major depressive disorder or generalized anxiety disorder, the effects of treatment with SSR149415 did not differ from the effects of the placebo (Roper et al., 2011; Griebel et al., 2012). Thus, further clinical studies are needed, likely with a different Avpr1b antagonist, to determine if manipulation of Avpr1b signaling may have some therapeutic benefit. Furthermore, the development of such a drug is likely to require genetic testing and biomarker identification to aid in identifying patients that are likely to be responsive to Avpr1b receptor antagonism.

INTEGRATIVE DISCUSSION

Clearly, the Avpr1b has an important, and conserved, function in the modulation of social behaviors as well as the hormonal stress response. Based on some very elegant work in preclinical models, as well as work in humans, it appears that at least one of the roles of the Avpr1b is to aid an animal in determining its social context. Plainly stated, social context is the physical and social setting in which an animal finds itself. Thus, the capacity of an animal to display an appropriate, context-specific, social behavior is often rooted in how that individual interprets their social environment. In the case of Avp signaling via the Avpr1b, the expression of the Avpr1b in the CA2 region of the hippocampus is hypothesized to be important for determining social salience, as its manipulation within this part of the brain impacts the acquisition of memories associated with social context as well as aggressive behaviors (Pagani et al., 2015; Caldwell and Albers, 2016; Smith et al., 2016). With regards to Avpr1b expression in the anterior pituitary, depending on the stressor, genetic disruption of the Avpr1b results in a blunted ACTH release compared to controls, but not always a reduced CORT response (Roper et al., 2011). Likewise, treatment with an Avpr1b antagonist has been found to reduce anxiety-like and depressive-like behaviors in rodents (Serradeil-Le Gal et al., 2005; Stevenson and Caldwell, 2012), and SNPs of the Avpr1b are associated with anxiety and depression in humans (van West et al., 2004; Dempster et al., 2007; Keck et al., 2008; Zai et al., 2012). As dysregulation of the HPA-axis can affect a variety of behaviors, including stress coping, this too shapes how an animal perceives its social environment and alters behavioral responses.

But how do these seemingly separate systems interact? We suggest that their interaction is dynamic and can be reinforcing. Specifically, it seems likely that the stress axis is affecting the interpretation of the social environment, but also that the social environment affects the stress axis. The CA2 region of the hippocampus represents a possible point of intersection of these two systems. It has already been established that the CA2 region is structurally and functionally distinct from other regions of the hippocampus (Lein et al., 2004, 2005). For instance, it is the only part of the hippocampus to receive input from the posterior hypothalamus (Borhegyi and Leranth, 1997; Vertes and McKenna, 2000; Bartesaghi et al., 2006) and the perforant pathway; which connects the entorhinal cortex to the hippocampal formation (Bartesaghi and Gessi, 2004). The entorhinal cortex receives input from the olfactory system, and its input into hippocampus is known to be important to the coding of olfactory-based memories (Petrulis et al., 2005; Sanchez-Andrade et al., 2005). This input to the hippocampus along with the Avp projection from the PVN may be involved in providing information about the social environment. Since the PVN is important for integrating numerous internal and external information and then serving as a control center that effects numerous autonomic functions, this seems plausible. But how would this occur? Perhaps via the glucocorticoid receptors that are expressed in the PVN, which are known to affect the expression of CRH, Avp (Sawchenko, 1987), as well as melanocortin receptors, i.e., MC3R

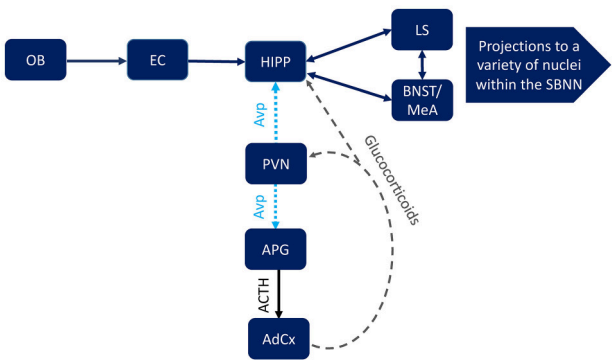

FIGURE 4 | Possible points of intersection between hippocampal (HIPP) Avpr1b, which are localized to pyramidal cells in the CA2 region, and the mammalian stress axis, which includes Avpr1b in the anterior pituitary gland corticotrophs (APG). An animal's social context and subsequent behavioral responses to that context require a complex interaction between the social behavior neural network (SBNN), including the lateral septum (LS), bed nucleus of the stria terminalis (BNST), medial amygdala (MeA), as well as numerous other brain region. Input from the olfactory system, specifically the olfactory bulb (OB) and entorhinal cortex (EC), can be transmitted directly to the hippocampus (HIPP). The paraventricular nucleus (PVN) integrates external and internal information that can be conveyed to the Avpr1b via arginine vasopressin (Avp) projections to the HIPP and the APG (corticotropin releasing hormone release would also be stimulated). The result of the latter projection is activation of the stress axis, including adrenocorticotropic hormone (ACTH) release from the APG and subsequent glucocorticoid release from the adrenal cortex (AdCx). The glucocorticoids in turn act on numerous neural substrates in the brain, including the PVN and HIPP.

(Roselli-Rehfuss et al., 1993). It is through these glucocorticoid receptors that the periphery could provide information about social context to Avp-ergic cells in the PVN, in turn altering Avp neurotransmission to the CA2 region. Likewise, input into the PVN directly affects the corticotrophs, which express the Avpr1b. Once the HPA axis is activated glucocorticoid receptors in the hippocampus (Reul and de Kloet, 1985; Aronsson et al., 1988; Arriza et al., 1988) may affect the input to, or from, the CA2 region (**Figure 4**). The interaction of these systems likely has wide-spread and context specific effects on neural targets, influencing a variety of behaviors, including anxiety-like, depression-like, and aggressive behaviors, which in turn has possible implications for numerous human neuropsychiatric disorders.

Based on what has been presented in this review it seems likely that the CA2 region represents a newly identified node in the SBNN, since this region appears to be a point of convergence for information about social context and perhaps social salience that then helps to influence behavioral output. The possibility that this brain area may represent a critical integrating site for where peripheral signals and the modulation of behavioral output occurs is quite exciting, but requires further study. That said, by improving our understanding of the connectivity of this system we may better understand species similarities and gain insights into, and improve therapeutics for, the numerous neuropsychiatric disorders that are characterized by abnormal sociability.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Vasopressin and Oxytocin Reduce Food Sharing Behavior in Male, but Not Female Marmosets in Family Groups

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Oxytocin (OT) is critical for lactation and maternal care, but OT and the related nonapeptide vasopressin are important for caregiving behaviors in fathers and alloparents as well. This experiment tested the effects of vasopressin and OT on food sharing in marmoset families. We treated caregivers (parents, siblings) with intranasal vasopressin, OT, or saline, and then paired them with the youngest marmoset in the family. Caregivers were given preferred food, and then observed for food sharing and aggressive behavior with young marmosets. OT reduced food sharing from male alloparents to youngest siblings, and fathers that received vasopressin refused to share food with their youngest offspring more often than when treated with OT. Vasopressin increased aggressive vocalizations directed toward potential food recipients in all classes of caregivers. These results indicate that vasopressin and OT do not always enhance prosocial behavior: modulation of food sharing depends on both sex and parental status.

Keywords: food sharing, provisioning, oxytocin, vasopressin, marmoset, sibling, parental care

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INTRODUCTION

In mammals, mothers begin providing nutritional support (i.e., lactation) immediately after the delivery of offspring, and this process is regulated by the nonapeptide hormone oxytocin (OT) and its cognate receptor (1). OT is also an important modulator of other maternal behaviors in addition to lactation, as demonstrated in multiple experimental approaches. OT administered intracerebroventricularly (i.c.v.) induces maternal behavior in estrogen-primed rats (2), and OT receptor (OTR) antagonists administered directly into the ventral tegmental area, or administered directly into the medial preoptic area block the normal expression of postpartum maternal behavior in rats (3, 4), indicating a causal role for OT in the onset of maternal behavior. Arginine vasopressin (AVP), a nonapeptide that is closely related to OT, also modulates maternal behavior. AVP and OT are highly similar nonapeptides, differing at only two amino acid positions, and each can bind and activate the others' receptors [reviewed in Ref. (5, 6)], but often AVP and OT affect different behavioral patterns associated with mother–offspring interactions. Pharmacological manipulations of AVP in the brain indicate that AVP is an important neuromodulator of "active" maternal behavior, including the enhancement of defensive aggression [(3, 4); c.f. (7, 8)]. Data from correlational studies investigating OT or AVP support a role for both nonapeptides in the regulation of maternal care (9–11), though there is some concern over whether peripheral measures of nonapeptides accurately reflect levels

in the central nervous system (12, 13). These experimental and correlational data show that OT and AVP are important neuromodulators of maternal behavior.

There is strong evidence that OT modulates behavior in caregivers other than the mother, including fathers (paternal care), as well as older siblings and unrelated, reproductively inexperienced males and females (alloparental care). OT-like compounds facilitate male parental care in several non-mammalian species [(14, 15); c.f. (16)]. OT induces maternal-like behavior in female sheep exposed to unrelated offspring and enhances pup care in reproductively naïve female rats, animals which would not otherwise provide care spontaneously (2, 17, 18). Moreover, OTR knockdown reduces spontaneous alloparental behavior in female prairie voles (19). Male caregiving behavior is affected by OT as well; i.c.v. OT enhances food provisioning behavior in marmoset monkey fathers (20), and intranasal OT increases responsiveness to infant stimuli in marmoset males (21). In humans, intranasal OT in fathers enhances infant touching and joint father–infant social gaze (22). Correlational data support these pharmacological studies in fathers and alloparents. In general, OT-system activity, measured both peripherally and centrally, increases with caregiving behavior in human fathers (9, 11, 23), non-human alloparents (24, 25), and fathers of biparental non-human species (24, 26–29). Taken together, these data show that OT is important for modulating the behavior of all caregivers.

Arginine vasopressin and its non-mammalian analogs also affect caregiving behavior in fathers and alloparents. In reproductively inexperienced male prairie voles, i.c.v.-administered AVP enhanced, and a V1aR antagonist inhibited, alloparental behavior (30). Similarly, AVP enhanced responsiveness to infant stimuli in female marmosets [including infant-naïve females (21)]. Correlational data also suggest that AVP-system activity enhances parental behavior. Exposure to young enhances AVP-system activity in the brain (27, 31–34), and enhanced paternal behavior is positively associated with AVP-system activity (9, 35). In other species and contexts though, AVP activity inhibits caregiving behavior by non-mothers. AVP administration reduced nest building in biparental old-field mouse fathers, and inhibition of AVP neuron activity enhances nest building in male and female laboratory mice (36). Similarly, V1aR mRNA is downregulated in biparental California mouse fathers, and increased V1aR mRNA expression in California mice is associated with longer latencies to approach pups (28). AVP-mediated inhibition of paternal behavior is present in non-mammals as well; intraperitoneal vasotocin inhibited paternal behavior in poison frogs and clownfish (14, 16). In other contexts, the relationship between AVP and caregiving behavior by non-mothers is less clear. AVP administration did not affect responsiveness to infant stimuli in male marmosets (21), and V1aR antagonist treatment in reproductively inexperienced male prairie voles was only effective at reducing alloparental behavior when it was co-administered with an OTR antagonist (37). Thus, the relationship between AVP and caregiving in non-mothers is less clear than the relationship between OT and caregiving in non-mothers, and it is less clear than the relationship between AVP and caregiving behavior in mothers.

Females are the primary provisioners early in mammalian development (*via* lactation) but in marmosets, mothers, fathers, and alloparents participate in food sharing behavior to infants both during and after weaning. Moreover, the relationship between increased urinary OT and food provisioning in marmosets strengthens during and after weaning (24). To date, only one study has shown that OT manipulation enhances food sharing behavior. Saito and Nakamura (20) treated marmoset fathers with i.c.v. OT and found that OT reduced food sharing refusals to young, a measure of enhanced food provisioning, but not older offspring. OT did not affect active food sharing in fathers, though. We sought to expand Saito and Nakamura's findings by investigating both OT- and AVP-mediated food sharing in all family members. In this experiment, we investigated the influence of AVP and OT on food sharing with juvenile family members by fathers, mothers, and older siblings (alloparents) in marmosets. We treated marmoset mothers, fathers, and alloparents with intransal AVP, OT, or saline control, and then tested their provisioning of rewards in a food sharing paradigm. In this paradigm, caregivers could choose to share or withhold preferred food items with the youngest member of the family. If AVP and OT affect food provisioning *via* general prosocial mechanisms, then we would expect both AVP and OT to increase food provisioning in all caregivers, regardless of sex or parental status (breeder vs. alloparent). Alternatively, if AVP and OT act *via* mechanisms specific to sex or parental status of food provisioners, then we would expect differential rates of food provisioning between AVP- and OT-treated mothers, fathers, and alloparents. Because AVP enhanced responsiveness to infant stimuli in marmoset females and OT enhanced responsiveness to infant stimuli in marmoset males (21), we expected a similar pattern with respect to food provisioning; we expected AVP to enhance food sharing behavior in mothers and female alloparents and OT to enhance food sharing behavior in fathers and male alloparents.

MATERIALS AND METHODS

Subjects

We used 17 marmosets (*Callithrix jacchus*) from three different family groups at the University of Nebraska at Omaha's Callitrichid Research Center as subjects. Twelve served as potential food provisioners (four adult parents and eight older sibling alloparents, ages 1.15–6.7 years) and five were juvenile marmosets (30–60 weeks of age) that served as potential food recipients. Breeding females were contracepted with cloprostenol (38) to prevent the confounding effects of the presence of nursing and dependent infants within family groups. Thus, all potential food recipients were the youngest animals in their family groups. **Table 1** provides demographic and social information on the animals included in the experiment. Marmosets were housed in large family enclosures (1.0 m × 2.5 m × 2 m), and each enclosure had two smaller holding areas (30 cm × 30 cm × 66 cm each) in which all food sharing trials occurred. Marmosets were fed a daily diet of commercial marmoset diet (Science Diet), at approximately 0900 h, and fresh fruits, eggs, mealworms, and yogurt, at approximately 1500 h. Further details on colony management

TABLE 1 | Marmoset family demographics and recipient pairings.

Family ID	Parents (ages)	Alloparent siblings (ages)	Recipient juveniles (sex/age)
C1	Mother (5.7 years) Father (6.0 years)		Juvenile 1 (F/0.7 years) Juvenile 1
C2	Mother (6.7 years) Father (6.7 years)	Male 1 (2.1 years) Female 1 (2.1 years) Female 2 (1.2 years) Male 2 (1.2 years)	Juvenile 1 (F/0.7 years) Juvenile 2 (F/0.7 years) Juvenile 1 Juvenile 2
C3	Mother[a] (5.7 years) Father[a] (3.4 years)	Female 1 (2.0 years) Female 2 (2.0 years) Male 1 (1.6 years) Male 2 (1.6 years)	Juvenile 1 (M/1.1 years) Juvenile 2 (M/1.1 years) Juvenile 1 Juvenile 2

[a]*Indicates these animals were removed from the study because they refused experimenter-provided food.*

and husbandry can be found in Ref. (39). All procedures were approved by the University of Nebraska at Omaha/University of Nebraska Medical Center IACUC (#15-005-04-FC).

Identification of Preferred Food Items

We wanted to identify foods that were preferred enough by marmosets to elicit consistent food begging by juveniles, but not so highly preferred that provisioners would refuse to share them. We surveyed our colony (four males, six females from **Table 1**, plus an additional male and an additional female) to identify preferred food items using a two-choice food preference test (40). The food items tested were Science marmoset diet, breakfast cereal (Honey Nut Cheerios©), apple, and marshmallows. Adult and subadult marmosets were presented with two food items on a tray, separated by 2.5 cm, and we recorded which food item was selected first among each food pair. All possible combinations of food item pairs were presented to each marmoset at least four times, with order of testing food pairs randomized and position of food items on the tray alternated between trials.

Food Sharing Test

At the beginning of each session, the marmoset serving as food provisioner was briefly manually restrained and treated intranasally with either the variant of OT native to marmosets (Pro^8-OT; approximately 150 μg/kg), vasopressin (approximately 133 μg/kg; ~80 IU), or a saline control. Intranasal treatments were applied dropwise in a volume of 50 μL per nostril. These doses have been shown to alter social behavior in marmosets and Titi monkeys (21, 41–43). Each provisioner was exposed to all three treatments in a counterbalanced order, with at least 48 h between treatments. Salivary OT in humans returns to near baseline levels in less than 7 h after intranasal administration (44). The marmoset was returned to the home cage, and a period of 20 min was given to allow uptake of the treatment (45, 46). After 20 min, the provisioner and recipient were moved to a holding area within the home cage, eliminating the potential for other family members to interfere with potential food provisioning. The provisioner and the recipient were briefly separated with a slotted barrier, and the provisioner was offered a piece of food in a dish. As soon as the provisioner obtained the food item, we removed the barrier, and interactions between the caregiver and recipient were recorded by a single observer who was blind to experimental treatment condition for the provisioner.

Specific behaviors of interest were begging, food sharing, food sharing refusals, and vocalizations. *Begging (count)* was recorded when the recipient marmoset made contact with the provisioner when attempting to take the apple or cereal. *Food sharing (count, latency)* was recorded when the provisioner transferred or allowed recipient to take part or all of the food provided. *Food sharing refusals (count)* were recorded when a beg occurred, but sharing did not. *Begging cries (count)* from the recipient and *aggressive "Ehr-Ehr" vocalizations (count)* by the provisioner were also recorded for each trial. To account for trial-by-trial differences in recipient behavior, we recorded if the recipient *did not see (yes/no)* food before it was eaten, recipient appeared to see food, but had *no interest (yes/no)*, and recipient *watched (yes/no)* caregiver eat food, but did not attempt to take food.

Each session of testing consisted of 20 1-min trials, and apple and cereal were alternated in successive trials. If the provisioner dropped the piece of food before the barrier between the provisioner and recipient was removed, an additional food item was given to the provisioner. Each provisioner:recipient pair was tested under all three experimental conditions (OT, AVP, saline).

Data Analysis

We used a trial-by-trial analysis to evaluate effects of treatment, sex, and caregiver parental status within the family (parent vs. alloparent). We used a Linear Mixed Model analysis, and nested food sharing trials within testing sessions, sessions within individual marmosets, and marmosets within families. This strategy allowed us to control for trial-by-trial differences in recipient and provisioner hunger status, motivation, or attention, as well as experiment-wide differences in recipient age and family size. Moreover, we were able to appropriately treat families, individuals, and testing sessions as non-independent entities. Our final model is described in Eq. 1. Significant main effects and interactions were explored using Fisher's *post hoc* tests, using a Satterthwaite approximation for degrees of freedom.

$$\begin{aligned}\text{Behaviour} = {} & \textbf{Caregiver Sex} \times \textbf{Treatment} \times \textbf{Parental Status} \times \textbf{Food Type} \\ & + \text{Family Size} + \text{Recipient Age} + \text{Session Number} \\ & + \text{Trial Number} + \text{Recipient Interest}^{a} \\ & + \text{error}(\text{FamilyID}) + \text{error}(\text{MonkeyID}) \\ & + \text{error}(\text{SessionID}) + \text{error}(\text{residual}) \end{aligned} \quad (1)$$

Equation 1. Template model for analysis of behavioral data. Bolded variables indicate primary tests of hypotheses. [a]*Recipient Interest* was composed of three separate variables and corresponding regression coefficients: recipient did not see food before it was eaten, recipient appeared to see food, but had no interest, and recipient watched caregiver eat food, but did not attempt to take food.

RESULTS

Food Preference

Adult marmosets showed a clear hierarchical preference profile for the four food items we tested. Standard diet was never preferred

over other foods, and marshmallows were always preferred over other foods. However, there was no overall preference for apples vs. cereal (**Table 2**, bolded), thus apples and cereal were intermediate in preference compared to diet and marshmallow. In order to maximize food begging while optimizing rates of food sharing (i.e., prevent floor or ceiling effects due to food preference), we chose apples and cereal as our food items in our food sharing test.

Food Sharing Test

Food sharing was associated with the parental status of the provisioner, and it was affected by the interaction between parental status and nonapeptide treatment. Mothers shared more often than fathers, but otherwise there were no differences in rates of food sharing among parents or alloparents [**Figure 1**; $F_{(1,\ 13.65)} = 6.23$, $p = 0.026$]. Mothers also had shorter latencies to share food than fathers and female alloparents [**Figure 2**; $F_{(1,\ 13.7)} = 7.28$, $p = 0.018$]. Male alloparents were the only family members whose rates of food sharing were altered by nonapeptide treatment. In male alloparents, Pro^8-OT reduced food sharing compared to AVP and saline [$F_{(2,\ 26.3)} = 3.45$, $p = 0.047$], but neither Pro^8-OT nor AVP changed rates of food sharing in mothers, fathers, or female alloparents nor did it affect latencies to share. Provisioners shared marginally, but not significantly, more often [$F_{(1,\ 11.29)} = 3.78$, $p = 0.07$; Table S1 in Supplementary Material] and faster to younger recipients than to older recipients [$F_{(1,\ 11.31)} = 4.31$, $p = 0.06$, Table S2 in Supplementary Material].

Food sharing refusals were also associated with the parental status of the provisioner with the family, and food sharing was also affected by the interaction between parental status and nonapeptide treatment (**Figure 3**). Just as mothers shared more often than other caregivers, mothers also refused to share less often than any other caregivers [**Figure 3**, brackets; $F_{(1,\ 42.4)} = 14.38$, $p < 0.001$]. Fathers were the only family members whose rates

TABLE 2 | Choice matrix for all food items paired with all other food items.

	Chosen food (%)			
Paired food	**Diet**	**Apple**	**Cereal**	**Marshmallow**
Diet	–	100[a]	97.9[a]	100[a]
Apple		–	**62.5**	70.8[a]
Cereal			–	70.8[a]
Marshmallow				–

Bold values indicate no significant preference for chosen food over paired food.
[a]Indicate percentage for chosen food was significantly different from 50% [t(11) > 2.41, p < 0.05].

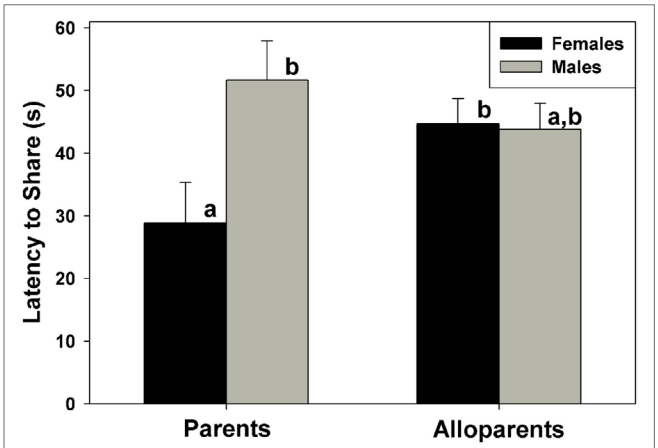

FIGURE 2 | Latency for caregivers (parents, alloparents) to share food to the youngest family members. Mothers shared significantly faster than fathers and female alloparents. Bars with differing letters indicate significant differences between social roles using a Fisher's *post hoc* test.

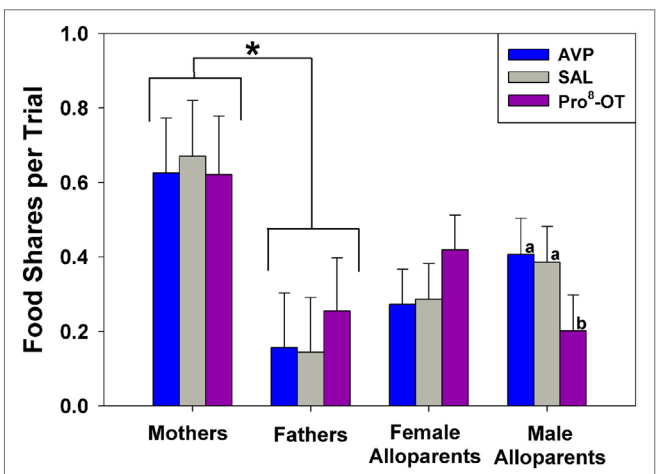

FIGURE 1 | Food sharing from caregivers (parents, alloparents) to the youngest family members. Mothers shared significantly more than fathers did. Male alloparents treated with Pro^8-oxytocin (OT) shared less than when they were treated with saline or arginine vasopressin (AVP). Asterisks indicate significant differences between social roles using a Fisher's *post hoc* test ($p < 0.05$). Bars with differing letters indicate significant differences across treatments within individuals using a Fisher's *post hoc* test.

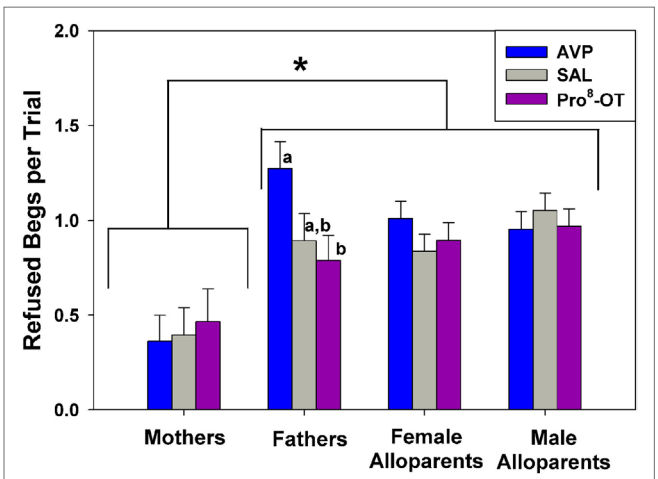

FIGURE 3 | Caregiver (parents, alloparents) food share refusals after a recipient food beg. Mothers refused to share food less often than any other caregiver group. Fathers treated with arginine vasopressin (AVP) refused more when treated with AVP compared to Pro^8-oxytocin (OT). Asterisks indicate significant differences between social roles using a Fisher's *post hoc* test ($p < 0.05$). Bars with differing letters indicate significant differences across treatments within individuals using a Fisher's *post hoc* test.

of food sharing refusal were affected by nonapeptide treatment. Fathers treated with AVP had higher rates of food sharing refusals than when treated with Pro^8-OT [**Figure 3**, letters; $F_{(2, 39.9)} = 3.24$, $p = 0.050$]. Recipient age did not affect food sharing refusals [$F_{(1, 33.04)} = 2.64$, $p = 0.11$].

Aggressive vocalizations (Ehr-Ehr) emitted by the provisioner during the food sharing test were associated with the parental status of the provisioner, as well as nonapeptide treatment. Alloparents emitted more aggressive vocalizations than parents did [$F_{(1, 694)} = 13.52$, $p < 0.001$; alloparents, M (± SEM) = 0.3 (0.03) vocalizations per trial; parents, M (± SEM) = 0.069 (0.05) vocalizations per trial]. Additionally, AVP increased aggressive vocalizations in provisioners compared to both Pro^8-OT and saline in both parents and alloparents [**Figure 4**; $F_{(2, 694)} = 4.49$, $p = 0.012$]. There were no sex differences in provisioner aggressive vocalizations [$F_{(1, 694)} = 0.02$, $p = 0.877$], and sex did not interact with nonapeptide treatment or parental status [F's < 0.31, p's > 0.640]. Provisioners emitted marginally, but not significantly, more aggressive vocalizations toward younger recipients than they did toward older recipients [$F_{(1, 694)} = 2.99$, $p = 0.08$, Table S4 in Supplementary Material].

Finally, we evaluated whether nonapeptide treatment of provisioners altered rates of begging cries emitted by recipients. Nonapeptide treatment of the provisioner did not affect recipient begging cries, nor did parental status of the provisioner or the interaction (F's < 2.24, $p > 0.05$). Begging cries did, however, vary by the recipient's age; older recipients exhibited fewer begging cries than younger recipients [$b = -0.0034$, $F_{(1, 11.2)} = 5.38$, $p = 0.040$].

DISCUSSION

In marmosets, all family members perform post-weaning caregiving behavior in the form of food sharing, and we showed that nonapeptide treatment altered food sharing behavior in some, but not all, caregivers. Overall, mothers consistently provisioned food to recipients more frequently than fathers or alloparents did, and food provisioning behavior by mothers and female alloparents was not altered by manipulations of AVP or OT. The food provisioning behavior of male alloparents and fathers, however, was altered by AVP and OT treatment. Contrary to our hypothesis, AVP decreased provisioning behavior in fathers, and OT decreased provisioning behavior in male alloparents.

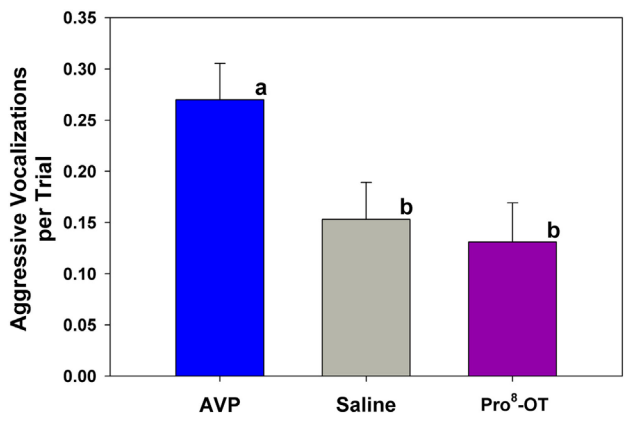

FIGURE 4 | Aggressive vocalization emitted by caregivers (parents, alloparents). Treatment with arginine vasopressin (AVP) increased aggressive vocalizations compared to saline, Pro^8-oxytocin (OT). Bars with differing letters indicate significant differences across treatments using a Fisher's *post hoc* test.

Previous studies in our lab indicated that AVP and OT enhance parental behavior and food sharing in marmosets. Food sharing among adults toward the pair mate was reduced following OTR *antagonist* treatment, suggesting that OT is important for prosocial food sharing behavior within the family (47). In a simulated infant distress paradigm, AVP enhanced responsiveness to infant stimuli in females, and OT enhanced responsiveness to infant stimuli in males (21), so we had expected to observe the same pattern with regard to food provisioning to juveniles; we expected AVP to enhance caregiving behavior in females, and OT to enhance caregiving behavior in males. However, in the context of maintaining monogamous pair bonds, previous work in our lab has provided evidence that OT may not always enhance prosocial behavior. OT did not enhance behavior directed toward the pair mate, but rather it reduced prosocial food sharing and sociosexual behavior directed toward opposite-sex strangers, thereby enhancing fidelity to the established pair mate (41, 43). Thus, AVP and OT may not enhance prosocial behavior generally, instead they may alter social decision-making based on context and social relationships.

We designed this experiment to expand upon the work done by Saito and Nakamura (20), who demonstrated that OT enhances food sharing in fathers toward younger (7–16 weeks), but not older (24–31 weeks) offspring. We expanded on the age range, and showed that OT does not alter paternal food sharing behavior toward older offspring (36–57 weeks). We used a different dose of OT and method of administration that Saito and Nakamura (20), and found no effect of OT on food sharing behavior toward older offspring in fathers. Escalating doses of OT and AVP produce differential behavioral effects in other species [e.g., Ref. (42, 48–50)] and it is likely that the same is true in marmosets. We used a single dose of each nonapeptide that affects adult pair-bonding behavior (41–43), but it may be the case that varying doses may have had differential behavioral effects in this context. With regard to fathers though, OT did not affect food sharing behavior at our intranasal dose or the i.c.v. dose used by Saito and Nakamura (20). An important distinction between our study and Saito and Nakamura's (20) is that the OT ligand used differed: Saito and Nakamura used the conserved mammalian variant of OT (Leu^8-OT), while we used the variant native to marmosets, Pro^8-OT. Pro^8-OT and Leu^8-OT differentially affect marmoset social behavior in some contexts of adult pair bonds (41, 43, 51, 52). We also treated marmosets with AVP in our food sharing task, and compared to treatment with Pro^8-OT. Like Saito and Nakamura, we found that OT at these doses did not affect paternal food sharing toward older offspring, and that AVP at this dose inhibited paternal food sharing behavior. We also included mothers and alloparents, and found that OT inhibited food sharing behavior in male alloparents. Our findings, combined with those of Saito and Nakamura, demonstrate that behavioral modification *via* AVP and OT is flexible; AVP- and OT-mediated

food sharing behavior depends on multiple factors, including offspring age, caregiver sex, and parental status.

Food sharing behavior in primates is the product of multiple demographic and contextual variables. The relationship between OT and caregiving behavior in marmosets change with offspring age (20, 24), suggesting that OT modulates caregiving behavior dynamically with changing offspring and caregiver needs. Moreover, in large marmoset families, offspring age, caregiver experience, sex, and parental status interact to produce differential food provisioning behavior. Tolerance for food begging in adult marmosets wanes as offspring mature (53), reflecting reduced responsivity to signals for continued care from older offspring. Food sharing behavior in alloparents is also modulated by multiple variables, including sex and experience. Previous experience in rearing infants is associated with improved food sharing in male, but not female alloparents during undisturbed conditions (54). In an experimental task in which a response provided food to a younger family member, mothers, fathers, and male alloparents all selectively provided food to younger family members, but female alloparents exhibited lower scores on this measure (55). There is some evidence supporting the role of OT in altering social decision-making depending on social context, rather than enhancing global prosociality. In macaques, OT increases the willingness of male macaques to reward another macaque, but only when the alternative is to reward no one. However, when choosing to reward the self or another, OT increased selfish choices (56). In pair-bonded adult marmosets, OT does not increase food sharing with the pair mate, it instead decreases food sharing with an opposite-sex stranger (43). OT also reduces food sharing in group-housed adult capuchin monkeys, and it was suggested that this was mediated by OT-induced increases in social distance (57). It is likely that interactions between older and younger siblings, neither of which are wholly dependent on caregivers, will yield some selfish decision-making that is altered by hormonal neuromodulators like OT and AVP. Our findings speak to the broader issue of whether OT and AVP enhance prosocial behavior generally, or whether they alter social behavior depending on social context. We found that OT and AVP inhibited food sharing behavior, suggesting that OT and AVP alter social behavior depending on characteristics of the caregiver, rather than global enhancement of prosociality.

Arginine vasopressin is known to affect a wide range of aggressive behaviors, including maternal aggression [(3, 4, 10); c.f. (7, 8)], as well as territorial aggression [reviewed in Ref. (58)]. In general, the association between AVP and defense of offspring is limited to females (reviewed in Section "Introduction"), though not always (31), while AVP-mediated modulation of territorial aggression is often limited to males [reviewed in Ref. (58)]. We found that AVP increased aggressive vocalizations during food sharing trials, in males *and* females, as well as in parents and alloparents. There are two explanations for our lack of a sex effect. First, food aggression, maternal aggression, and territorial aggression may be controlled by different endocrine mechanisms, including AVP and OT. There is some evidence for this, as AVP V1b receptor knockout mice display impaired maternal and territorial aggression, but predatory aggression remains intact, suggesting that food aggression is different from defending offspring or territory (59, 60). However, while V1b knockout mice do compete for food, they do not compete as aggressively as wild types (59), weakening this argument. An alternative explanation for our lack of a sex effect in AVP-mediated aggression is that AVP and OT may affect aggressive behavior differently in primates than it does in rodents. There is some evidence for this, V1b receptor genetic polymorphisms human children are associated with aggression in both boys and girls, though they are more robust in boys than in girls (61, 62). Our findings highlight the need for more continued study of AVP, OT, and aggression in non-human primate models.

Oxytocin and AVP are involved in the modulation of dyadic interactions that are dependent on the behavior of both individuals. In humans, intranasal OT treatment in fathers enhances social reciprocity between father and infant, it also causes an increase in infant salivary OT and duration of social gaze (22). Similarly, high paternal plasma and salivary OT in human mothers and fathers is associated with father–infant coordination of affect (23, 63). Both AVP and OT are associated with dyadic interactions involving responding to infant gaze (9). This work in humans suggests that OT and AVP in the caregiver can affect behavior in the recipient. Previous work in our lab has shown that the behavior of an untreated marmoset is altered by OT treatment of the pair mate, suggesting that nonapeptides might alter the social attractiveness of a social partner (52). There is an important dyadic component to our measure of food sharing refusals. AVP-mediated increases in refusals may be the result of stable rates of begging and increased rates of refusal, or it may be the result of both increased rates of begging *and* increased refusal. However, begging cries emitted by the recipient were unaffected by nonapeptide treatment, suggesting that the behavior of recipients did not change in response to altered stimulus properties or any unobserved behavior of the caregiver.

There is considerable overlap between the OT and AVP systems in terms of neuroanatomical distributions [Reviewed in Ref. (64)] and receptor affinity [Reviewed in Ref. (6)], and there are also often important sex and species differences in the effects of OT and AVP on behavior. Given the considerable variation in NWM species OTRs and V1aRs, interactions between Pro^{8}-OT and V1aR (or AVP and marmoset OTR) may be either reduced (i.e., greater receptor selectivity) or enhanced (i.e., greater receptor promiscuity) compared to humans, mice, and rats. Currently, the binding affinities and signaling potencies/efficacies of these ligand–receptor complexes is unknown. When AVP and OT are studied together, they provide valuable insights on these closely related systems, such as showing that OT and AVP act *via* one another's receptors, and that they affect behavior synergistically. For example, both AVP and OT induce territorial marking in Syrian hamsters, but OT-induced marking is blocked by AVP receptor antagonists, not OTR antagonists (65). Similarly, blocking both OTRs and V1aRs reduced alloparental behavior in male voles, but blocking only one of these receptor types did not, indicating that AVP and OT work in concert to modulate male vole parental behavior (37). We found that AVP increased food sharing refusals in fathers, but not in male alloparents. Instead, for male alloparents, OT reduced total food sharing. These examples show that more information and nuance are gained from studying AVP and OT together than the sum of what is gained from studying each individually. These studies highlight

the importance of comparing OT and AVP, especially in species with complex behavior and interindividual relationships.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the University of Nebraska Medical Center/University of Nebraska at Omaha Institutional Animal Care and Use Committee. The protocol was approved by the University of Nebraska Medical Center/University of Nebraska at Omaha Institutional Animal Care and Use Committee (protocol #15-005-04-FC).

AUTHOR CONTRIBUTIONS

JT, AI, and JF planned the experiment. JT and AI carried out the experiment under the supervision of JF. JT performed statistics, and JT, AI, and JF each contributed to writing and editing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fendo.2017.00181/full#supplementary-material.

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REVIEW
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Arginine Vasotocin, the Social Neuropeptide of Amphibians and Reptiles

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Arginine vasotocin (AVT) is the non-mammalian homolog of arginine vasopressin (AVP) and, like vasopressin, serves as an important modulator of social behavior in addition to its peripheral functions related to osmoregulation, reproductive physiology, and stress hormone release. In amphibians and reptiles, the neuroanatomical organization of brain AVT cells and fibers broadly resembles that seen in mammals and other taxa. Both parvocellular and magnocellular AVT-containing neurons are present in multiple populations located mainly in the basal forebrain from the accumbens–amygdala area to the preoptic area and hypothalamus, from which originate widespread fiber connections spanning the brain with a particularly heavy innervation of areas associated with social behavior and decision-making. As for mammalian AVP, AVT is present in greater amounts in males in many brain areas, and its presence varies seasonally, with hormonal state, and in males with differing social status. AVT's social influence is also conserved across herpetological taxa, with significant effects on social signaling and aggression, and, based on the very small number of studies investigating more complex social behaviors in amphibians and reptiles, AVT may also modulate parental care and social bonding when it is present in these vertebrates. Within this conserved pattern, however, both AVT anatomy and social behavior effects vary significantly across species. Accounting for this diversity represents a challenge to understanding the mechanisms by which AVT exerts its behavioral effects, as well are a potential tool for discerning the structure-function relationships underlying AVT's many effects on behavior.

Keywords: vasotocin, urodeles, anurans, amphibians, reptiles, communication, aggression

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INTRODUCTION

Mammalian vasopressin is one of a small group of nine-amino acid peptides whose wide and consistent distribution across animals indicates a long phylogenetic history (1). Both the peptides and their receptors are highly conserved in their structure and expression in neural and non-neural tissue (2, 3). Whereas invertebrates have a single nonapeptide differing slightly among taxonomic

Abbreviations: III, third ventricle; AH, anterior hypothalamus; BNST, bed nucleus of the stria terminalis; Dp, dorsal pallium; Lp, lateral pallium; Ls, lateral septum; MA, medial amygdala; Mp, medial pallium; Ms, medial septum; NAcc, nucleus accumbens; PAG, periaquaductal gray; POA, preoptic area; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; Str, striatum; Tel, telencephalon; Th, thalamus; VL, ventrolateral hypothalamus; VM, ventromedial hypothalamus.

groups, vertebrates have two such peptides. In mammals, these are vasopressin and oxytocin, and other vertebrates have two homologous peptides with similar structures. Amphibians and reptiles, like other non-mammalian vertebrates, express arginine vasotocin (AVT) rather than the arginine vasopressin (AVP) found in mammals. AVT differs from AVP by a single amino acid (**Figure 1**).

Despite vast differences in overall nervous system structure and in species-specific behavioral repertoires and physiology, vasopressin, and its peptide homologs demonstrate a remarkable conservation in their function. In all cases, peripheral physiological effects target osmoregulation and smooth muscle contraction, particularly in reproductive organs. In the nervous system, they have profound impacts on social behavior. Most importantly for behavioral neuroendocrinology, the AVT/AVP nonapeptide and its invertebrate homologs invariably modulate social communication, reproduction, and aggression. Amphibians and reptiles share this common feature with all other organisms along with a similar neuroanatomical distribution of AVT cells and fibers.

ANATOMY OF THE AVT SYSTEM IN AMPHIBIANS AND REPTILES

The locations of AVT/AVP cells have been described in all groups of vertebrates, including cartilaginous and bony fish, amphibians, reptiles, birds, and mammals. Although there are variations in the extent and position of neuronal populations expressing this peptide, the general anatomical organization of AVT/AVP populations and fibers is conserved across vertebrates (4, 5). Several magnocellular and parvocellular cell groups are located in the basal forebrain extending from the amygdala/nucleus accumbens (NAcc) through the preoptic area (POA) to the hypothalamus. These give rise to a neurosecretory pathway to the posterior pituitary as well as an extensive AVT fiber system throughout the central nervous system with particularly heavy innervation of the limbic system. Anatomical abbreviations used in text, tables and figures are found in the abbreviation section; **Table 1** summarizes the locations of AVT cells across amphibian and reptilian species.

AVT in Urodeles (Salamanders and Newts)

Arginine vasotocin has a widespread distribution from forebrain to hindbrain in salamanders and newts [for reviews see Ref. (30, 31)]. We employ the nomenclature of Gonzalez and Smeets in this article. In all three urodele species (*Taricha granulosa*, *Pleurodeles waltl*, and *Plethodon shermani*) for which brain AVT distribution has been studied to date (7, 8, 12) AVT cells occur in the bed nucleus of the stria terminalis (BNST) and POA. In addition, in *T. granulosa* and *P. shermanii* AVT cell populations have been identified in pallium [dorsal pallium and medial pallium (Mp)], and subpallial limbic areas [medial septum (Ms) and medial amygdala (MA)], the suprachiasmatic nucleus (SCN), ventral thalamus, nucleus isthmi, optic tectum, and torus semicircularis (inferior colliculus). In addition, in *P. waltl* and *T. granulosa*, AVT neurons have been identified in the ventromedial hypothalamus (7, 12). A feature common to several of these nuclei is their involvement in the social decision-making network, a group of brain nuclei that modulate behaviors related to socially salient stimuli (32).

Arginine vasotocin cells from these nuclei send projections to many different brain regions forming an extensive network of fibers. In *T. granulosa* and *P. waltl* (7, 12) fibers are present from the olfactory bulb to the cervical segments of the spinal cord. AVT fiber density is not homogeneous throughout the brain. Denser fiber aggregations of variable thickness are found mainly in the vicinity of the lateral pallium, Mp, and POA.

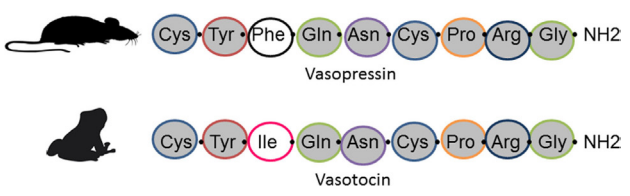

FIGURE 1 | Amino acid sequence in vasopressin (top) found in mammals and arginine vasotocin (bottom) found in amphibians, reptiles, and other non-mammalian vertebrates.

AVT in Anurans (Frogs and Toads)

A larger number of studies have investigated the presence of AVT cells in the brain of anurans as compared to urodeles and includes descriptions in more species from different families (6, 9–14, 16, 17). Comprehensive whole-brain AVT immunocytochemical studies have been conducted in five species: *Xenopus laevis*, *Rana catesbeiana* (=*Lithobates catesbeianus*), *Rana ridibunda* (=*Pelophylax ridibundus*), *Acris crepitans*, and *Hyla cinera*. In all five species, AVT cells are located in the MA, POA, SCN, and VM. In four of the species listed above AVT cells also occur in the striatum and NAcc, but this was not reported in *X. laevis* (6). **Figure 2** shows AVT cells in the MA and POA of the South American frog *Pleurodema thaul*. For simplicity, we refer to the entire region as the POA, as finer distinctions are often not made in amphibian neuroanatomy papers. However, when subregions are identified, the amphibian POA is usually divided along the rostral–caudal axis into anterior, magnocellular, and posterior areas. Most magnocellular AVT-containing neurons with projections apparently going to the median eminence are located in the magnocellar division of the POA where they are interspersed with AVT-positive parvocellular neurons. Some magncellular neurons are also located in other parts of the POA. We assume that the magnocellular POA is equivalent at least in part to the PVN of other tetrapods, but published reports have generally not identified it as such. AVT cells are also present in midbrain and hindbrain nuclei of some of these species (**Table 1**); however, AVT cells at these levels are not as abundant as in forebrain regions (6, 12, 13). In *L. catesbeianus*, AVT cells are also found in the Ms; this frog species has the largest number of AVT nuclei identified to date (13). A thorough comparative analysis of anuran AVT cell populations that takes into account connections, cell morphology, and molecular markers is needed to clarify AVT cell group homologies in the amphibian POA and

TABLE 1 | Brain regions containing arginine vasotocin (AVT) cells in different amphibian and reptile species.

Group	Species	Family	Dp	Mp	Lp	Ms	Ls	NAcc	Str	Ma	BNST	POA/ PVN-SON	SCN	AH	VM/VL	Midbrain	Hindbrain	Reference
Amphibians																		
Urodela (newts and salamanders)	*Pleurodeles waltl*	Salamandridae									x	x					x (1)	González and Smeets (6)
	Taricha granulosa	Salamandridae	x	x		x				x	x	x			x	x (2) (3)	x (1) (4)	Lowry et al. (7)
	Plethodon shermani	Plethodontidae	x	x	x	x				x	x	x				x (5)	x (1) (6)	Hollis et al. (8)
	T. granulosa	Salamandridae	x	x		x	x			x	x	x			x	x (5) (2)	x (1) (6)	Hollis et al. (8)
Anura (toads ad frogs)	*Rana temporaria*	Ranidae										x						Vandesande and Dierickx (9)
	Rana esculenta (=*Pelophylax bedriagae*)	Ranidae										x						Vandesande and Dierickx (9)
	Bubo bufo	Bufonidae										x						Vandesande and Dierickx (9)
	Bubo japonicus	Bufonidae										x						Jokura and Urano (10, 11)
	Xenopus laevis	Pipidae								x	x	x	x		x	x (7)		González and Smeets (6, 12)
	Rana ridibunda (=*Pelophylax ridibundus*)	Ranidae						x	x	x	x	x	x		x	x (3)	x (8)	González and Smeets (6)
	Rana catesbeiana (=*Lithobates catesbeianus*)	Ranidae				x		x	x	x	x	x	x				x (9)	Boyd et al. (13)
	Acris crepitans	Hylidae						x	x	x		x	x					Marler et al. (14)
	Hyla cinerea	Hylidae						x		x		x						O'Bryant and Wilczynski (15)
	H. cinerea	Hylidae						x	x	x		x	x		x			Lutterschmidt and Wilczynski (16)
	H. cinerea	Hylidae						x	x	x		x	x		x			Howard and Lutterschmidt (17)
Reptiles																		
Squamata (lizards)	*Lacerta muralis*	Lacertidae										x						Bons (18)
	Acanthodactylus paradis	Lacertidae										x						Bons (18)
	Acanthodactylus boskianus	Lacertidae										x						Bons (18)
	Tarentola mauritanica	Gekkonidae										x						Bons (18)
	Gekko gecko	Gekkonidae									x	x					x(10)	Stoll and Voorn (19)
	G. gecko	Gekkonidae									x	x					x(10)	Thepen et al. (20)
	Anolis carolinensis	Dactyloidae				x						x		x	x	x (3)		Propper et al. (21, 22)
	A. carolinensis	Dactyloidae										x		x				Hattori and Wilczynski (23)
	Anolis sagrei	Dactyloidae									x	x						Kabelik et al. (24)
	Cnemidophorus uniparens (=*Aspidoscelis uniparens*)	Teiidae									x	x		x				Hillsman et al. (25)
	Urosaurus ornatus	Phrynosomatidae										x	x					Kabelik et al. (26)
Squamata (snakes)	*Natrix maura*	Natricidae										x						Fernández-Llebrez et al. (27)
	Python regius	Pythonidae									x	x						Smeets et al. (28)
	Bothrops jararaca	Viperidae										x						Silveira et al. (29)
Testudines (turtles)	*Pseudemys scripta* (=*Trachemys scripta*)	Emydidae									x	x						Smeets et al. (28)
	Mauremys caspica	Geoemydidae										x						Fernández-Llebrez et al. (27)

The presence of AVT cells is indicated by x under the corresponding nuclei, which are listed in rostrocaudal order. POA includes the entire preoptic area for amphibians. We combined POA with the more caudal nuclei the PVN and SON in a single column, as reports in amphibians do not generally distinguish separate POA/PVN/SON areas; and whereas recent work on lizards indicate AVT-immunoreactive cells to be in distinctly separate PVN and SON nuclei, earlier studies in reptiles do not always clearly make that distinction. In the midbrain and hindbrain columns, the numbers in parentheses indicate specific regions in which AVT cells are found: (1) nucleus isthmi, (2) torus semicircularis (inferior colliculus), (3) interpeduncularis nucleus, (4) lateral auricle area, (5) optic tectum, (6) eminentia trigemini, (7) an area dorsolateral to oculomotor nucleus, (8) nucleus of the solitary tract, (9) pretrigeminal nucleus, (10) nucleus reticularis. We emphasize that the lack of reported AVT cells and fibers should be considered very cautiously, as it can be due to any number of technical differences across species and labs and could reflect the variable nature of AVT densities related to particular social or seasonal conditions (see Anatomical Variation by Group, Sex, and Season) rather than a strict species trait, or simply that the particular published study did not investigate that area. See abbreviation section for abbreviations used.

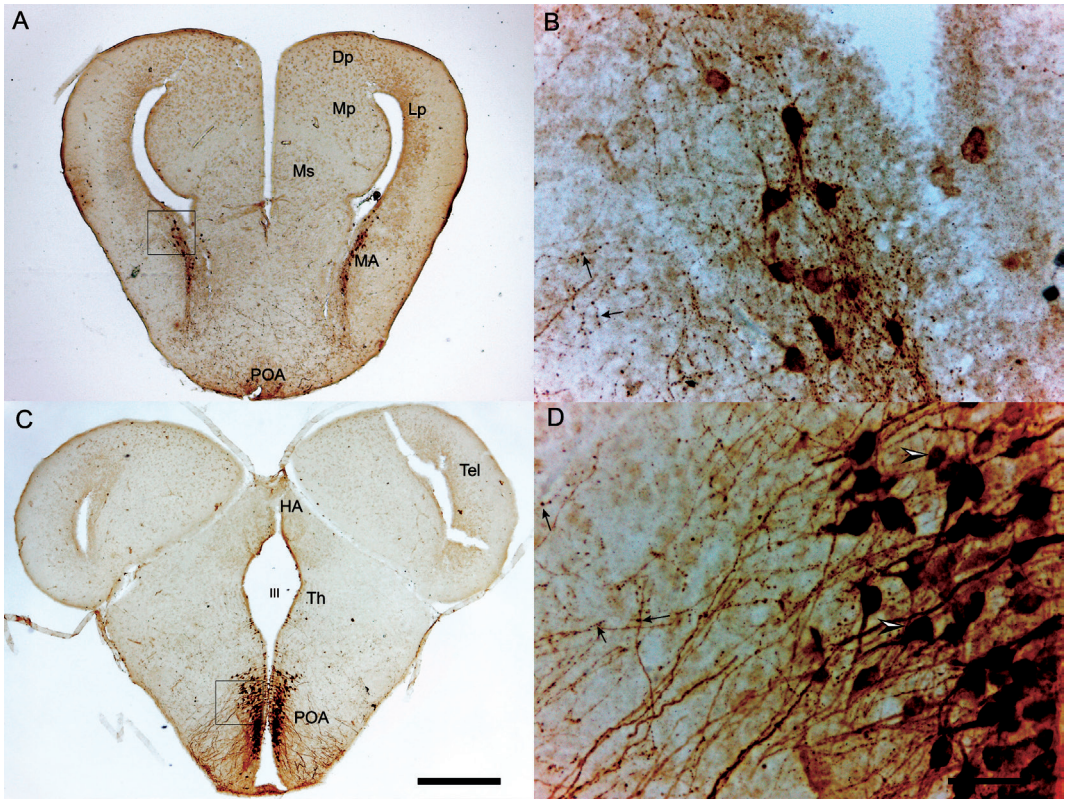

FIGURE 2 | Transverse sections through the brain of a *Pleurodema thaul* male showing arginine vasotocin (AVT)-immunoreactive cells at the level of amygdala **(A,B)** and preoptic area (POA) **(C,D)**. **(A)** AVT cells in the medial amygdala (MA). AVT cells in the inset are magnified in **(B)**; arrows indicate axonal varicosities characteristic of AVT neurons in many vertebrates. **(C)** AVT cells in the caudal POA. AVT cells in the inset are magnified in **(D)**; arrowheads show parvo- (upper) and magnocellular (lower) neurons, which are interspersed in this magnocellular region of the POA. Scale bars on panel **(C)** (0.5 mm) and **(D)** (50 μm) also apply to **(A,B)** respectively. See abbreviation section for abbreviations used.

other areas so that they can be compared with those in other vertebrates.

As in urodeles, AVT fibers occur in regions along the entire anuran brain, from olfactory bulb to spinal cord (6, 12, 13). The POA contains the most dense plexus of AVT fibers, apparently originating in AVT cells located in this same nucleus. Other extra-hypothalamic regions with an ample distribution of AVT fibers are Ms, NAcc, BNST, and MA in the forebrain and also the optic tectum, torus semicircularis, tegmentum, and pretrigeminal nucleus at the midbrain and hindbrain levels. The torus, tegmentum, and pretrigeminal nucleus are regions that participate in reception or production of calls by frogs (33, 34), and therefore, even though these nuclei generally lack AVT cells, the presence of AVT fibers could be relevant for AVT effects on frog vocal behavior (see Effects of AVT on Male Reproductive Behavior).

AVT in Reptiles

The reptile AVT system has been described in lizards (18–26), turtles (27), and snakes (27–29). The supraoptic nucleus (SON) and PVN are the most conspicuous AVT nuclei in reptiles and are considered homologous to the AVT-containing magnocellular POA in amphibians (30). All reptile species investigated to date have AVT cells in these nuclei. It is noteworthy that, unlike in anurans, AVT cells have not been reported in NAcc or MA in reptiles.

In all lizards so far examined, with the exception of *Gekko gecko* (19, 20), numerous AVT cells also occur in the POA rostral to the area identified as equivalent to the mammalian SON plus PVN (18, 21, 23–26). This area is sometimes termed the anterior hypothalamus (21, 23, 25). Smaller groups of AVT cells have been reported in the BNST (19, 20, 24, 25). AVT cells have also been reported in the hindbrain in *G. gecko* (19, 20), and another unique case is the occurrence of AVT cells in the Ms and interpeduncularis nucleus in *Anolis carolinensis* (22).

In comparison to lizards, turtles and snakes have fewer AVT-containing nuclei, and only the SON and PVN have been reported to have the neuropeptide in the turtle *Mauremys caspica* and in the snakes *Natrix maura* and *Bothrops jararaca* (27, 29). The BNST also contains AVT cells in the turtle *Pseudemys scripta elegans* and the snake *Python regius* (28).

The distribution of AVT fibers in the brains of different reptiles is comparable to amphibians in its extent. Detailed studies in lizards (19, 20, 22, 26) as well as in turtles and snakes (27–29) report AVT fibers extending from forebrain to spinal cord, passing through olfactory bulb, lateral septum (Ls), Dc, NAcc, MA, periaquaductal gray (PAG), and locus coeruleus.

Anatomical Variation by Group, Sex, and Season

Although AVT cells have been reported to occur in multiple nuclei within the urodele, anuran, and reptile central nervous systems, the reported areas do vary. For example, in frogs, but not urodeles or reptiles, AVT cells are present in the NAcc, and AVT cells are present in the cortex of reptiles and homologous pallium in urodeles, but not in anurans. Because the reports span many years and many different labs, often using slightly different nomenclature, it is difficult to reconcile these reports, and as always the reported absence in an area must be considered with caution. Despite these difficulties, important common patterns are apparent across amphibians and reptiles, and between these herpetological groups and mammals. Both magnocellular and parvocellular AVT neurons are found, with the former indicated as the neurosecretory AVT cells. The magnocellular cells are found in a discrete population mixed with parvocellular cells in the POA in a region called the amphibian POA (**Figure 2**) or sometimes the magnocellular POA to distinguish it from the more rostral anterior POA. Distinct PVN and SON nuclei with AVT-immunoreactive (AVT-ir) cells are now recognized with a more distinctly separate magnocellular population in the PVN [e.g., see Ref. (26)]. Parvocellular AVT populations are found in multiple regions of the hypothalamus and forebrain limbic system and in all species an extensive AVT fiber system extends throughout the brain. A clearer understanding of the homologous relationships of AVT/AVP populations across vertebrates awaits additional comparative work, and the different functions each cell group may have in regulating social behavior or other processes needs further investigation as well.

Sex differences in immunoreactive cell number or fiber density are apparent in amphibians and reptiles. Usually these favor males, but in some areas females show more AVT. Relative to females, *T. granulosa* males have more AVT cells in the POA, MA, and BNST (13, 35) and higher AVT concentrations in the optic tectum and tegmentum (36). Remarkably, during the breeding season, *T. granulosa* females possess a higher number of AVT cells in the hypothalamus relative to males, but out of the breeding season, no such difference is expressed (36). Male-biased sexual dimorphisms also occur in frogs (13, 14, 16, 35). For example, male bullfrogs have a higher number of AVT cells and denser or more numerous AVT fibers in the MA and habenula; in contrast, females have larger AVT cells than males in the SCN (13). Boyd and Moore (35) reported AVT concentrations, as measured with radioimmunoassay, to be higher in males than in females in the La, optic tectum, and tegmentum, but in the dorsolateral nucleus of the mesencephalon AVT concentration was higher in females. Less work on AVT sex differences has been done in reptiles, but males have been reported to have a higher density of AVT fibers than females, particularly in the PAG and Ls (19, 20, 28), Dc (22), and in the amygdala, BNST, NAcc, and POA in addition to the Ls (26) in a variety of species.

Arginine vasotocin patterns also vary seasonally and with sex steroid hormone levels in all three groups. In *T. granulosa*, higher concentrations of AVT are found in the optic tectum during the breeding season than out of it, whereas in the dorsal POA AVT concentrations are higher out of the mating period (37). Boyd et al. (13) reported evidence for AVT seasonal variation in bullfrogs, with the vocal premotor pretrigeminal nucleus expressing AVT-ir cells only during the fall. Gonadectomy in bullfrog males and females decreases AVT concentration in most of the nuclei containing AVT cells, and treatment with gonadal steroids increases it (38). Seasonal differences in AVT fiber density have been found in the tree lizard (*Urosaurus ornatus*), with higher densities in peak and late breeding seasons compared to early in the season (26). There are relatively few studies of hormonal effects. Kabelik et al. (26) reported that testosterone implants in males increased soma size in both magnocellular and parvocellular AVT cells as well as higher fiber densities in the PVN and more rostral limbic areas. Whiptail lizards (*Cnemidophorus uniparens* and *C. inornatus*) treated with testosterone implants have a larger number of AVT neurons in the POA, but not other brain areas, compared with animals castrated and with blank implants (25). However, a correlation between AVT cell number and naturally circulating testosterone levels is not apparent in green anoles (*A. carolinensis*) (23). More work on reptiles in this area is needed, both for identifying conserved regulatory features impacting AVT across vertebrates, and for supporting the behavioral endocrinology work on lizard aggression and social hierarchy formation.

EFFECTS OF VASOTOCIN ON BEHAVIOR IN URODELES

Investigations of AVT's modulation of social behavior in herpetological taxa started with work by Diakow on release calls in frogs (reviewed in Section "Effects of AVT on Female Reproductive Behavior") and expanded with Frank Moore's extensive research program that established salamanders as a model organism for AVT research [reviewed in Ref. (39, 40)]. Most of the now classic research in salamanders has focused on an early phase of courtship behavior, namely the clasp response of males on females during which the male positions himself on the female's back and tightly grasps her around her pectoral region, a behavior called "amplexus." Amplexus is an important component of courtship, especially in species with internal fertilization, comprising above 90% of urodeles (41).

More recent studies have explored AVT's modulation of signaling and sensory capabilities relevant for breeding interactions between males and females. Salamanders and newts have evolved chemical communication as an important mediator of courtship and mating behavior (42). Visual stimuli are also important, and tactile stimulation operates during courtship contact episodes between males and females (40, 43, 44). All are targets of AVT's modulation.

Effects of AVT on Male Courtship Behavior

Most of the studies of AVT's effects on male urodeles has been conducted in *T. granulosa,* from the family Salmandridae. This species has internal fertilization, and during courtship males pursue female to amplex them. Intraperitoneally injected AVT stimulates amplextic clasping in males exposed to receptive females (45). Moore and Miller (46) reported the same effect in males that received intracerebroventricular (ICV) injections of

the neuropeptide; the effect on clasping behavior was stronger and the doses required were lower. Furthermore, ICV injections of the AVT receptor antagonist Manning Compound reduced clasping. This early study (46) and a later one by Toyoda et al. in the Japanese newt *Cynops pyrrhogaster* (47) are the only direct evidence to date in this species or any other amphibian or reptile that AVT can exert its action at the central nervous system level rather than depending solely on a peripheral, systemic action as proposed previously in early studies on the AVT stimulation of anuran release calls (48).

In addition to modulation of the clasping response, AVT influences male responses to and production of sexual signals. Male *T. granulosa* show more approaches and time in proximity to models scented with female sex pheromones after intraperitoneal AVT injections, relative to saline injected controls (43). AVT also affects the production of other types of courtship signals. The Japanese newt *C. pyrrhogaster* uses tail vibrations during courtship that occur concomitant with release of sodefrine from the male abdominal gland. Both displays are enhanced in AVT-injected males relative to controls, both in systemic and ICV administration and in a dose-dependent manner (47). The deposition of the male spermatophore is also activated in a dose-dependent manner. Recent studies have been conducted on another Asian salamander *Hynobious leechi*, an external fertilization breeder that does not perform amplexus. Males in this species produce body undulations which generate water waves that stimulate egg laying by conspecific females. AVT-injected *H. leechi* males show higher undulation rates both during the breeding season and out of the breeding season, relative to controls (49). Interestingly, AVT-injected animals show larger undulation rates in the presence of female scent than unexposed animals, indicating AVT modulation of olfactory sensitivity as well as signal production in this species. In both species, treatment with vasopressin V1 receptor antagonists reduced the behavior (47).

Early work related AVT levels in various brain regions to behavioral states as indirect evidence for AVT's role in urodele social behavior (50, 51). However, many more neural studies conducted in this group of amphibians have emphasized neurophysiological approaches focusing on motor and sensorimotor areas of the brainstem where AVT fibers terminate (40, 52). This emphasis arises from the tradition of investigating AVT modulation of male clasping in this amphibian group, that is, specifically on the motor component of courtship. In *T. granulosa* males, neurons in the rostral medulla reticular formation respond to cloacal pressure, a signal relevant for clasping behavior. Neurons there increase their spontaneous firing as well as their response to clasp-triggering cloacal stimulation after males are injected with AVT (53). This suggests that modulation of the sensitivity of this group of neurons by the neuropeptide could be relevant for AVT's stimulation of clasping behavior.

Arginine vasotocin innervation of sensory areas is also robust, reaching multiple olfactory areas including the olfactory bulb and midbrain visual areas (52). In many sensory areas, AVT fiber density is sexually dimorphic and higher in males (35). Both modalities are important in male mate searching, and AVT treatment enhances responses to female pheromones as well as broadly increases responses to visual stimuli (43, 44).

Effects of AVT on Female Behavior

In contrast to the body of work on AVT effects in male urodeles, there has been little investigation of its modulation of female behavior. AVT treatment does elicit oviposition in female *T. granulosa* (54). However, it is not clear whether this is simply a result of AVT stimulation of oviduct contraction, a well-known phenomenon in multiple amphibians and reptiles (55). It also increases a female-typical clasping behavior that in nature is used to attach the inseminated eggs to underwater foliage and other objects. AVT has no effect in ovariectomized females unless they are subsequently treated with estrogen, indicating that AVT alone is not sufficient to trigger this behavior. Interestingly, implanting ovariectomized females with dihydrotestosterone shifts female clasping preferences from foliage to other females (54). It is not at all clear how "male-like" this female-to-female clasping is. However, the work is instructive in showing that the female's steroid environment interacts with AVT to direct the target of the individual's response. A similar interaction related to olfactory investigation was described by Thompson and Moore (44). Female *T. granulosa* do not approach female scented models as do males, and AVT alone does not influence such olfactory or visual sexual exploratory behavior in intact or estrogen-implanted females. However, females that were ovariectomized and received testosterone implants and an AVT injection displayed the male-like olfactory investigation behavior.

EFFECTS OF AVT ON BEHAVIOR IN ANURANS

In contrast to the predominantly chemosensory-based salamanders, anurans depend strongly on acoustic signals to communicate. During the breeding season, male frogs and toads converge in chorusing aggregations where they signal their presence to nearby females and neighboring rival males by means of advertisement calls (56, 57). Conspecific females are attracted by these reproductive vocalizations and neighboring males typically engage in antiphonal calling contests (56, 58). Furthermore, different types of calls may be emitted depending on the behavioral context, such as aggressive calls during agonistic encounters between males or release calls emitted by males and unreceptive females to reject the amplexus of males (59). Although other sensory channels may also be employed by some species, including chemical signals and visual displays (60–62), acoustic communication is undoubtedly the leading sensory modality used by anurans in sexual and social interactions.

Effects of AVT on Male Reproductive Behavior

Studies conducted on salamanders have focused on a restricted number of species from the families Salamandridae and Hynobiidae (see Effects of AVT on Male Courtship Behavior). In contrast, the behavioral effects of AVT have been tested on a diversity of frog species belonging to seven different families

(**Table 2**). Overall, studies have mainly addressed the influence of AVT on male frogs' signaling, revealing that AVT favors the display of reproductive behaviors by promoting the emission of advertisement calls (**Figure 3**). Systemic AVT treatment induces three main changes in the vocal activity of male frogs, relative to control saline injections: (1) increased number of calls emitted per time unit (i.e., call rate), (2) increased likelihood to resume calling after experimental treatment (measured as the proportion of males that resumes calling after AVT injection), and (3) reduced latency to resume calling after disturbance or experimental manipulation (63–71). All three effects suggest that AVT increases the motivation to call. In addition, despite the conspicuous differences between the acoustic structure of advertisement calls emitted by different frog species, studies have shown that exogenous AVT administration modifies fine-scale temporal and spectral properties of these signals (14, 67, 69, 72–75).

Although evidence for AVT-mediated modifications of the vocal behavior of male frogs is compelling, the significance of these alterations for intra- and inter-sexual communication is poorly understood. Experiments conducted on gray treefrogs (*Hyla versicolor*) have provided valuable insights into the influence of AVT on male–male social interactions. In this species, AVT increases both the number of pulses and the duration of male advertisement calls (73). Also, AVT-injected males placed in the proximity (within 10 cm) of another conspecific calling male are more likely to emit advertisement vocalizations and, furthermore, take over the calling site of the resident male without engaging in physical aggression (79). This is an uncommon outcome, as in other frog species resident males usually retain calling sites when confronted by intruders (83, 84), suggesting that AVT may either increase the aggressiveness of *H. versicolor* advertisement calls or, alternatively, make them more competitive by improving their attractiveness (73). Intriguingly, AVT-induced changes in the calls of male gray treefrogs occur only in the presence of other nearby calling males, as these modifications are not evident when males are placed farther than 2 m away from other vocally active males (74). Altogether, these results indicate that the influence of AVT on male frogs' reproductive behavior are relevant for intrasexual communication, and, most notably, that AVT-induced modifications of male signaling may be shaped by the surrounding social (acoustic) context. Similar conclusions can be derived from experiments conducted on male cricket frogs (*A. crepitans*) (67). The significance of the social context highlighted by these studies pinpoints a fundamental issue to be considered in future studies. Research aimed at evaluating the effects of AVT on frogs' reproductive behavior should provide a careful characterization of the acoustic environment in which experiments are conducted. In addition, the acoustic context could be homogenized by using playback designs [e.g., Ref. (69)].

Arginine vasotocin-induced changes on male calls can also have implications for female responses, an issue that has been considered central to elucidate the relevance of AVT modulation

TABLE 2 | Effects of arginine vasotocin (AVT) on anuran social behavior.

Sex	Family	Species	Behavior	Effect of AVT	Reference
Male	Ranidae	*Rana catesbeiana* (=*Lithobates catesbianus*)	Release calling	Increase	Boyd (76)
			Advertisement calling	Increase	Boyd (38, 64)
		Rana (=*Lithobates*) *pipiens*	Release calling	Decrease	Raimondi and Diakow (77)
	Leptodactylidae	*Physalaemus* (=*Engystomops*) *pustulosus*	Advertisement calling	Increase	Kime et al. (69)
			Phonotaxis	Increase	Baugh and Ryan (78)
	Hylidae	*Hyla versicolor*	Advertisement calling	Increase	Semsar et al. (79), Tito et al. (72)
			Advertisement calling	No effect	Klomberg and Marler (73), Trainor et al. (74)
			Aggressive calling	No effect	Tito et al. (72)
			Release calling	No effect	Tito et al. (72)
		Hyla cinerea	Advertisement calling	Increase	Penna et al. (63), Burmeister et al. (68)
		Acris crepitans	Advertisement calling	Increase	Marler et al. (65), Chu et al. (67)
	Eleutherodactylidae	*Eleutherodactylus coqui*	Advertisement calling	Increase	Ten Eyck (80)
			Aggressive calling	Increase	Ten Eyck and ul Haq (70)
			Parental care	No effect	Ten Eyck and ul Haq (70)
	Bufonidae	*Bufo* (=*Anaxyrus*) *cognatus*	Advertisement calling	Increase	Propper and Dixon (66)
			Amplexus	No effect	Propper and Dixon (66)
	Dendrobatidae	*Ranitomeya imitator*	Parental care	Decrease	Schulte and Summers (81)
	Pipidae	*Xenopus tropicalis*	Advertisement calling	Increase	Miranda et al. (71)
			Amplexus	No effect	Miranda et al. (71)
Female	Ranidae	*R. catesbiana* (=*Lithobates catesbeianus*)	Release calling	Decrease	Boyd (76)
			Phonotaxis	Increase	Boyd (38, 64)
		Rana (=*Lithobates*) *pipiens*	Release calling	Decrease	Diakow (82), Diakow and Nemiroff (48), Raimondi and Diakow (77), Raimondi and Diakow (77)
	Leptodactylidae	*Physalaemus* (=*Engystomops*) *pustulosus*	Phonotaxis	Increase	Baugh and Ryan (78)
	Dendrobatidae	*R. imitator*	Parental care	No effect	Schulte and Summers (81)

For those species in which vocal behavior was studied (i.e., advertisement, aggressive, and release calling), "Increase" refers to an augmentation of the probability of calling or calling rate, or a reduction in latency to call (see text for further descriptions). Studies that measured any of these calling parameters but did not find an effect of AVT are labeled as "No effect." For the studies evaluating the effects of AVT on phonotaxis, "Increase" refers to an increase in sexual arousal, measured as a shorter latency to approach a speaker playing advertisement calls, or increased choice behavior.

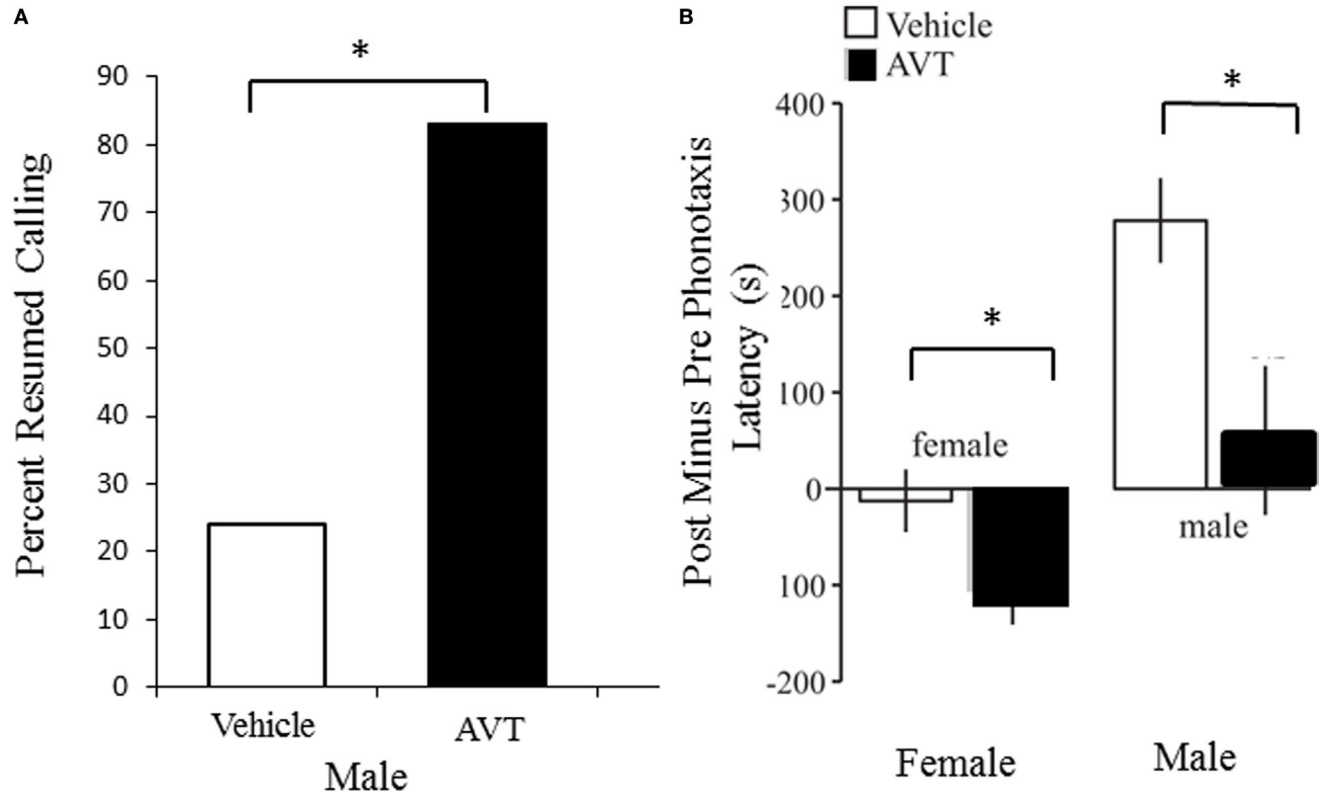

FIGURE 3 | Arginine vasotocin (AVT) modulates reproductive social behavior in both male and female túngara frogs. **(A)**. Treating males with 25 mg of AVT (IP injection; *N* = 18) significantly increases the probability that males will resume calling after treatment compared to saline vehicle injection (*N* = 17). Data are from Kime et al. (69). **(B)**. Treatment with 25 mg of AVT (IP injection) decreases latency to choose and reach a speaker playing a conspecific male advertisement call in both females (left) and males (right), compared to saline vehicle injection. Data are expressed as the difference between pre and post injection latencies; *N* = 12 individuals of each sex per treatment. Note that saline injections themselves increased latency to respond in males, but AVT treatment mitigated this effect. Data are from results illustrated in Baugh and Ryan (78).

on anuran reproductive behavior (85). Some studies have found that AVT modifies call properties likely to be relevant for female choice (65, 73); nevertheless, female preference for AVT-modified male calling has only been tested in túngara frogs [*Physalaemus (=Engystomops) pustulosus*]. The advertisement calls emitted by male túngara frogs consist of a frequency-modulated note called a "whine," which can be followed by one or more secondary notes termed "chucks." Males emit shorter whines with higher initial frequency and add more chucks to their calls following AVT administration. As females exhibit a strong preference for calls having chucks (86, 87), Kime et al. (69) initially hypothesized that AVT improves the attractiveness of male vocalizations. To evaluate this possibility, Kime et al. (75) conducted two-speaker female phonotaxis trials with natural male calls emitted before and after AVT treatment. These experiments showed that females consistently preferred whines (chucks were initially cutoff for these female choice experiments) emitted before males were administered AVT. When females were tested with complete calls (i.e., whines including subsequent chucks), there was no difference in female preference between calls emitted before and after AVT injection. Together these results indicate that AVT renders the obligatory part of the male advertisement call (the whine) unattractive to females, and that call attractiveness is at least not enhanced by AVT when attractive notes (chucks) are added to the vocalizations. Because of the few studies on female responses to male AVT-modified calls it is not clear whether social context or female reproductive state would change the results. However, given that male responses appear to be context dependent, the environmental settings in which experiments with females are conducted should certainly be considered.

An alternative approach to treatment with exogenous AVT is to assess the relationship between brain AVT levels and behavioral state. AVT cell populations are widespread in the anuran brain [see AVT in Anurans (Frogs and Toads)], and AVT expression levels in one such nucleus has been related to the display of male reproductive behaviors. Silent satellite cricket frogs (*A. crepitans*) present more AVT staining in the nucleus NAcc relative to sexually active males (i.e., calling individuals) (14). This forebrain region is thought to be involved in weighting the salience of social stimuli across vertebrates (88). As AVT administration increases the motivation to call in this species (65, 67), the authors suggested that the emission of vocalizations is associated with an increase of brain AVT release (14), thus resulting in a decreased level of observed NAcc AVT due to

depletion by this increased secretion. In consonance with this interpretation, early in the breeding season of the frog *Hyla cinerea*, when the vocal activity of males is typically high, AVT-ir cells in the NAcc of males are smaller and less abundant relative to males measured once the mating season is over (15). Neither study found evidence of an association between calling activity and AVT cell number or size in other brain nuclei. It is possible that some other metric of brain AVT level or activity [e.g., high performance liquid chromatography with fluorescence detection as has been applied to measure fish brain AVT levels (89, 90)], would reveal relationships with calling in other areas. However, it should be noted that AVT/AVP has multiple effects on social behavior across vertebrates beyond social signaling, as well as effects on physiological processes. It is possible that these other frog AVT centers are associated with one or more of those other functions, as Kabelik et al. (24) found in *Anolis* lizards (see Effects of Vasotocin on Behavior in Reptiles).

There is evidence that AVT may modulate other aspects of male amphibian reproductive behavior beyond signal production. For example, some frogs rely on acoustic cues (e.g., male advertisement calls) to orient toward breeding aggregations (91), and a recent study reported that the phonotactic responses of male túngara frogs to conspecific advertisement calls is increased by AVT administration (78), highlighting the role of this peptide in the recruitment of males to mating assemblages. Studies have also evaluated the effect of AVT on the amplectic clasping of male frogs. Similarly to the courtship behavior of rough-skinned newts, male frogs embrace females for effective mating and receptive females subsequently release their eggs, which are externally fertilized by males' sperm (57). Despite the behavioral similarities between frog and *T. granulosa* amplexus, differences have been found in AVT's modulation of clasping behavior among these amphibians. While AVT strongly induces amplectic behavior in *T. granulosa* (45), experiments conducted with western clawed frogs (*Xenopus tropicalis*) and great plain toads [*Bufo* (=*Anaxyrus*) *cognatus*] have failed to elicit amplexus through systemic AVT treatment (66, 71). Still, the influence of AVT on frog amplectic behavior cannot be dismissed, as male *X. tropicalis* attempt to clasp females more often after being injected with a combination of human chorionic gonadotropin (hCG) plus AVT relative to frogs receiving hCG only (71).

Effects of AVT on Male Aggression

In general, agonistic encounters among male frogs occur when they defend calling territories from other neighboring males. These aggressive interactions are usually solved by means of the emission of aggressive vocalizations (59), although conflicts may escalate to physical aggression in some species [e.g., Ref. (92)]. In frogs, agonistic vocal interactions are generally modulated by androgens and corticosterone (93, 94), but AVT is also important. However, the influence of this neuropeptide on anuran aggression is not as clear as its influence on the emission of advertisement signals.

One species where this was examined is the cricket frog (*A. crepitans*), where males have a "graded communication" system (57, 59, 95, 96) in which they modify their pulsed advertisement call in a graded fashion in order to signal increasing levels of aggressiveness to challenging conspecific calls rather than switch to a different aggressive call (97–101). Relative to normal advertisement calls, aggressive-like vocalizations include a combination of temporal (i.e., increased call duration and number of pulses, among others) and spectral (i.e., lower dominant frequency) modifications (98, 102). By means of a set of field experiments and detailed acoustic analyses, Marler et al. (65) demonstrated that treatment with AVT increases the probability of calling and also induces call modifications typical of less aggressive males when experimental subjects are stimulated with the natural surrounding chorus. A later study suggested that the apparent reduction in aggression levels found by Marler et al. is probably a by-product of an increased motivation to call (67). Whether or not this is the case, the calls emitted by AVT-injected cricket frogs are likely to be perceived as less aggressive variants by other neighboring conspecifics. In contrast, a study with male *H. versicolor*, which have separate advertisement and aggressive calls rather than using a graded call system, failed to find changes in the emission of aggressive calls after AVT treatment (72). Furthermore, different effects of AVT have been described for Puerto Rican coquí frogs (*Eleutherodactylus coqui*). After mating, male coquí frogs stay near the egg clutch providing parental care, a period during which their vocal activity is typically low (103). Both male and female coquí frogs emit distinct aggressive calls in response to the intrusion of other conspecifics into their shelters (104). In paternal *E. coqui*, the probability of emitting aggressive calls is increased following AVT administration (70). In addition, AVT treatment stimulates the emission of advertisement vocalizations in non-calling satellite male *E. coqui* (80).

These studies reinforce the notion that the effects of AVT on anuran behavior are species- and context-dependent, and, in the case of coquí frogs, dependent on the reproductive status of the males. The mechanisms responsible for such dependency are unknown, but one possibility is that the behavioral effects of AVT depend on the overall hormonal states associated with different behaviors. Differences in androgen and corticosterone levels have been reported between calling and silent male frogs (105, 106) and paternal *E. coqui* have lower circulating testosterone levels than calling males (107). This is a promising avenue for future research, as the behavioral effects of AVT have been shown to be dependent on the administration of androgens (63). Moreover, gonadal steroids (38, 108) and melatonin (16, 17) have been shown to modulate the expression of AVT and its putative receptor in brain areas. These effects may be part of the mechanism by which these other hormones influence frog social behavior.

Effects of AVT on Female Reproductive Behavior

Arginine vasotocin modifies female reproductive behavior as well as male calling (**Table 2**; **Figure 3**). For instance, female bullfrogs and túngara frogs approach a loudspeaker playing male calls faster following AVT treatment (64, 78). Whether AVT affects females' responsiveness to different call variants, such as attractive and unattractive vocalizations, is unknown.

Calling behavior, including the production of female advertisement calls, is not absent in female frogs, and it has been described in numerous species belonging to over 10 families (33, 109, 110). AVT's effect on natural female advertisement vocalizations has not been tested. However, an interesting set of experiments indicates that AVT does have the ability to unlock female calling. Normally, female *H. cinerea* frogs do not emit calls; however, hormonal manipulations have successfully elicited vocalizations in this species. AVT promotes the emission of calls in testosterone-implanted female *H. cinerea* stimulated with conspecific calls, while non-implanted intact females remain silent. In contrast, saline injections do not evoke vocalizations in intact and testosterone-implanted females (63). The spectral and temporal characteristics of female vocalizations were similar to the advertisement calls emitted by intact males, except for the low frequency peak, which was about 350 Hz higher in females. These results demonstrate that testosterone and AVT act synergistically to induce mating-like vocalizations in female *H. cinerea*. This is a puzzling result, as reproductive vocalizations have not been reported for female Hylids [see Ref. (111) for an updated survey of female frogs' vocalizations], yet the circuitry for calling is apparently present and modifiable by AVT.

One type of social signal produced by females as well as by males in many frog species is the "release call" (57). Release calls are produced by vibrating trunk muscles in response to another individual's grasping, as males normally do with females during amplexus. This display can have both acoustic and vibratory components as is the case in some Bufonidae species (112, 113), or only trunk vibrations [e.g., *Pleurodema thaul* (114)]. The release signal communicates a lack of receptivity (in females) or inappropriate clasping (in males), and hence it signals the amplexing individual to release the signaler. The first study of AVT effects on anuran social signaling was in fact done on release calls. Diakow (82) reported that a systemic AVT injection reduced release calling in female bullfrogs [*R. catesbeiana (=Lithobates catesbeianus*)], which would mean that AVT increases receptivity. Boyd (76) confirmed the female effect in leopard frogs [*Rana (=Lithobates) pipiens*]. These effects on release calling are consistent with AVT's enhancement of female phonotaxis to male advertisement calls (64, 78).

Arginine vasotocin modulation of male release calling is inconsistent and difficult to interpret, as this neuropeptide has been reported to decrease release calling in both male and female leopard frogs (77), but increases release calling in male bullfrogs while it decreases this response in females (76). Furthermore, these changes occurred only in the spring; AVT treatment in the fall had no effect on either sex. In addition AVT has no effect on release calling in male *H. versicolor* other than to decrease the duration of individual release calls (72). The significance of these changes for natural male sexual behavior are not clear, as a decrease in male release calling apparently lacks an adaptive role, and it is not known how male reproductive state correlates with natural release calling. Overall it is difficult to interpret the significance of the opposite effects that AVT has on release calling in closely related Ranid species, or even if the experimentally induced change in release calling is an epiphenomenon of AVT effects on calling, social motivation, or systemic physiological regulation.

Unanswered Questions

Research evaluating the influence of AVT on anuran behavior has largely focused on male behavior, particularly on the emission of acoustic signals under various social contexts. These studies prompt two general conclusions: (i) AVT promotes the emission of reproductive signals and (ii) the modulation of male sexual and aggressive behaviors by AVT is dependent on the social context. All of this work has focused on acoustic communication. The influence of AVT on the emission of non-acoustic signals now arises as an important question for future research, as a recent study demonstrated that the emission of agonistic visual displays (foot-flagging) is modulated by androgens in Bornean rock frogs (*Staurois parvus*) (115). Also, male dwarf African clawed frogs (*Hymenochirus* sp.) have breeding glands that produce odorants effective for female attraction (116), but influences of androgens or peptides on such secretions have not been explored.

Male-biased AVT research is intriguing but female behavior has been neglected. Female frogs also display many of the behaviors that have been studied in males, including aggression (117) and even the emission of different kinds of vocalizations (33). None have been examined for AVT modulation. AVT effects on female phonotaxis, the most conspicuous reproductive behavior of this sex, have been rarely studied until recently (78). One additional social behavior that females and males display in some species is parental care (81, 118). Shulte and Summers (81) recent study indicates that AVT might influence some aspects of egg-care behavior, although decreasing some aspects while increasing others. The more complex social behaviors seen in some frog species deserve more attention to determine if the conserved functions of the nonapeptides extend to these types of amphibian behaviors.

Finally, although the behavioral effects of AVT are clearly documented, what remains unclear is what neural mechanism might account for these changes. In urodeles, AVT research has extended to studies of its influence on modification of the elemental sensory and motor components of social communication systems in this group (see Effects of AVT on Male Courtship Behavior). That might explain at least some of the facilitation of this nonapeptide on urodele social behavior. This type of research is almost wholly lacking in anurans (and reptiles). The one exception is early work by Penna et al. (63) that examined midbrain auditory thresholds after manipulations of both testosterone and AVT. They found AVT reduced midbrain sensitivity to mid- and high-frequency sound, but only in males, following castration with or without testosterone implant. The authors noted that this was puzzling, given that the same treatment stimulated calling in the testosterone-implanted males. This is only one paper and, therefore, only begins to address how AVT is modifying neural systems, but it does at least indicate that changes on the sensory side of the communication system may be induced by AVT, and suggests that further work like this is warranted, perhaps modeled on the neurophysiological experiments on sensory and sensory–motor centers done in urodeles.

EFFECTS OF VASOTOCIN ON BEHAVIOR IN REPTILES

Compared to work in amphibians and other vertebrates, there have been relatively few studies in reptiles directly linking AVT to behavior. This is true despite the long history of research on lizard aggression, male displays, and social hierarchy formation (119–121), all of which are prime targets of AVT/AVP research in other vertebrates. What research has been done focuses mainly on social behavior (aggression and to a lesser degree courtship) rather than on social signal production *per se*. Although few in number, behavioral endocrinology studies of reptile AVT shows that the general function of this nonapeptide in modulating male aggression is conserved in reptiles. As in amphibians, the effects of AVT in lizards are complex and context dependent.

The majority of research that links brain AVT function with aggression or other social behaviors in reptiles has taken the indirect approach of relating AVT cell number or staining density to individual or group behavioral profiles. Within-sex differences indirectly implicate brain AVT in social regulation. Male green anoles held in laboratory conditions will, when paired, interact with aggressive displays and ultimately form stable dominant/subordinate dyads (121–123). Hattori and Wilczynski (23) found that after 10 days of stable paired housing, dominant males had a higher number of AVT cells in the POA than did subordinate males, with no difference apparent in other AVT populations. Although not specified in this publication, the area affected most likely includes, but may not be limited to, cell population equivalent to the PVN of mammals and reptiles. Singly housed control males had POA AVT cell counts intermediate between dominants and subordinates, a pattern that suggests a rise in POA AVT in dominants and a decline in subordinates as a function of this ongoing difference in social state.

Kabelik et al. (24) used a more sophisticated double labeling immunocytochemistry approach to identify the participation of various AVT populations in different types of social behavior in brown anole lizards (*Anolis sagrei*). They double-labeled neurons for AVT and the immediate early gene *Fos*, a marker of neural activity, in males engaged in aggressive or sexual interactions and found differential activation of brain AVT populations. Paraventricular nucleus (PVN) AVT cells were active during aggressive encounters with another male, with a positive correlation between aggression levels and *Fos* activation. In contrast, AVT cells in the POA were preferentially active during sexual interactions (courtship and copulation) with a female. SON and BNST AVT cells were active in both social situations. The relationship between the activation results of Kabelik et al. and the state-dependent differences reported by Hattori and Wilczynski have not been reconciled. One might have hypothesized a greater dominant/subordinate activity difference in the aggression-activated PVN AVT cells, given that differences in aggressive behavior and displays are the preeminent causes and consequences of social status differences in anoles (121, 123–125). However, as is the case in many vertebrates, dominant male anoles differ in many behavioral and physiological traits beyond aggression (121, 124, 126), any of which could be related to AVT changes in different nuclei. Furthermore, a slowly emerging, stable state difference in the brain following long term experience in a social status is a different characteristic than immediate activation during a specific behavior. Although much work remains to be done in order to understand the role of various AVT populations in social behavior and their modification as a consequence of that behavior, the results of these studies suggest a complex network of forebrain AVT cell populations participating in a variety of male social behaviors.

Few studies have taken the more direct approach by treating males with exogenous AVT. Dunham and Wilczynski (127) did this using intraperitoneal injections of AVT in male green anoles. AVT injections decreased, rather than increased, aggressive displays to a mirror (**Figure 4**). Although at first this may seem

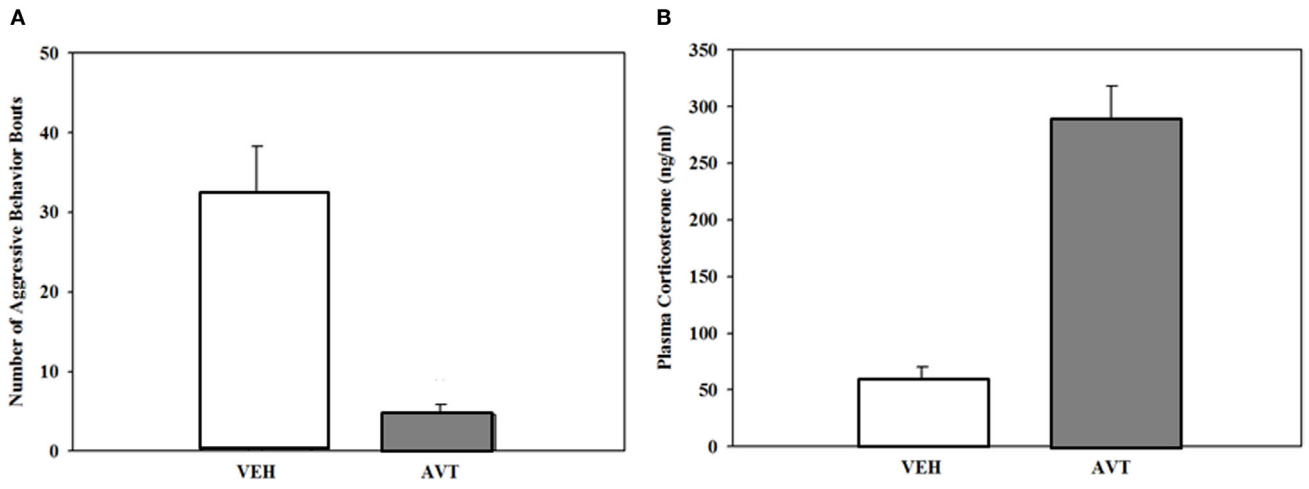

FIGURE 4 | **(A)**. Reduction in aggressive responses (mean + SEM) to a mirror in male green anoles following intraperitoneal injection of arginine vasotocin (AVT) (15 μg AVT/50 μl reptile Ringer; *N* = 10) or vehicle (*N* = 6). **(B)**. Increase in plasma corticosterone in the same animals following treatment (mean + SEM). From data in Dunham and Wilczynski (127).

contrary to expectations, in fact AVT has been found to have opposite effects depending on species and context. Green anoles are territorial, and most consistently across species of birds and fish AVT tends to suppress aggressive behavior in territorial individuals, species, or morphs while stimulating it in non-territorial or socially gregarious animals (4, 128). AVT did not, however, influence aggression or the outcome when AVT treated male anoles interacted with size-matched saline injected males. This is of course a more complicated situation where the interaction between the two males determines the level of behavior in each as well as the outcome of the encounter. AVT treatment effects put the increased POA AVT cell number in dominant males into perspective. This dominant-subordinate difference may represent lower release of the aggression-suppressing peptide in the more aggressive dominants. Note that this is similar to the argument regarding AVT cell variation in male cricket frogs, that is, that the higher release of the call-stimulating peptide results in smaller sized and a lower number of AVT-containing cells in calling vs. satellite males (14).

Interestingly, AVT treatment of male anoles does not change the number of courtship displays to a novel female; however, females were significantly more responsive to AVT-injected males (127). AVT must have modified some aspect of male courtship signaling, either through subtle changes in the form of the observable visual displays not yet documented, or through some modulation of signals not readily apparent such as pheromonal signals. Chemical signaling is poorly understood in anoles but is present in many lizards (129). AVT does influence responses to conspecific odors in male European Common Lizards (*Zootoca vivipara*) (130), where it suppresses attraction to odors of other conspecific males. The effect is restricted to smaller males, highlighting again the complex context and class dependence of AVT's effects. Whether females are similarly affected by AVT is unknown.

Social bonding, which is a function of nonapeptides in mammals (1), is usually considered beyond the realm of reptiles, and in fact most lizards, turtles, and snakes do not pair-bond or show parental care. Some viviparous snakes do, however, show mother–offspring brooding-like behavior and defense of their offspring. Pigmy rattlesnakes (*Sistrurus miliarius*) are an example, and a recent paper (131) reported that blocking AVT/AVP V1a receptors eliminated mothers' preferences for aggregating with their offspring. It is worth noting that in addition to many species of snakes, skinks in the order Scincidae also show parental care, and crocodilians are virtually bird-like in their nesting behavior. This species diversity provides ample opportunities for extending the investigation of reptilian AVT into areas of social behavior beyond simple aggression and courtship responses, just as the expected diversity of frog social behavior beyond male advertisement calls and female responses does for amphibians.

PERIPHERAL EFFECTS OF VASTOCIN IN AMPHIBIANS AND REPTILES

In addition to work on AVT's behavioral effects, a great deal of research has been done on the peripheral physiological and endocrinological functions of this peptide in both amphibians and reptiles. In reptiles, AVT, like AVP in mammals, is a peripherally acting peptide hormone influencing osmoregulation, which in reptiles is also tied to thermoregulation, and stimulating smooth muscle contraction, including oviduct contractions associated with oviposition (55, 132–136). Similarly, osmoregulation is an important peripheral function of amphibian AVT (137, 138). Frogs "drink" water through their ventral skin, which is the main way in which they stay hydrated. Treatments with exogenous AVT stimulate water absorption in both anurans (138) and urodeles (139). Frog ventral skin expresses AVT receptors (140) verifying the peripheral action of AVT there. ICV AVT injections also stimulate water absorption (141) indicating that AVT also acts as a central nervous system modulator of hypothalamic centers regulating water retention. Given the importance of water for survival and egg laying in amphibians, it is possible that AVT's osmoregulatory effects may have an unrealized influence on their behavior. Diakow and Nemiroff (48) in fact suggested that the AVT triggered decrease in female release calling was due to water absorption, leading to abdominal extension mimicking a large egg mass. This may have contributed to her experimental results, but it is not at all clear how this would account for the enhancing effects of AVT on release calling in males.

Particularly important in the context of its effects on social behavior, AVT is a potent stimulator of adrenal gland steroid hormone secretion in both amphibians (**Figure 5**) and reptiles (68, 127, 142), just as AVP is in mammals. High cortisol levels can dramatically change social behavior in many vertebrates by various mechanisms including interaction with other hormones and, although less well studied, possibly through glucocorticoid effects on glucose metabolism and related energetic functions. Although peripheral AVT action is less a concern in studies investigating natural variation of brain AVT measures with behavior, those peripheral effects are problematic for experiments employing systemic treatment with exogenous AVT or vasopressin receptor blockers, as these procedures will influence bodily physiological functions in addition to, and perhaps more than, AVT signaling in the brain. In some cases, such as the decrease

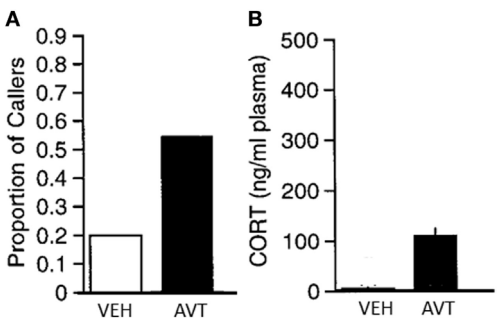

FIGURE 5 | **(A)** Significant increase in the proportion of male green treefrogs that resumed spontaneous calling following and IP injection of 20 μg arginine vasotocin (AVT) compared to vehicle (saline) injection. **(B)** Significant increase in plasma corticosterone (mean + SEM) following injection with 20 μg AVT compared to vehicle (saline) injection. Results show that AVT increases calling even when stimulating corticosteroid release. *N* = 10 males per group. From data in Burmeister et al. (68).

in aggression seen in anoles after systemic AVT treatment (127), it is possible that the coincident rise in corticosteroids might contribute to the AVT's effect in decreasing aggression. On the other hand, the fact that AVT treatment in frogs increases calling rates even though it also increases corticosteroids (68) (**Figure 5**) suggests that AVT positively influences calling independent of, and in spite of, any negative effects of elevated corticosteroids. Understanding the role of AVT's peripheral endocrinological and physiological effects on social behavior remains a critical, but largely overlooked, component of AVT's behavioral endocrinology.

SUMMARY AND DISCUSSION

Arginine vasotocin's anatomy and function in amphibians and reptiles are similar to that of AVP in mammals, both in their general features and in the questions they raise. AVT-containing cells, consisting of both magnocellular and parvocellular neurons, are found mainly in limbic forebrain and hypothalamic areas with a widespread fiber distribution throughout the central nervous system marked by a particularly heavy innervation of the limbic Social Decision Network and associated social communication areas of the brain. There is generally a male-biased sexual dimorphism in the anatomy, although with several reported exceptions. Finally, AVT has both peripheral physiological effects common across vertebrates and modulatory influences on social behavior and communication. These behavioral effects are most consistently observed in males, but are now seen as modulating female social behavior as well in the few cases where it has been investigated. Within this general framework, significant diversity within and across species is apparent, leading to the first and most fundamental question regarding AVT's (and AVP's) modulation of social behavior: what is the mechanism by which this important neuropeptide exerts its effects?

Various studies on amphibians and reptiles point to multiple points at which AVT could influence an organism's responses to, and production, of social signals as well as modulate other aspects of socially cued behavior. There is evidence that AVT modifies chemical, visual, and auditory processing in urodeles and anurans (39, 63), channels used in various species for their communication. There is also direct physiological evidence as well as suggestive neuroanatomical data that implicate action on motor areas (40). Moreover, the cellular location and dense AVT innervation of the NAcc points to an impact on signal salience or social reward, and more broadly the presence of AVT cells or fibers in multiple areas of the Social Decision Network and on limbic pallial/cortical areas suggests an impact on motivational and higher cognitive functions. Furthermore, AVT's influence on peripheral physiology and endocrinology is significant; how this does or does not account for any of the experimental results of AVT treatment remains an important issue to address. Of course, it is possible that AVT acts in multiple, and perhaps independent, ways, both centrally and peripherally, or that AVT in some way acts to bind these areas together by modifying a functional network linking them. An important step toward understanding exactly what this peptide is doing would be formulating truly testable hypotheses explaining the mechanisms by which changes in peptide levels result in changes in specific aspects of social behavior.

The diversity seen in the AVT system represents both a challenge to understanding its mechanism of action and a potential tool to investigate it. Although the widespread fiber distribution is common across species, species variation in the location of AVT cells themselves is apparent in **Table 1**. This diversity needs to be viewed cautiously, as reports of AVT cell location in urodeles, anurans, and reptiles, go back over 30 years, and systematic, well controlled comparative studies remain to be done with modern methods. Nevertheless, if variation in the anatomical distribution of AVT neurons is real, an analysis of whether or not this is functionally relevant could make an important contribution to understanding the structure-function relationship of AVT and other neuroactive peptides.

Explaining the diversity of AVT's effects remains a major challenge as well as an opportunity to delve deeper into the mechanisms by which this peptide operates. The AVT system is a dynamic one, sensitive to multiple factors within and across individuals. In addition to a consistent sex difference within species, AVT characteristics vary within individuals based on seasonal, social, and hormonal state. The influence of steroid hormones on AVT levels and possibly action is particularly profound. This diversity has already been used to confirm AVT's modulation of various social behaviors, and this provides a foundation for deeper explorations of AVT's mechanisms. The influence of melatonin on AVT neurons shown in anurans (16) is also an aspect that deserves further explorations in the other groups. The variation in AVT's influence across species is also significant. Whereas AVT consistently influences social communication and aggression across species, what is influenced and how it is modulated can vary greatly. For example, AVT influences chemical signaling in urodeles, vocal signaling in anurans, and visual displays in reptiles; in the first two taxa, AVT increases the propensity to signal, whereas in reptiles, at least in an aggressive context, it decreases visual signaling (spontaneous display behavior has not been assessed in lizards). Moreover, all possible effects of AVT on male frog aggressive calling have been reported: decreased aggressiveness (65), increased aggressiveness (70) and no effect (72). Why this is so remains an enigma; however, the species- and context-dependence of AVT's effects on aggression is a phenomenon observed in other taxa as well. How the link between AVT function and behavioral output can vary so dramatically remains an important gap in our knowledge. Although a challenging issue, the species diversity across urodeles, anurans, and reptiles in social behavior also represents an opportunity to dissect the way in which AVT acts on the neural systems responsible for social behavior. Anuran species, for example, range in calling sex dimorphism from vocal advertisement signaling being strictly a male behavior, to females producing audible calls other than advertisement calls, to both males and females producing advertisement calls. Visual displays are an important adjunct to vocal signals in some anuran species, but not others. Release signals produced by both males and females have both audible and vibratory components in some species, but only vibratory in others. How is this diversity within and between sexes reflected in either the anatomical or physiological characteristics of brain

AVT? Extending the type of structure-function analysis used to implicate AVT function within species to the diversity seen among species could provide real insight into how social peptides evolve and operate.

AUTHOR CONTRIBUTIONS

All authors discussed and planned the paper. Each author was the primary author for approximately 25% of the paper. All authors reviewed and edited all sections of the paper, which was then compiled by the corresponding author.

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REVIEW
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Vasopressinergic Neurocircuitry Regulating Social Attachment in a Monogamous Species

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The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent species that forms a lasting connection between mates, known as a pair bond. The pair bond is primarily characterized by three distinct behaviors: partner preference, selective aggression, and biparental care of the young. The presence of these behaviors in the prairie vole and their absence in closely related non-monogamous species makes the prairie vole an important model of social relationships and facilitates the study of the neurobiological mechanisms of social affiliation and attachment. The nona-peptide arginine-vasopressin (AVP) is an important neuromodulator of social behavior and has been implicated in the regulation of the pair bond-related behaviors of the prairie vole, through activation of the AVP receptor subtype 1a (AVPR1a). Modulation of AVPR1a activity in different regions of the prairie vole brain impacts pair bond behavior, suggesting a role of AVP in neurocircuitry responsible for the regulation of social attachment. This review will discuss findings that have suggested the role of AVP in regulation of the pair bond-related behaviors of the prairie vole and the specific brain regions through which AVP acts to impact these unique behaviors.

Keywords: vasopressin, prairie vole, pair bond, partner preference, aggression, parental care

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INTRODUCTION

The prairie vole (*Microtus ochrogaster*) is a small, mouse-sized rodent native to the Midwest region of the United States. This species performs behaviors related to social monogamy, a trait seen in fewer than 5% of mammalian species (1). This social exclusivity is beneficial in areas where populations of animals are spread out such as in the vast Midwest grasslands, as it may be difficult to encounter potential new mates. In fact, opposite-sex pairs of prairie voles are caught together more frequently in areas where the population is less dense (2). The pair bond is a unique, strong affiliative connection between mates of a socially monogamous species. This bond is characterized primarily by behavioral and physiological hallmarks, including preference for a social partner over unfamiliar conspecifics, selective aggression toward intruding conspecifics, nesting together during gestation, and displaying biparental care of offspring, distress, and social-seeking behavior during periods of separation or social loss, and stress alleviation among reunion and consoling behaviors (1, 3). The prairie vole exhibits these distinctive characteristics (2), but closely related non-monogamous species such as the meadow vole (*Microtus pennsylvanicus*) and the montane vole (*M. montanus*) do not (4, 5). Thus, manipulation and description of these behaviors in the prairie vole, as well as cross-species comparison with the meadow vole or montane vole, allows for the study and understanding of the neural mechanisms behind pair bond-related behaviors. For these reasons, the prairie vole has

become an attractive model for studying the neurobiological basis of behaviors related to social affiliation and attachment that are not easily modeled in other laboratory species.

Social behavior is regulated by a number of neuromodulators, such as the neuropeptide arginine-vasopressin (AVP). AVP is a key regulator of a number of social behaviors, including social recognition (6, 7), aggression (8, 9), and maternal care (10). Furthermore, it has been determined that the AVP system in the vole brain functions as a neuromodulator of a number of social behaviors critical for the establishment and maintenance of the pair bond between breeding pairs, including partner preference, selective aggression, and paternal care.[1] AVP primarily acts through three G protein-coupled receptors: the AVP receptor subtype 1a (AVPR1a), subtype 1b (AVPR1b), and AVP receptor type 2 (AVPR2). The distribution of AVPR1a in the prairie vole brain has been well established (11–17). However, the distribution of AVPR1b and AVPR2 in the prairie vole brain has not been characterized, and thus the regulation of social behavior by AVP is primarily attributed to AVPR1a action. Comparative studies of AVPR1a distribution in closely related *Microtus* species have revealed an expression pattern in prairie voles that is similar to the monogamous pine vole (*M. pinetorum*) (11) and distinct from non-monogamous species such as the montane vole and the meadow vole (12–14). As these distribution patterns correlate with unique patterns of social organization and behavior, it has been theorized that expression of AVPR1a has some role in the neurobiological basis of social affiliation and attachment. The first study to investigate the role of central AVP administration in both prairie voles and montane voles found that AVP promoted pair bond-related behavior, namely selective aggression, in the prairie vole, but not in the montane vole (12). In addition, this study found that AVPR1a binding distribution correlated with *avpr1a* mRNA expression levels. Transgenic mice expressing the prairie vole *avpr1a* gene not only display more affiliative behaviors but also have a "prairie vole-like" distribution pattern of AVPR1a that is distinct from that of wildtype mice (18). These findings suggest a relationship between the *avpr1a* gene and AVPR1a protein distribution patterns, thus prompting investigation into the genetic basis of the role of AVPR1a in social behavior.

While both monogamous and non-monogamous vole species share 99% sequence homology of the *avpr1a* gene, prairie vole *avpr1a* is preceded by an extended 5′ flanking microsatellite region that is not present in non-monogamous species (18). It was originally suggested that this microsatellite region contains *cis*-regulatory elements, controlling *avpr1a* gene expression through binding of transcription factors or secondary DNA structure formation, and promotes species differences in AVPR1a expression and social behavior. However, there are incongruences in the reported relationship between microsatellite length and neuronal and behavioral phenotypes (19). For example, variation in microsatellite length has contrasting correlates with variation of AVPR1a binding in several brain regions and bond-related behaviors of the prairie vole (19–25). Furthermore, insertion of either prairie or meadow vole microsatellite structure ahead of the mouse *avpr1a* coding region leads to measurable differences in AVPR1a density in mice brains, though these results do not fully explain the distribution variability observed among vole species (26). Recent work has expanded beyond microsatellite length into other sources of genetic variation. Single-nucleotide polymorphisms within regulatory sequences have been demonstrated to be good predictors of individual differences in cortical AVPR1a expression, sexual fidelity, and spatial use (23, 27, 28), though this is a weaker relationship in wild-caught voles compared with laboratory-reared animals potentially due to increased variation in the developmental environment in wild populations. Further research is needed to explain how *cis*-regulatory variants and other regulatory elements affect individual and species level AVPR1a distribution patterns and social behavior. Nevertheless, the relationship between AVPR1a distribution patterns and pair bond-related behaviors remains, and thus, it is appropriate to investigate the role of AVPR1a in the modulation of such behaviors unique to monogamous species such as the prairie vole. This review will discuss the role of general and site-specific AVPR1a activity in regulation of three key pair bond-related behaviors of the prairie vole: partner preference, selective aggression, and paternal care of the young.

PARTNER PREFERENCE

One of the defining characteristics of pair bond behavior in prairie voles is a preference for contact with the mate over an opposite-sex stranger, also known as partner preference (2, 29). In the lab, partner preference is measured using a three-chamber social interaction test in which the subject may choose to spend time by itself in a neutral chamber or interact with either the partner or a novel opposite-sex conspecific. If the subject shows a selective preference for contact with the partner rather than with the stranger during a 3-h assessment period, it is determined that a partner preference has been established (30). Male prairie voles will establish a partner preference after 24 h of cohabitation with a new mate (29, 31), and females exhibit this behavior as well (30, 32). This partner preference is enduring and lasts for at least 2 weeks of separation from the mate (33, 34). This preference is not infallible, however, and is diminished after 4 weeks of separation from the mate (33) and may be interrupted if the breeding pair is reproductively unsuccessful (35).

Recognition and affiliation are vital components of partner preference formation, and AVP has been implicated as necessary for these behaviors in mice (6, 7). Winslow and colleagues first demonstrated that male prairie voles will form a partner preference when receiving a central infusion of AVP during a short, non-mated cohabitation but not if receiving a central administration of a selective AVPR1a antagonist (AVPA) immediately prior to a long, mated cohabitation with a female (29). Another study later corroborated these results and revealed that AVP is involved in partner preference formation in female prairie voles as well (32). It has also been suggested that AVP is not only important in the formation of partner preference but also its expression (36). The display of a partner preference is inhibited in male prairie

[1] While AVP is an important neuromodulator of stress physiology [e.g., functioning as a secondary hypothalamic-pituitary-adrenal (HPA) axis secretagogue], it has yield to be determined the role that AVP serves in separation distress, consoling behavior, or social buffering.

voles that received centrally administered AVPA at the start of a 24-h mated cohabitation or immediately prior to behavioral testing. However, this was not the case for control subjects or those that received AVPA following the 24-h mated cohabitation period in which partner preference was assessed 3 d later, indicating that each administration of AVPA prior to cohabitation and the partner preference test was uniquely responsible for suppression of partner preference behavior. While these studies have suggested both the necessity and sufficiency of AVP in modulating partner preference, they do not suggest which regions of the brain may be involved in AVP-mediated partner preference neurocircuitry. There are a number of regions within the prairie vole brain with high levels of expression of AVPR1a that have been quantified (11–16). Of these regions, AVP signaling in the lateral septum (LS) has been thought to modulate social behavior and organization, and shows different AVPR1a binding levels between monogamous and non-monogamous vole species (11). In addition, the ventral pallidum (VP) shows differential AVPR1a binding between monogamous and non-monogamous vole species, and AVPR1a in this region promotes partner preference (37, 38). Thus, the LS and VP have become regions of interest in the study of the role of AVP in the modulation of partner preference behavior (**Figure 1**).

Lateral Septum

Activity of AVP in the LS is known to be vital for social recognition in rats and mice (43–46). Gene transfer of prairie vole AVPR1a in the septum of the rat brain improves recognition of familiar juveniles and promotes more active social interaction behavior (47). Similarly, higher expression of AVPR1a in the LS of male prairie voles is correlated with higher levels of investigatory behavior in response to a novel female (48). In addition, while AVPR1a expression is lower in the LS of male prairie voles compared with male meadow voles, vasopressinergic fiber density in the LS of male prairie voles is significantly higher than that of male meadow voles (39). This suggests that distinct and, potentially, more robust AVP signaling from presynaptic neurons into the LS, and despite lower postsynaptic receptor expression may serve as a mechanism of partner preference behavior. The LS receives vasopressinergic signaling from the bed nucleus of the stria terminalis (BNST) (39, 49), and AVP mRNA expression in the BNST is increased in male prairie voles

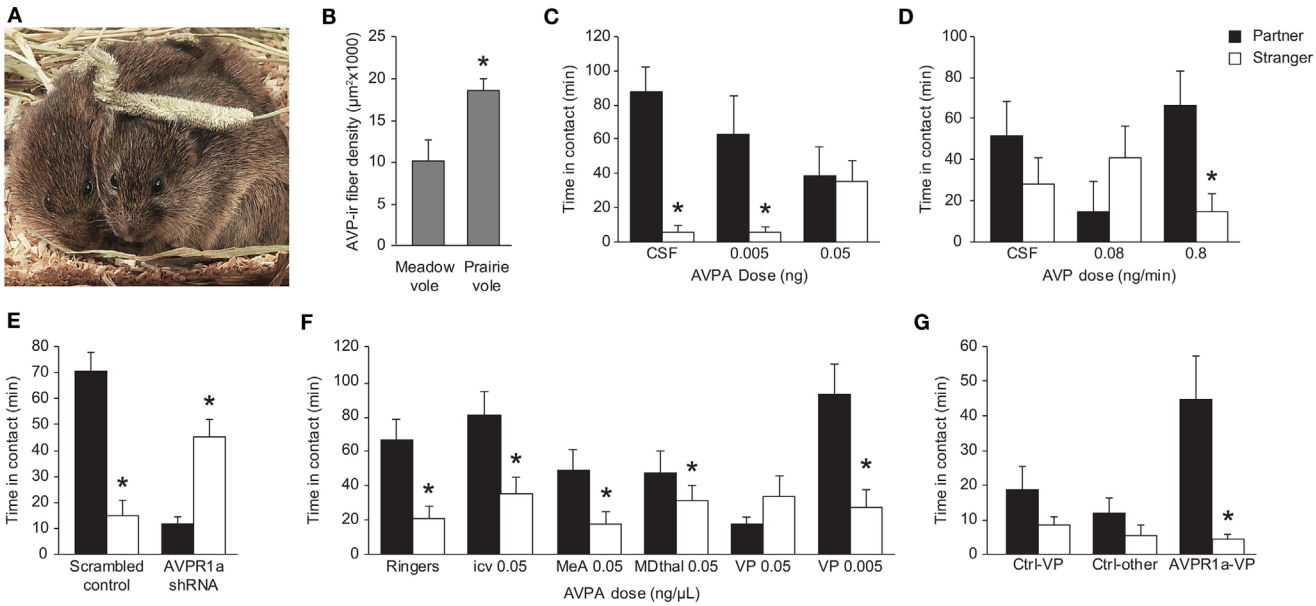

FIGURE 1 | Role of AVP in modulation of prairie vole partner preference. **(A)** A prairie vole breeding pair in direct side-by-side contact. Duration of this kind of contact is a typical marker for affiliation in a partner preference test. Photo credit: Charles Badland. **(B)** Male prairie voles have significantly greater AVP-ir fiber density in the LS compared with male meadow voles. *$p < 0.05$. Females of both species do not show robust AVP-ir fiber density in this region (data not shown). **(C)** Direct administration of AVPA into the LS of male prairie voles prior to a 24-h cohabitation with a sexually receptive female inhibits partner preference formation in a dose-dependent manner. *$p < 0.01$ versus duration in contact with the partner. **(D)** Direct infusion of AVP into the LS during a 6-h cohabitation with a non-receptive female induces partner preference in a dose-dependent manner. *$p < 0.05$ versus duration of contact with the partner. **(E)** Male prairie voles receiving a scrambled shRNA in the VP show a preference for the mate, but shRNA knockdown of AVPR1a in the VP leads to a preference for the stranger. *$p < 0.05$ between contact with the partner versus the stranger. **(F)** Administration of AVPA into the VP of male prairie voles inhibits partner preference formation. This effect is not seen in animals receiving Ringer's solution, i.c.v. administration of AVPA, administration of AVPA into the medial amygdala or mediodorsal thalamus, or administration of a 10-fold lower dose of AVPA into the VP. *$p < 0.05$ versus duration of contact with the partner. **(G)** Meadow voles overexpressing AVPR1a in the VP show a preference for the partner over the stranger following 24-h cohabitation with a sexually receptive female. This effect is not seen in control animals or stereotactic misses. *$p < 0.01$ versus duration of contact with the stranger. AVP, arginine-vasopressin; AVPA, AVPR1a antagonist; AVPR1a, AVP receptor subtype 1a; i.c.v, intracerebroventricular; ir, immunoreactive; LS, lateral septum; VP, vasopressin. Adapted/reproduced from Wang (39) and Liu et al. (40) with permission from American Psychological Association, Barrett et al. (41) and Lim and Young (42) with permission from Elsevier, and Lim et al. (38) with permission from Nature Publishing Group.

following a 3-d cohabitation with a female (50). This increase in AVP mRNA expression upstream of the LS is not seen in the non-monogamous meadow vole. Administration of AVPA into the LS of prairie voles during a 24-h mated cohabitation blocks partner preference, and AVP activation of AVPR1a in the LS during a 6-h non-mated cohabitation induces partner preference (40). While similar AVPR1a expression in the LS is observed in both male and female prairie voles (11, 12), AVP innervation into this region is significantly higher in males than it is in females (39, 51, 52). In addition, AVP innervation in the LS of male prairie voles varies over the course of cohabitation with a female, but this effect is not seen in females (53). Taken together, these findings not only implicate a role AVP activity in the LS in partner preference in male prairie voles but also suggest sexual dimorphism in the impact of AVP on partner preference.

Ventral Pallidum

The VP is located within the basal ganglia and is known to play a role in reward and motivation (54–56). In male prairie voles, it has been determined that AVP signaling and AVPR1a expression in the VP are important in partner preference formation. After 17 h of cohabitation with a non-receptive female, males overexpressing AVPR1a in the VP, but not in the caudate putamen or those treated with LacZ vector, exhibited a significant partner preference (37). A more recent study investigated the role of AVPR1a expression in the VP in modulating partner preference by using short hairpin RNA knockdown of the receptor in this region (41). Male prairie voles with reduced levels of AVPR1a in the VP showed a complete elimination of partner preference behavior. Pharmacological data have also determined that AVPR1a expression in the VP, but not all regions associated with reward or sociosexual neurocircuits, is specifically necessary for partner preference formation. Introduction of AVPA into the VP, but not the medial amygdala or mediodorsal thalamus, prior to a 22-h cohabitation with a receptive female blocked partner preference formation (42). AVPR1a expression in the VP of the prairie vole is considerably higher than in the VP of promiscuous cousins such as the montane vole and the meadow vole (*M. pennsylvanicus*) (13, 38), and overexpression of AVPR1a via virally mediated gene transfer into the VP of the meadow vole induces partner preference formation, a behavior not normally observed in this promiscuous species (38).

Expression of AVPR1a in the VP of female prairie voles varies depending on pair bond and reproductive status. Pair-bonded females exhibit elevated AVPR1a expression in the VP relative to single females, and AVPR1a expression in the VP drops during pregnancy (57). However, AVPR1a levels in the VP are actually elevated immediately following fertilization, and drop back down to pre-pregnancy levels as parturition approaches (58). These findings may also suggest a reason why earlier studies required a higher dose of AVPA in order to manipulate female partner preference behavior (32, 34). Pharmacological treatment of females early in pregnancy may yield different results. However, the behavioral effects of AVP in female prairie voles are not well characterized, and thus further study is needed to understand the behavioral significance of this variation in VP AVPR1a expression.

SELECTIVE AGGRESSION

Sexually naïve prairie voles are highly affiliative and socially tolerant, and will rarely act aggressively to unknown conspecifics. However, after mating has occurred and a pair bond has been established, prairie voles will display robust levels of aggression toward conspecifics entering their territory, but remain highly affiliative to their mates (29, 31, 59–63). This behavior is known as "selective aggression," which is a type of mate guarding (64, 65) that is specifically a result of mating and the formation of a pair bond. Male prairie voles that have a 24-h cohabitation and mating period with a female will display selective aggression, but prairie voles cohabitating with a same-sex conspecific or for a brief time with a female without mating will not (31, 59). In addition, the promiscuous montane vole, which does not form pair bonds, does not exhibit selective aggression (4). This selective aggression behavior is not limited to intruders of the same sex, as pair bonded male prairie voles will attack unknown female strangers (59–61, 63). Similar to other rodent species, female prairie voles are aggressive during pregnancy and following parturition (2). However, female prairie voles stand apart from females of other rodent species in that female prairie voles also display pair bond induced selective aggression, just as males do (66, 67). Similar to partner preference, selective aggression endures even after a week of separation from the mate (29), but is diminished after 4 weeks of separation (33).

Arginine-vasopressin has been suggested to have a role in aggression in the prairie vole. Administration of AVP via intracerebroventricular (i.c.v.) infusion increases aggressive behaviors of sexually experienced and reproductively successful male prairie voles toward unknown conspecifics (12). This effect is not seen in the non-monogamous montane vole. Infusion of AVPA into the lateral ventricles of male prairie voles that had experienced a 24-h mated cohabitation with a female reduces aggressive behaviors to pre-mated levels, suggesting that AVPR1a is the mediator of this effect (29). In addition, i.c.v. administration of AVP induces selective aggression in sexually naïve males. These findings suggest that AVP has a role in not only general aggressive behaviors but the formation of selective aggression as a form of mate guarding. However, central AVPA administration does not reduce aggression in established breeders, suggesting that full-brain modulation of AVPR1a activity is not sufficient to understand the role of AVP in established selectively aggressive behavior. Therefore, it is necessary to investigate the site-specific regulation of selective aggression by AVP in order to understand its role in expression and maintenance of this behavior.

Anterior Hypothalamus

Arginine-vasopressin in the anterior hypothalamus (AH) has been found to regulate aggression in hamsters (68, 69). Similarly, recent work by Gobrogge and Wang has established AVPR1a expression in the AH that is modulated by pair bonding, and its activation is important in the regulation of selective aggression in the prairie vole (**Figure 2**). AVPR1a binding levels are elevated in the AH of pair-bonded male prairie voles relative to non-pair-bonded animals (61). Intriguingly, virally mediated overexpression of AVPR1a in the AH facilitates selective

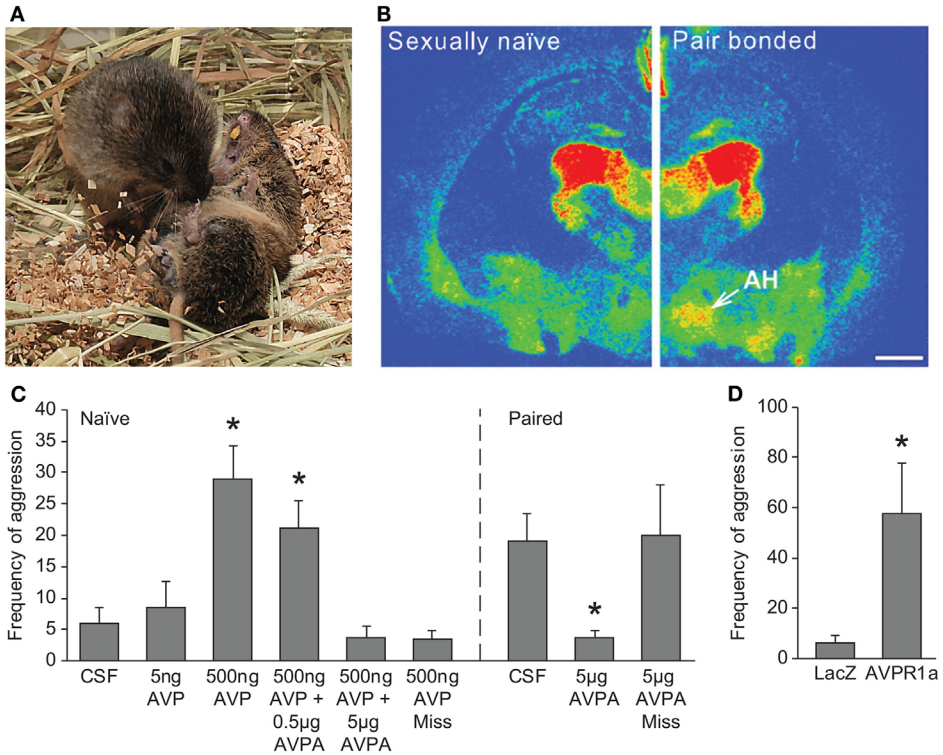

FIGURE 2 | Role of AVP in regulation of selective aggression in prairie voles. **(A)** Aggressive behavior is seen in prairie voles following cohabitation and mating with an opposite-sex animal. Photo credit: Charles Badland. **(B)** A significant increase in AVPR1a binding is observed in the AH of pair-bonded male prairie voles compared with sexually naïve male prairie voles. Scale bar is 1 mm. **(C)** Direct administration of AVP into the AH of sexually naïve male prairie voles induces aggressive behavior, and this effect is blocked by coadministration with AVPA. An increase in aggression is not observed with a stereotactic miss of the AH. Similarly, administration of AVPA into the AH of pair-bonded male prairie voles blocks bond-induced aggressive behavior. *$p < 0.05$ versus CSF-treated levels. **(D)** Virally mediated gene transfer of AVPR1a into the AH of sexually naïve male prairie voles induces aggressive behavior. *$p < 0.05$ versus LacZ vector control. AH, anterior hypothalamus; AVP, arginine-vasopressin; AVPA, AVPR1a antagonist; AVPR1a, AVP receptor subtype 1a; CSF, Cerebrospinal fluid. Adapted/reproduced from Gobrogge et al. (61) with permission from Proceedings of the National Academy of Sciences.

aggression in sexually naïve male prairie voles, suggesting that the increase in receptor expression following pairing may prime the selective aggression observed in male prairie voles. Certainly, exposure of either a female or male stranger, but not the partner, to a pair-bonded male increases neural activation, measured by Fos-immunoreactive (ir) labeling, in the AH (60). This information is supported by the finding that AVP release in the AH is positively correlated with aggression and negatively correlated with affiliation (61). Similar to the i.c.v. studies described above, site-specific AVP administration into the AH induces aggression toward novel females in naïve male prairie voles, and AVPA into the AH of pair-bonded animals reduces aggressive behavior toward stranger females (61). In addition, real-time infusion of AVPA into the AH of a pair-bonded male prairie vole while in the presence of a novel female reduces aggression and increases affiliation, and similar treatment with AVP while in the presence of the partner induces aggression toward the partner (63). These results suggest that AVPR1a in the AH is important not only for the formation of selective aggression but also the decision between aggressive or affiliative behaviors toward the partner or a novel female. Modulation of selective aggression by AH-AVPR1a has not yet been investigated in female prairie voles. Still, similar to a newly pair-bonded male, an increase in AH-AVPR1a has been observed in pregnant pair-bonded female prairie voles relative to pregnant non-pair-bonded female prairie voles (57). This may suggest that the role that AH-AVPR1a plays in regulating selective aggression in female prairie voles may be quite complex, involving both pair bond and pregnancy status.

PATERNAL CARE

Prairie voles stand out from many mammalian species in parental care of young. Other than nursing, prairie vole fathers are just as involved in the rearing of pups as mothers are, performing parental behaviors such as nest building, licking, grooming, huddling, and pup retrieval (70). In addition, juvenile and sexually naïve male prairie voles display alloparental care of neonates (51, 71–73). While juvenile females will display alloparental behaviors (74), sexually mature but inexperienced females are often neglectful of pups or even infanticidal (71, 72) unless raised to adulthood with the parents (75). For this reason, studies of alloparental care are often performed using male prairie voles. Administration of AVP into the lateral ventricles of sexually naïve male prairie voles does not increase parental behaviors above untreated and

vehicle-treated levels (76); this may be the result of a ceiling effect, since sexually naïve male prairie voles are already highly parental. In fact, central administration of AVP can diminish infanticide and promote paternal behavior in male meadow voles that were previously non-paternal, but does not affect paternal behavior in already paternal males (77). However, a high dose of AVPA leads to a higher frequency of pup attack, which is normally a rare behavior. Coadministration of AVPA with an oxytocin receptor (OTR) antagonist significantly reduces parental behaviors as well as further increases the incidence of infanticidal behavior, suggesting that AVPR1a and OTR may work in tandem to promote alloparental behavior.

Lateral Septum

Several studies have established a correlation between AVP-ir fiber expression in the LS and parental behaviors in both male and female prairie voles (**Figure 3**). AVP-ir fiber density is significantly higher in the LS of male prairie voles than female prairie voles (39, 51). In addition, an increase in AVP-ir fiber density in the LS following estrogen replacement is correlated with an increase in the incidence of maternal behavior in normally infanticidal, ovariectomized females (71). These findings are supported by pharmacological manipulation of AVPR1a in the LS; administration of AVP into the LS promotes parental behavior in naïve male prairie voles, and this effect is prevented by administration of AVPR1a prior to pup exposure (78). Not only does AVP in the LS regulate parental behavior, but AVP fiber density in the LS of male prairie voles is affected by cohabitation and the birth of the first litter as well. Male prairie voles have significantly fewer AVP-ir fibers in the LS than sexually naïve males shortly after mating, as well as 6 days following parturition (51, 53). This decrease in AVP fiber immunoreactivity may reflect an increase of AVP release that has not been recovered. This idea is supported by the finding that AVP mRNA expression in the BNST of male prairie voles is increased as a result of cohabitation with a female (50). These changes in AVP fiber density in the LS and mRNA expression in the BNST are not observed in female prairie voles or meadow voles of either sex (50, 51, 53), suggesting a role of AVP in the LS specifically in paternal behavior.

Although acute antagonism of AVPR1a in the LS leads to a decrease in paternal responsiveness (78), elimination of AVP-ir fibers in the LS as a result of castration does not lead to a decrease in parental behavior (71). This implies that AVP action alone in the LS is not responsible for the modulation of

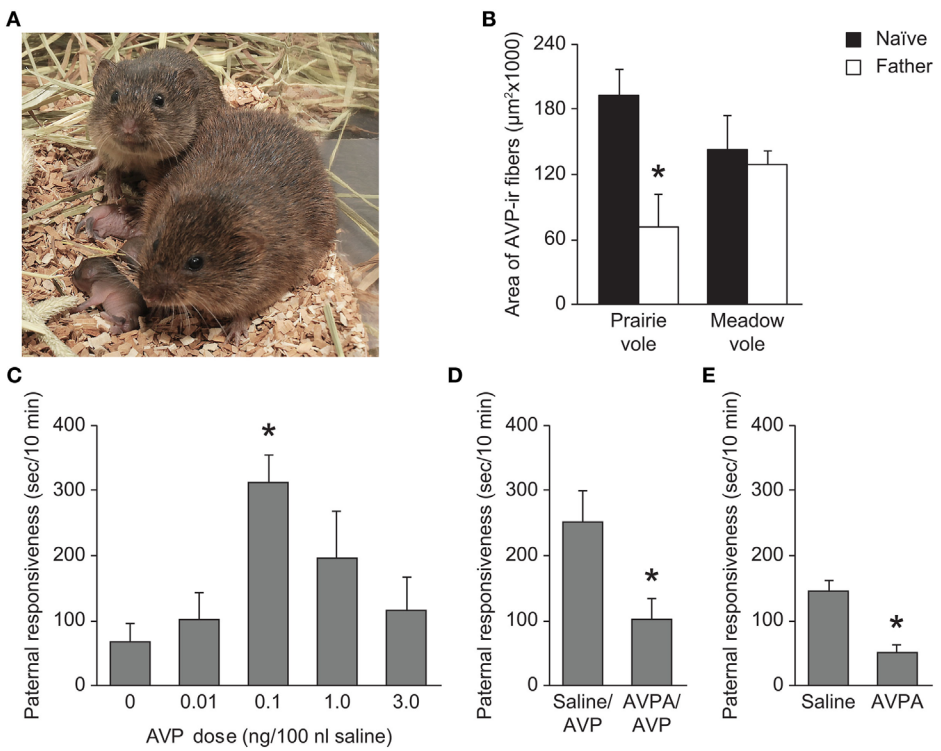

FIGURE 3 | Impact of AVP activity in paternal behavior of prairie voles. **(A)** Prairie voles are a biparental species, and with the exception of nursing, fathers perform all of the same parental behaviors as mothers such as nest building, huddling, and grooming of the young. Photo credit: Charles Badland. **(B)** Prairie vole fathers show a significant decrease in AVP-ir fiber density in the LS relative to sexually naïve prairie voles. This effect is not seen in meadow voles. *$p < 0.01$ versus naïve voles. **(C)** Administration of AVP into the LS of sexually naïve prairie voles increases paternal responsiveness. *$p < 0.0001$ versus saline-treated animals. **(D)** Administration of 1 ng AVPA in the LS reduces the AVP-induced (0.1 ng AVP) increase in paternal responsiveness. *$p < 0.05$ versus saline/AVP-treated animals. **(E)** Administration of 1 ng AVPA reduces baseline levels of paternal responsiveness. *$p < 0.001$ versus saline-treated controls. AVP, arginine-vasopressin; AVPA, AVPR1a antagonist; LS, lateral septum. Adapted/reproduced from Bamshad et al. (51) with permission from Karger Publishers and Wang et al. (78) with permission from Proceedings of the National Academy of Sciences.

parental behaviors, such that dampened AVP signaling can affect parental behavior transiently while other modulating signals compensate for a prolonged AVP deficit. The onset of paternal behavior may be independent of hormonal regulation, as paternal behavior is spontaneous and observed even in sexually immature juveniles (73). As AVPR1a activity in the LS promotes social recognition in rats and prairie voles (45, 46, 48) and promotes partner preference behavior (40), AVPR1a activation in the LS may induce recognition of a pup as a non-threatening and familiar conspecific that should be cared for. In addition, more recent research has found that only central administration (i.c.v.) of both AVPA and an OTR antagonist together, but not either antagonist alone, is sufficient to reduce parental behavior in naïve male prairie voles (76). This is in contrast to some of the site-specific pharmacology results with AVPA only, which may be due to the quite low concentration of AVPA used in the study, but it does raise the intriguing possibility that AVP and OXT act as redundant and compensatory signals to promote paternal behavior in voles. Moreover, OTR is expressed in the LS (17, 79) and OTR expression in LS is correlated with absence or presence of female alloparental care (74). Therefore, although AVP activity in the LS may promote recognition and reduce infanticide, OTR and AVPR1a in the LS may work in tandem to promote paternal care of the young, though this is only speculation.

DISCUSSION

The studies discussed above have supported a role of AVP in the formation, expression, and regulation of the pair-bond-related behaviors of the prairie vole. This role of AVP is absent in non-monogamous vole species that do not normally exhibit these behaviors. However, genetic manipulation of AVPR1a expression in specific regions of the brain can induce similar behavior in non-monogamous species, or conversely, eliminate these behaviors in the prairie vole. With this, and site-specific pharmacological manipulation of AVPR1a activity and subsequent behavioral consequences, there is strong evidence supporting a role of AVPR1a distribution and function in the neurobiological basis of social attachment. Central administration of AVP or an AVPR1a selective antagonist regulates behaviors such as partner preference, selective aggression, and paternal care (12, 29, 76). AVP regulation of social behaviors in the prairie vole may be acting through distinct neurocircuits with different roles in relationship formation and maintenance.

The regions in which AVP regulation of partner preference has been characterized, the VP and LS, play important roles in motivation and social recognition in prairie voles (37, 40, 48) and other rodent species (44, 55). In these regions, AVP has a role in regulating affiliative behavior such as partner preference and paternal response. Upregulation of AVPR1a activity in the VP promotes partner preference (37, 42), and reduction of AVPR1a expression in this region drives male prairie voles to prefer an unknown female over the mate (41). In addition, an increase in AVPR1a expression in this region in the non-monogamous meadow vole induces partner preference behavior (38). Upregulation of AVPR1a activity in the LS promotes partner preference (40) and increases paternal behaviors (78). This signaling may have an important role in the formation of affiliative relationships through promotion of recognition of social stimuli, such as a new partner or pup, as conspecifics that should be affiliated with or cared for.

Conversely, AVP signaling in the AH regulates behavior in a manner distinct from VP and LS modulation of positive social relationships. In the AH, AVP signaling regulates selective aggression, and the decision-making process between affiliation toward partners and aggression toward strangers is advantageous in the maintenance of an established bond. Manipulation of AVPR1a activity in the AH not only regulates aggressive behavior in male prairie voles (60, 61), but also appears to regulate the decision to be aggressive or affiliative to a conspecific (63). This regulation of selective aggression occurs as a result of various neurochemicals and brain regions signaling to the AH to either promote or inhibit AVPR1a activity. Overall, burgeoning evidence support a role of AVP in the formation and maintenance of social bonds in prairie vole, possibly through distinct neurocircuitry responsible for social recognition and social decision-making.

Despite this knowledge, some questions remain unanswered. For example, while the *cis*-regulatory element, specifically single-nucleotide polymorphisms and microsatellites, of the prairie vole *avpr1a* gene has been implicated in the regulation of AVPR1a distribution patterns, the specific mechanisms of control of protein expression have been poorly studied. Chromatin remodeling at the *avpr1a* gene has been implicated in the regulation of partner preference behavior. Administration of a histone deacetylase inhibitor into the nucleus accumbens of female prairie voles, but not male prairie voles, upregulates AVPR1a expression and promotes partner preference behavior (80, 81). These findings suggest a sex-specific mechanism of epigenetic modulation of pair bond-related behaviors, and further investigation in this direction could give valuable insight into regulation of social behavior at the transcriptional level. Second, the downstream signaling of AVPR1a and its impact on the regulation of social behavior in the prairie vole has been hardly investigated. One study has examined site-specific induction of phosphoinositol, a second messenger of $G_{\alpha q}$ signaling, following introduction of AVP into the brains of prairie voles and montane voles, and found species differences in regional induction (11). This study, however, did not investigate the impact of site-specific phosphoinositol induction or reduction on social behavior in these species, nor did it investigate induction of other secondary messengers such as cAMP. While AVPR1a has been characterized as a $G_{\alpha q}$-GPCR in hepatocytes (82), it has been suggested to have $G_{\alpha s}$ action in neurons (83). Thus, its signaling cascade should not be assumed.

In addition, one of the shortcomings of not only the studies discussed above, but of prairie vole research in general, is the use of the standard 24-h cohabitation to study neurobiological regulation of pair bond-related behaviors. While this length of cohabitation plus the presence of mating is usually sufficient to induce partner preference and selective aggression, two characteristic behaviors of a pair bond, this model only gives insight into the early stages of pair bond formation. A longer cohabitation period on the scale of days to weeks would allow for investigation of the maintenance of this bond and the behaviors associated with it. Finally, one of the major benefits of the prairie vole model is the similarity of behavior between males and females (29, 30).

This allows for investigation of sexual dimorphism in the neurobiological basis of these behaviors. However, much of the research of the impact of AVP on pair bond-related behaviors use male voles and neglect to study any potential impact of AVP in female voles. While it does seem males may be more sensitive to AVP and females to oxytocin (34), there does still appear to be some impact of AVPR1a in the pair bond-related behaviors of female prairie voles (32, 57). Therefore, it is worth including female prairie voles in any study investigating the impact of AVP in social behavior, and if no impact is found, it can be reported so that sexual dimorphism can be noted. In conclusion, while much has been established about the impact of AVP and its action through AVPR1a in the regulation of social behavior, much remains to be discovered. The prairie vole will continue to be a useful model in answering these questions.

SUMMARY

One of the cognitive mechanisms underlying the formation of a pair bond is theorized to be the learned association between the memory of a partner and reward. Intriguingly, AVP in the LS serves to promote social recognition in rats and prairie voles (45, 46, 48) and partner preference behavior in prairie voles (40). In addition, partner preference formation is facilitated by AVP signaling and AVPR1a expression in the VP (37), a brain region known to play a role in reward and motivation. It is possible that these two neuronal inputs, one regulating recognition and the other reward, converge to promote the selective partner preference. If so, the AVP signal functions as a multimodal neuromodulator of this cognitive mechanism that triggers such bond formation. The AVPR1a distribution in the brain contributes to the social structure of the prairie vole, and genetic variation of the *cis*-regulatory elements of the *avpr1a* gene appear to contribute to these patterns. Thus, as more is learned about the genetic variants that contribute to AVPR1a distribution in the brain, as well as a stronger effort to determine downstream signaling of AVPR1a, this will lead to a coherent framework of the genetic and cellular basis of the AVP system on individual and species level differences in social behavior.

AUTHOR CONTRIBUTIONS

MT and AS conceived the review, acquired and critically analyzed the literature, and wrote and critically revised the manuscript.

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REVIEW
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Sex Differences in the Regulation of Offensive Aggression and Dominance by Arginine-Vasopressin

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Arginine-vasopressin (AVP) plays a critical role in the regulation of offensive aggression and social status in mammals. AVP is found in an extensive neural network in the brain. Here, we discuss the role of AVP in the regulation of aggression in the limbic system with an emphasis on the critical role of hypothalamic AVP in the control of aggression. In males, activation of AVP V1a receptors (V1aRs) in the hypothalamus stimulates offensive aggression, while in females activation of V1aRs inhibits aggression. Serotonin (5-HT) also acts within the hypothalamus to modulate the effects of AVP on aggression in a sex-dependent manner. Activation of 5-HT1a receptors (5-HT1aRs) inhibits aggression in males and stimulates aggression in females. There are also striking sex differences in the mechanisms underlying the acquisition of dominance. In males, the acquisition of dominance is associated with the activation of AVP-containing neurons in the hypothalamus. By contrast, in females, the acquisition of dominance is associated with the activation of 5-HT-containing neurons in the dorsal raphe. AVP and 5-HT also play critical roles in the regulation of a form of social communication that is important for the maintenance of dominance relationships. In both male and female hamsters, AVP acts *via* V1aRs in the hypothalamus, as well as in other limbic structures, to communicate social status through the stimulation of a form of scent marking called flank marking. 5-HT acts on 5-HT1aRs as well as other 5-HT receptors within the hypothalamus to inhibit flank marking induced by AVP in both males and females. Interestingly, while AVP and 5-HT influence the expression of aggression in opposite ways in males and females, there are no sex differences in the effects of AVP and 5-HT on the expression of social communication. Given the profound sex differences in the incidence of many psychiatric disorders and the increasing evidence for a relationship between aggressiveness/dominance and the susceptibility to these disorders, understanding the neural regulation of aggression and social status will have significant import for translational studies.

Keywords: gender differences, agonistic behavior, social status, social communication, oxytocin, serotonin, V1a receptors, V1b receptors

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AGGRESSION

Aggression is a complex phenomenon that has many different forms and adaptive functions (1–4). Aggression has been classified in a variety of ways, but the three forms of aggression that have been studied most extensively and that may be most closely linked with reproductive success are offensive aggression, defensive aggression, and parental aggression (the most frequently studied form of parental aggression is maternal aggression). Offensive aggression has been defined as aggression involving

response to challenge over important resources; defensive aggression as attack in defense of an individual's own integrity; and maternal aggression as aggression aimed at intruders threatening the offspring of a lactating female. As noted by Blanchard et al. (5), "the distinction between offensive and defensive aggression is based on a number of aspects of behavior, including antecedent conditions, organismic variables, attack topography (target of attack on the opponent's body), and typical outcomes." The ethograms for offensive and defensive aggression are unique (6). Maternal aggression appears to include elements of both offensive and defensive aggression and probably represents a mixed category (7). Therefore, based on these criteria, the current consensus is that offensive, defensive, and maternal aggression represent different categories of aggression, although similar behaviors can, at times, be observed in all three categories. In this review, we will focus on the role of arginine-vasopressin (AVP) in mammalian offensive aggression and how this role relates to the formation and maintenance of social relationships [for reviews of the role of AVP in maternal aggression see Ref. (7–9)]. When aggression occurs during a social encounter between strangers, both offensive and defensive aggression is observed. Winners and losers are rapidly determined and winners become dominant and losers subordinate. Along with the formation of dominant/subordinate relationships, aggressive behavior declines and other agonistic behaviors that serve to reinforce social status occur in increasing frequency (e.g., social communication). In the second section of this review, the role of AVP in the formation and maintenance of social status will be discussed.

AVP and the Neural Control of Offensive Aggression in Males

Historically, AVP has been well known for its many physiological actions in the periphery, including water reabsorption and cardiovascular homeostasis (10). More recently, however, AVP has been identified as a critical neurochemical signal in the neural circuitry regulating offensive aggression. Neuroanatomical studies have demonstrated that in addition to the well-known AVP-containing hypothalamic projections to the neurohypophysis, there is an extensive neural network of AVP-containing projections throughout the brain (11–13) (**Figure 1**). For example, in Syrian hamsters, AVP neurons originating from hypothalamic nuclei, such as the medial supraoptic nucleus (mSON), nucleus circularis (NC), and paraventricular nucleus (PVN), project to many brain regions thought to be involved in regulating social behavior. There is increasing evidence that this AVP circuitry is engaged during social encounters. During agonistic encounters, enhanced activation of AVP-containing neurons in the mSON and NC is observed in male hamsters compared to controls (13). Cross-fostering male California mice with the less aggressive and less territorial white-footed mouse reduces adult aggression and the amount of AVP- immunoreactivity (ir) in the mSON and the bed nucleus of the stria terminalis (BNST) (14). After an agonistic encounter between female pigs the expression of AVP mRNA is enhanced in the medial amygdala, septum, and BNST of aggressive compared to non-aggressive individuals (15).

The first evidence that AVP is involved in the control of aggression came from the finding that injection of an antagonist of the V1a AVP receptor (V1aR) into the anterior hypothalamus (AH) of male hamsters inhibits aggression (25, 26). Subsequent studies in hamsters and voles have found that AVP administered within the AH can stimulate high levels of aggression (27–30). Interestingly, however, the ability of AVP to stimulate aggression within the AH appears to depend on an upregulation in the number of V1aRs in the AH. This upregulation appears to occur as the result of specific types of social experience. For example, AVP is effective in increasing aggression following its injection into the AH in male hamsters that had previously been trained to fight but not in hamsters housed in stable social groups (27, 31). Socially isolated male hamsters also display higher

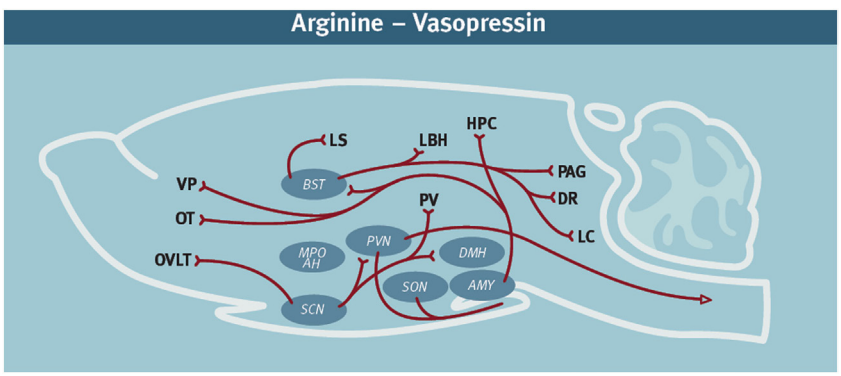

FIGURE 1 | Diagram of the arginine-vasopressin (AVP)-containing neural network in rodents. It is noteworthy that AVP immunoreactivity can vary by species, sex, age, and social experience (16, 17). These diagrams represent a compilation of the major AVP projections from several rodent species. In addition to the cell bodies indicated in the diagram, there are also accessory nuclei that likely also play an important role. AVP network (11, 18–23). Abbreviations: AMY, amygdala; DR, dorsal raphe; HPC, hippocampus; LS, lateral septum; LC, locus coeruleus; MPO AH, medial preoptic area—anterior hypothalamus; OT, olfactory tubercle; OVLT, organum vasculosum laminae terminalis; PVN, paraventricular nucleus; PAG, periaqueductal gray; PV, periventricular nucleus hypothalamus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; VP, [figure modified from Ref. (24) with permission].

levels of aggression and greater numbers of V1aRs in the AH than socially housed males (32, 33).

The upregulation of V1aRs in the AH also appears to be important in AVP regulation of male aggression in voles. Following pair bonding, male prairie voles engage in high levels of aggression and have significantly more V1aRs in the AH than sexually naïve voles (29, 34). There is also significantly more aggression and higher levels of AVP released in the AH in pair bonded males exposed to novel females than in males exposed to their female partners. It has been suggested that AVP is necessary for the transition to increased aggressiveness following pair bonding but not for the expression of aggressive behavior (34). In support of this idea is the finding that V1aR antagonists block the induction of partner preference but not the expression of aggression displayed by breeder male voles with established selective aggression (34). On the other hand, induction of large numbers of V1aRs in the AH by viral vector-mediated gene transfer significantly increases aggression in non-pair bonded males (29). In summary, social experience can modulate the number of V1aRs in the AH and the number of V1aRs in the AH can regulate the amount of male offensive aggression. Nevertheless, the precise role of V1aRs in regulating aggression remains to be determined and it is important to note that high levels of aggression can occur in the absence of V1aR activation at least in males with prior aggressive experience.

Further support for the role of AVP and V1aRs in the control of aggression comes from studies examining how drugs of abuse can stimulate aggression. Anabolic steroids (AAS) administered to adolescent male hamsters produce high levels of aggression that is inhibited by a V1aR antagonist injected into the AH (35). AAS also increases AVP fiber density and content within the AH without altering V1aR binding (36–38). Conversely, after 18 days of withdrawal from AAS, there is a reduction in both aggressive behavior and AVP fiber density in AAS-treated male hamsters (39). Chronic, low-dose cocaine treatment during adolescence increases adulthood aggression and electrically stimulated AVP release in the AH of male hamsters (40, 41). In male prairie voles, administration of amphetamine increases aggression and increases V1aR binding in the AH (29). In summary, drugs of abuse can act within the AH to alter aggression by influencing the amount of AVP innervation and/or by altering the number of V1aRs.

Another hypothalamic region where AVP plays an important role in male aggression is the ventrolateral hypothalamus (VLH). Injection of AVP into the VLH increases aggression in gonadally intact male hamsters and castrated males given testosterone but not in castrated males without testosterone replacement (42). The effects of testosterone on AVP-stimulated aggression are likely mediated by the effects of testosterone on V1aR number in the VLH. Castration reduces V1aR binding in the VLH and pre-castration levels of V1aR binding can be restored by the administration of testosterone (43–45).

There is also evidence for a relationship between aggression and AVP in other regions of the limbic system. In male California mice, aggression is positively correlated with the number of AVP-ir neurons in the posteromedial BNST (46). In the septum, a negative correlation between AVP fiber density and male aggression has been observed in strains of rats and mice bred for varying levels of aggression (47, 48). In other studies, employing rats selected for low or high anxiety, the release of AVP in the septum is considerably lower in the much more aggressive low anxiety rats than in the less aggressive, high anxiety rats. Interestingly, however, injection of AVP or a V1aR antagonist into the septum does not alter the expression of aggression in the low aggressive or high aggressive rats (49). Thus, although relationships between aggression and AVP-ir and release exist in the LS, there is no direct evidence that AVP acts in the septum to modulate aggression.

Gene targeting approaches have also been used to study the role of AVP receptors in male aggression. Surprisingly complete knockout of V1aRs has no effect on aggression in male mice and produces only a slight deficit in olfaction (50). By contrast, knockout of AVP V1b receptors (V1bR) significantly reduces aggression (51, 52). Further the reduction in offensive aggression in V1b knockout mice is due to deficits in social motivation and not in deficits in olfactory function (53, 54). Interestingly, knockout of the V1bR gene produces deficits specific to forms of aggression directed toward a conspecific (55). Further support for a role of V1bRs in aggression come from pharmacological studies where peripheral administration of a V1bR antagonist reduced aggression in male hamsters (56). V1bRs in the hippocampus likely play a prominent role regulating aggression. When V1bR function is restored in the CA2 region of the hippocampus in male knockout mice by lentiviral delivery of the V1bR gene, offensive aggression is partially restored (57). The ability of mice to express aggression in the absence of V1aRs is surprising given the considerable pharmacological data in other species indicating the importance of V1aRs in male aggression. The simplest explanation for the continued aggressiveness of these knockout mice might be a developmental compensation for the life-long loss of V1aRs. Perhaps the V1bR acts as such a compensatory mechanism that preserves aggressive behavior in the V1aR knockout mice.

AVP and the Neural Control of Offensive Aggression in Females

Little is known about the neurobiology of offensive aggression in females. The emphasis of competitive behaviors, such as offensive aggression in sexual selection in males, has resulted in little attention being paid to the neural control of offensive aggression in females (58). It is clear, however, that females as well as males compete for resources and mates to achieve reproductive benefits and that female competition has a significant role in evolution in mammals (59–61). Females compete for resources such as food, nest sites, and protection using a number of different strategies, including intergroup aggression, dominance relationships, and territoriality. As such, there are similarities in many of the competitive behaviors expressed by males and females (e.g., offensive aggression). Although many of the competitive behaviors displayed by males and females are similar, the neural mechanisms controlling them may be fundamentally different. Evolutionary biology would suggest that the social strategies used by females and males evolved in response to very different selective pressures. As a result, it seems likely that some of these behavioral similarities arose as the result of convergent evolution in the neural mechanisms

controlling social behavior. Indeed, the likelihood that there are sex differences in the neural mechanisms regulating aggression highlight the importance of a better understanding of how these mechanisms function in males and females. Given the substantial sex differences in the incidence of many psychiatric disorders, understanding the sex differences in the neural mechanisms regulating social behavior has the potential for substantial translational significance (62).

Another reason for the absence of data on the neurobiology of offensive aggression in females is the choice of species used to study the physiological mechanisms controlling aggression. Studies of the physiology of aggression have been conducted primarily in laboratory rats and mice (5, 7). Because laboratory rats and mice rarely display female offensive aggression, few studies on the physiological regulation of these critically important forms of aggression have been conducted (63, 64). Another laboratory species, Syrian hamsters, provide an outstanding model with which to study female competitive behavior. Female hamsters, like many female primates, display a number of different competitive strategies such as the expression of spontaneous offensive aggression, the rapid formation of hierarchical dominance relationships, and the ability to inhibit the reproductive capacity of other females (65–67).

Studies in hamsters provide the first evidence that there are fundamental sex differences in the neural circuitry controlling offensive aggression and that some of these sex differences involve AVP. AVP in the AH has opposite effects on the expression of aggression in males and females. Injection of AVP into the AH of female hamsters reduces aggression, whereas injection of a V1aR antagonist in female hamsters increases aggression (68, 69). While social isolation increases aggression in female hamsters, as it does in male hamsters, there is no increase in V1aR density in female hamsters as there is in males (33).

There are also some interesting sex differences in the developmental effects of AVP on adult aggression. AVP administered during the early postnatal period influences the expression of adult aggression in a sex-dependent manner (70, 71). Male but not female prairie voles exhibit significantly higher levels of aggression as adults when administered AVP peripherally during the early postnatal period. Taken together, the existing data indicate that AVP can play a critical, but opposite role in the regulation of aggression in males and females.

There is only a limited amount of data on the role of AVP in aggression in primates. In chimpanzees, polymorphisms of the V1aR gene are associated with enhanced or reduced aggressive behaviors (72). In humans, AVP cerebrospinal fluid levels positively correlate with a life history of aggression in individuals who meet DSM-IV criteria for personality disorder (73). Other studies in humans while not directly addressing the effects of AVP on aggression have identified some striking differences in the effect of AVP on social cognition. Intranasal administration of AVP in humans has sex-dependent effects on the social valence of stimuli (74). In women, AVP enhanced the perception of friendliness in the faces of unfamiliar women and stimulated affiliative facial motor patterns. In men, AVP reduced the perception of friendliness in the faces of unfamiliar men and stimulated agonistic facial motor patterns.

AVP Interactions with Other Neurochemical Signals in the Control of Aggression

Arginine-vasopressin interacts with several neurochemical signals to regulate offensive aggression. In males, serotonin (5-HT) has potent inhibitory effects on the expression of aggression in species ranging from fish to primates and at least some of these effects of 5-HT are mediated by its interactions with AVP (30, 75). One site where AVP and 5-HT likely interact to regulate aggression is the AH. The AH receives AVP-containing projections from the mSON and NC as well as 5-HT-containing projections from the raphe (13, 76). Co-infusion of AVP and the 5-HT1a receptor (5-HT1aR) agonist 8-OH-DPAT into the AH of male hamsters produces higher levels of aggression than 8-OH-DPAT alone and lower levels of aggression than AVP (69, 77). Systemic administration of the selective serotonin reuptake inhibitor, fluoxetine, decreases aggression in male hamsters (27) and pre-treatment with fluoxetine blocks AVP-induced aggression in both the AH and VLH (42, 77, 78). Chronic administration of fluoxetine during male adolescence increases both adulthood aggression and AVP fiber innervation in the AH (79, 80).

Surprisingly, although the effects of 5-HT have been investigated in hundreds of studies in males, not until recently have the effects of 5-HT been examined in females (81, 82). By contrast, to the striking inhibitory effects of 5-HT on aggression in males, injection of 8-OH-DPAT into the AH produces a dose-dependent increase in aggression in females. Co-infusion of AVP and 8-OH-DPAT into the AH of female hamsters produces lower levels of aggression than 8-OH-DPAT alone and higher levels of aggression than AVP (69, 77). Taken together, these studies indicate that AVP and 5-HT act in opposite ways within the AH to regulate offensive aggression in males and females.

Oxytocin (OT) is very similar in structure to AVP sharing seven of nine amino acid sequences (83, 84). In addition, OT and AVP receptors are very similar in structure and can respond in a relatively unselective manner to both neuropeptides (24). Indeed, OT can have effects on aggression similar to those of AVP. Like AVP, OT injected into the AH reduces aggression in a dose-dependent manner in female hamsters (85). Injection of an OT antagonist into the AH increases aggression in female hamsters (85). Total OT knockout male and female mice have higher levels of aggression than controls as do total male OT receptor (OTR) knockouts (86–88). There is increasing evidence that OT and AVP can influence a number of different social behaviors by acting on each other's receptors. OT or AVP induce social communication behavior by activating V1aRs but not OTRs, while OT or AVP can enhance social recognition and social reward by activating OTRs but not V1aRs (24, 89–92). While both OT and AVP can influence the expression of aggression the roles of OT and AVP receptors in mediating these effects remains to be fully understood.

Glutamate is also capable of interacting with AVP in the neural control of aggression. In a model of heightened male aggression in which male hamsters are chronically exposed to AAS during adolescence, glutamate receptor subunit type 1 density

is increased in the VLH and vesicular glutamate transporter 2 density is increased in the AH, two brain regions where AVP acts to control aggression (93, 94). Interestingly, AVP can induce the release of glutamate from astrocytes through binding of both V1aRs and V1bRs (95). AVP-induced release of glutamate provides a potential mechanism through which offensive aggression could be facilitated within the AH and VLH.

SOCIAL STATUS

For most mammalian and non-mammalian species, the formation and maintenance of dominance relationships rely on agonistic behaviors, particularly aggression and social communication. Although aggression has been commonly characterized as a negative social interaction, aggression plays a very constructive role in the formation of these important social relationships. In nearly all mammals, dominance relationships represent social relationships that have many adaptive functions (e.g., resource distribution) ultimately resulting in a reduction of social conflict (96). Most dominance relationships are determined rapidly by aggression but are primarily maintained by social communication (e.g., scent marking, vocalization, non-contact aggression or harassment, etc.) thereby reducing the dangers of continual, intense conflict (97). Success in maintaining these relationships depends on social skills that are also hallmarks of psychiatric health, such as effective social communication. If, for example, social communication is dysfunctional, then the social interactions become maladaptive, resulting in continuously high levels of social conflict (98). Importantly, dominance relationships have different consequences for the winners and the losers. Winning is rewarding (99, 100) and losing is stressful (101), particularly if subordinate status is imposed continually over time (102, 103). Indeed, losing is known to be a potent and ethologically relevant stressor and has become a leading model for investigation of the neural circuits and behavioral phenotypes (104) that are activated by social stress (105–107) and that have been widely shown to be useful models of stress-related mood and anxiety disorders (108–110).

AVP and the Neural Control of Social Status in Males

Social relationships among animals commonly take the form of hierarchical dominance relationships. Although the focus of this review is on the role of AVP in social status in mammals, there is considerable evidence that the non-mammalian homolog of AVP, arginine-vasotocin plays an important role in dominance relationships in birds, reptiles, amphibians, and fish [for reviews, see Ref. (111–115)]. Given its role in aggression, it is not surprising that AVP is also involved in the acquisition and maintenance of dominance relationships. In male hamsters, AVP-ir neurons in the mSON and NC display significantly higher levels of activation (as indicated by increased AVP-ir/fos-ir) in winners (i.e., dominants) than in losers (i.e., subordinates) or controls after a single agonistic encounter (69) (**Figure 2**). Differences in social status are associated with differences in AVP-ir and V1aR binding in the hypothalamus. Subordinate male hamsters in stable dominance relationships have significantly fewer AVP-ir neurons in the NC compared to dominant and control males (116) and dominant male hamsters have significantly more V1aR binding than subordinate or control hamsters in the hypothalamus (117). V1bR knockout male mice can establish dominance hierarchies suggesting that V1bRs may not be essential for the formation of these relationships. Non-dominant V1bR knockout mice do, however, engage in less offensive aggression than wild-type controls (118).

Social status is associated with different amounts of AVP-ir in several hypothalamic regions. In mandarin voles, dominant males have significantly more AVP-ir in the PVN, SON, LH, and AH than subordinates (119). Interestingly, no differences were observed in OT-ir between dominant and subordinate male voles in any of these brain regions except the PVN where OT-ir was lower in dominant than in subordinate males. By contrast, in male mice subordination seems to be associated with higher levels of AVP-ir in the PVN. In male California mice, a single social defeat significantly increases the activation of AVP neurons (i.e., AVP-ir/fos-ir) in both the PVN and SON (46). In addition, more than 10 weeks after social defeat AVP mRNA and AVP-ir were significantly reduced in the PVN. In an inbred strain of mice, chronic defeat increases AVP mRNA expression in the PVN of males (120). Adolescent male hamsters that are chronically defeated are more aggressive in adulthood toward smaller, weaker hamsters and less aggressive toward larger, stronger hamsters (121). Social subjugation also results in a 50% reduction in AVP-ir fiber density in the region of the AH involved in regulating aggression (121).

Several studies have also examined the effects of the porcine form of vasopressin, lysine-vasopressin (LVP), on dominant and subordinate behavior. LVP differs from AVP in that lysine is substituted for arginine in the eighth amino acid position. In male mice, administration of LVP increases submissive behavior when given prior to social defeat, immediately after social defeat, or immediately prior to a social interaction the day following social defeat (122, 123). By contrast, LVP administration prior to social defeat has no effect on dominance behavior (122–124). Interestingly, LVP binds to OTRs with a higher affinity than to AVP receptors suggesting the possibility that the ability of LVP to enhance submissive and not dominance behavior is mediated by OTRs (125).

A key element in the maintenance of dominance relationships is effective social communication (3, 97, 98). In hamsters, an important form of social communication is a type of scent marking called flank marking (126). Flank marking is used to communicate a variety of socially important information including social status (98, 126, 127). AVP plays a critical role in regulating flank marking by its actions within the hypothalamus as well as several other structures including the lateral septum and periaqueductal gray (128–132). Indeed, injection of AVP into the hypothalamus induces high levels of flank marking and injection of a selective V1aR antagonist blocks the expression flank marking (90, 133–136). After an initial agonistic encounter between two hamsters where dominance is defined by the winner of aggressive interactions, the levels of aggression rapidly decline and the expression of flank marking increases (98). Interestingly, flank marking increases in both the dominant and subordinate hamster, although the amount of flank marking

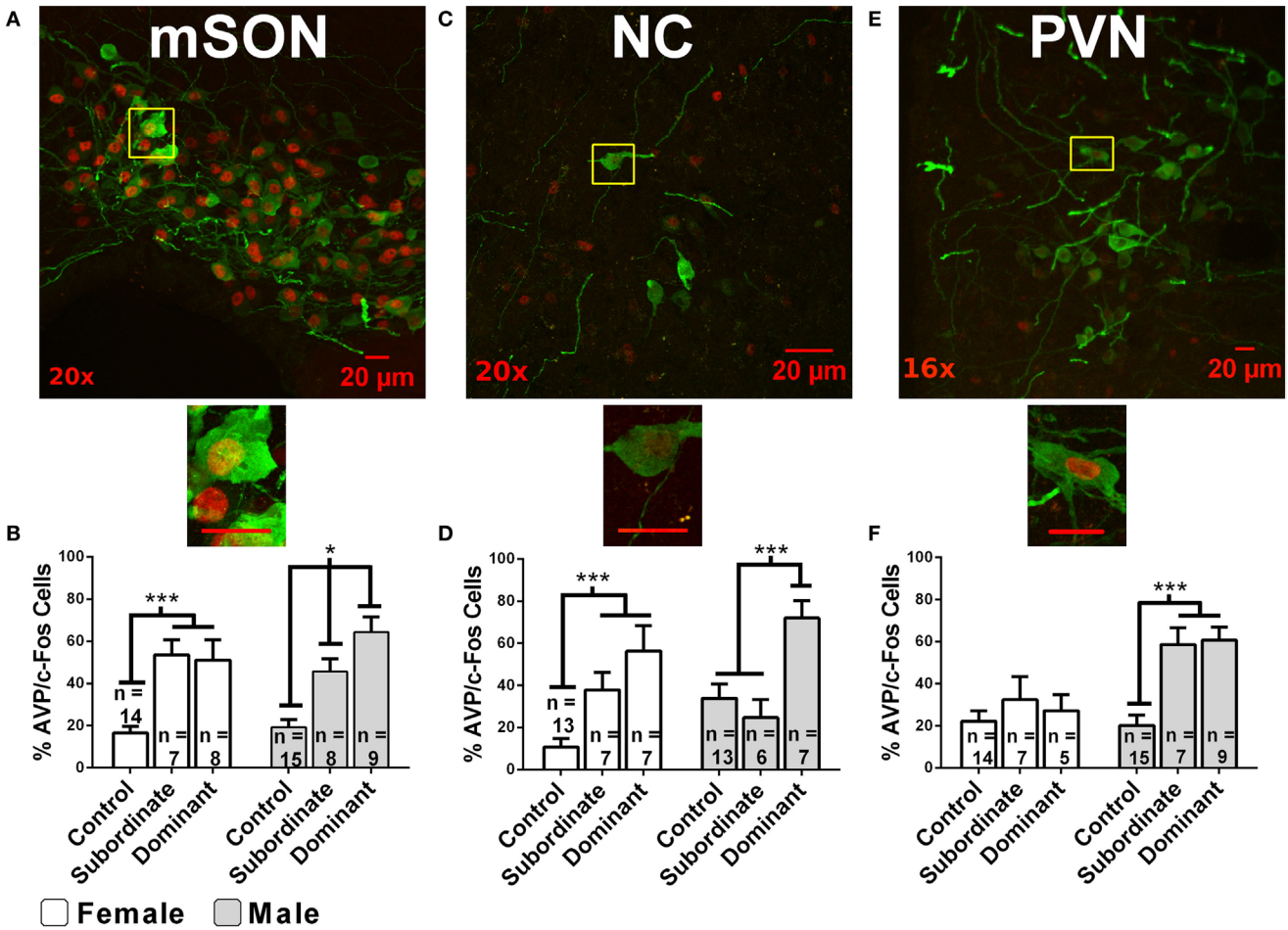

FIGURE 2 | Immunofluorescent colocalization of AVP-ir (green) and fos-ir (red) in cells within the following hypothalamic nuclei: the medial supraoptic nucleus [mSON; **(A,B)**], nucleus circularis [NC; **(C,D)**], and medial paraventricular nucleus [PVN; **(E,F)**]. Magnification is indicated in the bottom left corner. Yellow boxes indicate subregions magnified to 40×. Scale bars are 20 µM. Graphs indicate the percentage of AVP-ir cells that colocalize with fos-ir cells (% of activated AVP cells) as a function of acquired dominance status and sex in the mSON, NC, or PVN. White arrows indicate AVP-ir cells colocalized with fos-ir. Error bars indicate SEM. * indicates $p < 0.05$ and *** indicates $p < 0.01$ [figure modified from Ref. (69) with permission].

is substantially higher in the dominant. When the dominant hamster's flank marking is inhibited by hypothalamic injection of a V1aR antagonist the subordinate increases its flank marking (137). Interestingly, inhibition of flank marking in dominant hamsters by the injection of a V1aR antagonist while simultaneously stimulating flank marking in the subordinate by the injection of AVP for several consecutive days does not produce a reversal of social status. On the day immediately after the injections are terminated, the initially dominant hamster again flank marks significantly more than its subordinate partner (**Figure 3**). Thus, while flank marking communicates dominance status, it does not determine dominance status.

AVP and the Neural Control of Social Status in Females

Comparatively little is known about the role of AVP in the neural mechanisms regulating dominance relationships in females. One approach has been to examine the relationship between the numbers of AVP-ir neurons and social status. In mandarin voles, dominant females have significantly more AVP-ir cells in the AH and LH than subordinates (119). No differences were found in the number of AVP-ir cells in either the PVN or SON. Significant sex differences in the number of AVP-ir cells were also observed. Dominant females have significantly fewer AVP-ir cells in the PVN, SON, AH, and LH than dominant males and subordinate females have significantly lower levels of AVP-ir than subordinate males. No differences were observed in OT-ir between dominant and subordinate females in any of the brain regions examined.

Another approach has been to examine the relationship between social status and the activation of AVP-containing neurons. Following social defeat, female California mice, like males, have significantly more activated AVP neurons (i.e., AVP-ir/fos-ir) in both the SON and PVN (46). While the acute of effects of defeat are similar in females and males the long term effects of defeat on AVP neurons is stronger in males than in females. In hamsters, neuronal activation in AVP-containing neurons (i.e., AVP-ir/fos-ir)

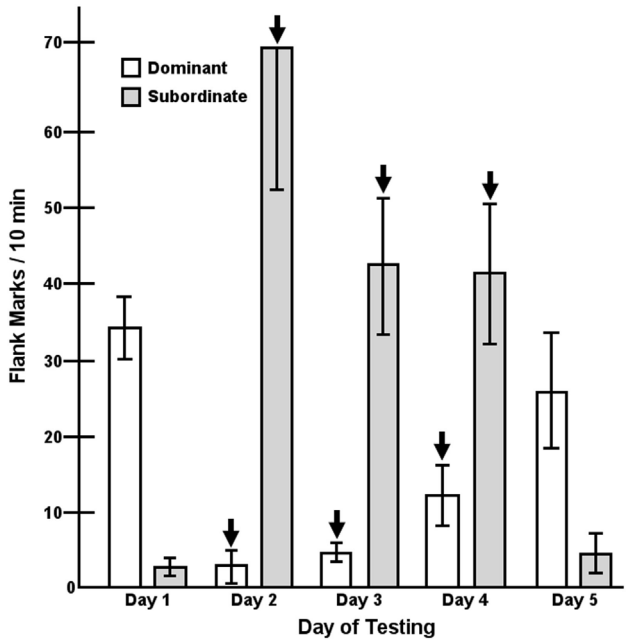

FIGURE 3 | Flank marking in pairs (n = 5) of dominant/subordinate male hamsters in response to the microinjection of dPTyr(Me)AVP [V1a receptor (V1aR) antagonist] and arginine-vasopressin into dominant and subordinate members, respectively, over three consecutive days (arrows, days 2–4). Each bar represents the mean ± SEM of the number of flank marks made in a 10-min test over five consecutive days. A two-way analysis of variance resulted in a significant treatments by trials interaction, $F(4,32) = 24.04$, $p < 0.001$ [figure modified from Ref. (98) with permission].

in the NC and mSON is higher in both male and female hamsters following an agonistic encounter than in controls (**Figure 3**). In females, however, similar levels of activation are seen in dominant and subordinate hamsters, while dominant male hamsters display significantly higher levels of activation as compared to subordinate males (69). Thus, there is a substantial sex difference in the relationship between social status and the activation of AVP-containing neurons within the hypothalamus.

Arginine-vasopressin plays an important role in the communication of social status in female hamsters as it does in males (3). Perhaps surprisingly given the opposite effects of AVP on aggression in the AH in males and females, AVP induces flank marking at high levels in both males and females (133). Indeed, the dose–response relationship between the hypothalamic injection of AVP and flank marking are almost identical in male and female hamsters (138). It, therefore, seems likely that although V1aRs mediate the effects of AVP on aggression and flank marking there are important differences in the circuitry controlling these behaviors. Whether separate populations of V1aRs in the AH control aggression and flanking marking is not known, there is some evidence to support this possibility. Exposure to short "winter-like" photoperiods significantly reduces, but does not eliminate V1aR binding in the AH of male hamsters (139). Interestingly, short photoperiod significantly reduces the ability of AVP injected into the AH to increase aggression but has no effect on the ability of AVP to induce flank marking (28, 139). As such, there may be a short photoperiod sensitive subpopulation of V1aRs in the AH that mediate aggression and a short photoperiod in-sensitive subpopulation of V1aRs in the AH that mediate flank marking.

AVP Interactions with Other Neurochemical Signals in the Control of Dominance

Several neurochemical signals can interact with AVP to modulate the communication of social status. In female hamsters, injection of norepinephrine significantly reduces AVP-induced flank marking by its actions in the AH (140). In male hamsters, injection of galanin into the AH significantly reduces AVP-induced flank marking (141, 142). Glutamate receptors within the AH appear to be necessary for the induction of flank marking by AVP at least in male hamsters (143). Co-administration of the glutamate receptor antagonists AP-5 or GAMS significantly inhibits the expression of AVP-induced flank marking. Further support for the hypothesis that glutamate has a critical role in AVP-induced flank marking comes from evidence that AVP can induce glutamate release from astrocytes (95).

There is considerable evidence that 5-HT can modulate AVP-induced flank marking. As discussed earlier, both AVP- and 5-HT-containing projections terminate in the AH so the AH is a likely site for AVP–5-HT interactions (13, 76). When 5-HT or 5-HT agonists are combined with AVP and injected into the AH, the ability of AVP to stimulate flank marking is substantially reduced in both males and females (142, 144, 145). The 5-HT receptor subtypes mediating the effects of 5-HT on flank marking in the AH are not fully defined but there is evidence for the potential involvement of 5-HT1a, 5-HT1b, 5-HT7, and 5-HT4 receptors (146). Systemic administration of fluoxetine can also reduce flank marking produced by injection of AVP into the AH or VLH so 5-HT could be acting a multiple brain sites to inhibit flank marking (144, 147).

The raphe likely plays an important role in mediating how AVP and 5-HT interact to regulate social status given its substantial projections to the AH. Indeed, in female hamsters, 5-HT-ir neurons in the dorsal raphe (DRN) display significantly higher levels of activation (as indicated by increased 5-HT-ir/fos-ir) in winners (i.e., dominant) than in losers (i.e., subordinates) or controls after a single agonistic encounter (69). By contrast, a similar relationship between social status and the activation of 5-HT neurons in the DRN is not seen in males. Despite these major sex differences, there is also a more nuanced relationship between the activation of DRN neurons and social status in males and females. Subordinate males, but not females, display more activation of 5-HT neurons in the ventral subregion of the anterior DRN than dominant or control males and dominance status alters the activation of 5-HT neurons in the dorsal subregion of the anterior DRN in both males and females (148).

There is additional support for a relationship between 5-HT and social status in males. 5-HT1aR mRNA is higher in the DRN of dominant male hamsters in established dominance relationships compared to subordinates (148). Injection of a 5-HT1aR agonist into the DRN of male hamsters, either prior to social defeat or prior to testing for conditioned defeat, reduces submissive and

defensive behaviors and infusion of a 5-HT1aR antagonist into the DRN increases submissive and defensive behaviors (149). Systemic injection of a 5-HT2a receptor agonist in male hamsters after social defeat and prior to testing for conditioned defeat decreases submissive behavior toward a non-aggressive stimulus animal (150). Taken together, the DRN is involved in regulating social status in both males and females, although there are major sex differences in the activation of 5-HT neurons during the formation of dominance relationships.

SUMMARY AND CONCLUSIONS

Arginine-vasopressin plays an important role in the establishment of dominance relationships *via* its effects on aggression and in the maintenance of dominance relationships by its effects on social communication. Although much remains to be learned about the neural circuitry controlling aggression and social status, the hypothalamus is a key site for the actions of AVP. V1aRs in the hypothalamus appear to be critical for the modulation of aggression and for social communication by AVP. The number of V1aRs in the hypothalamus can be influenced by a variety of factors including social experience and gonadal hormones. In males, AVP-induced aggression appears to require an upregulation of V1aRs. Although the most direct evidence for a role of AVP in modulating aggression has been in the hypothalamus there is a relationship between AVP and aggression in several other limbic structures, including the BNST and septum. Although AVP has a substantial role in regulating male aggression it is not clear if AVP promotes aggression in the same manner in all circumstances. For example, it remains to be determined if AVP drives male aggression directly and/or whether AVP is necessary for the transition from a non-aggressive state to aggressiveness.

In females, there have been fewer studies examining the role of AVP in regulating aggression. Nevertheless, there appear to be striking sex differences in the hypothalamic effects of AVP on aggression. While AVP stimulates aggression in males, it inhibits aggression in females. Despite the opposite effects of AVP on aggression in males and females the effects of AVP on aggression are mediated by V1aRs in both sexes. There are also sex differences in how V1aRs can be regulated in the hypothalamus. In males, social isolation increases the number of V1aRs in the AH, but in females social isolation does not change the number of V1aRs despite the fact that social isolation increases aggressiveness in both males and females. Interestingly, the hypothalamic effects of 5-HT on aggression complement the effects of AVP. While activation of 5-HT1aRs in the AH inhibits aggression in males, activation of these receptors stimulates aggression in females.

While aggression plays a critical role in the formation of dominance relationships, it becomes less important after the winners and losers have been determined. In most cases, dominance relationships remain comparatively stable over time as communication is used as a "reminder" of an individual's social status. In hamsters AVP has a central role in the social communication of dominance status. Interestingly, although AVP has the opposite effects on aggression in males and females, AVP potently stimulates flank marking in both males and females. These similarities and differences in the effects of AVP on flank marking and aggression are particularly interesting because both appear to be mediated by V1aRs. It seems likely that different subpopulations of V1aRs mediate the effects of AVP on aggression and flank marking even though they are found in overlapping regions of the hypothalamus. It is also interesting that 5-HT has the same inhibitory effects on AVP-stimulated flank marking in males and females.

It has been proposed that aggressiveness, dominance, and active coping strategies may represent traits that result in more resistance to psychiatric disorders (151, 152). In addition, a role for the vasopressinergic system in psychiatric disorders is receiving increasing attention (153). Importantly, however, sex differences in the effects of AVP on social behavior and in particular on aggression have received little attention. Given the substantial sex differences in the effects of AVP and 5-HT on aggression and dominance and the dramatic sex differences seen in the incidence of psychiatric disorders, it will be important to determine the extent to which these phenomena are linked. Further examination of the AVP system as a target for clinical intervention is particularly timely because of the advent of AVP-active drugs that can be administered orally (154, 155).

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JIT, CFF, and HEA all wrote and revised this manuscript.

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Arginine Vasotocin Preprohormone Is Expressed in Surprising Regions of the Teleost Forebrain

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Nonapeptides play a fundamental role in the regulation of social behavior, among numerous other functions. In particular, arginine vasopressin and its non-mammalian homolog, arginine vasotocin (AVT), have been implicated in regulating affiliative, reproductive, and aggressive behavior in many vertebrate species. Where these nonapeptides are synthesized in the brain has been studied extensively in most vertebrate lineages. While several hypothalamic and forebrain populations of vasopressinergic neurons have been described in amniotes, the consensus suggests that the expression of AVT in the brain of teleost fish is limited to the hypothalamus, specifically the preoptic area (POA) and the anterior tuberal nucleus (putative homolog of the mammalian ventromedial hypothalamus). However, as most studies in teleosts have focused on the POA, there may be an ascertainment bias. Here, we revisit the distribution of AVT preprohormone mRNA across the dorsal and ventral telencephalon of a highly social African cichlid fish. We first use *in situ* hybridization to map the distribution of AVT preprohormone mRNA across the telencephalon. We then use quantitative real-time polymerase chain reaction to assay AVT expression in the dorsomedial telencephalon, the putative homolog of the mammalian basolateral amygdala. We find evidence for AVT preprohormone mRNA in regions previously not associated with the expression of this nonapeptide, including the putative homologs of the mammalian extended amygdala, hippocampus, striatum, and septum. In addition, AVT preprohormone mRNA expression within the basolateral amygdala homolog differs across social contexts, suggesting a possible role in behavioral regulation. We conclude that the surprising presence of AVT preprohormone mRNA within dorsal and medial telencephalic regions warrants a closer examination of possible AVT synthesis locations in teleost fish, and that these may be more similar to what is observed in mammals and birds.

Keywords: nonapeptide, arginine vasopressin, arginine vasotocin, behavior, preoptic area, amygdala, hippocampus

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INTRODUCTION

A fundamental aspect of studying animal physiology and behavior is understanding the pathways and mechanisms by which they are regulated. Many studies have focused on understanding how certain neurochemicals, such as neurotransmitters or neuromodulators, influence behavior. One such family of neurochemicals, a class of nine amino acid molecules known as nonapeptides, is

of particular interest. Nonapeptides are highly conserved across vertebrates and play crucial roles in numerous physiological functions and behaviors (1). Their exact effects vary widely between species for reasons that are not fully clear, making them the subject of studies spanning taxa, sexes, social contexts, brain regions, and scientific fields.

One of the nonapeptides, arginine vasopressin (AVP; also known as antidiuretic hormone, ADH), is of particular interest in the study of social behavior across animals. AVP is a highly conserved nonapeptide that has a wide range of modulatory effects across vertebrates (2). Most vertebrate classes possess the ancestral nine amino acid peptide form, arginine vasotocin (AVT; AVP has a phenylalanine substitution of isoleucine in position 3) (3). Originally identified for its role in osmoregulation, cardiovascular function, and stress hormone release (4–6), AVP/T has also been shown to play a key role in modulating social behavior such as courtship and aggressive behavior in fish (7–9), amphibians (10–12), birds (13–16), and in mammals (17–19). AVP/T has also been shown to modulate territoriality and space use [reviewed in Ref. (20)] and alternative reproductive phenotypes in teleost fish (21–29). These effects are mediated by sex, social context, and the neural expression of the nonapeptide and its receptors (2, 3).

AVP/T is synthesized in magnocellular neurons of the hypothalamus in animals and is produced from prohormones that also encode a carrier protein, neurophysin. There are two types of neurophysin: the prohormone proxyphysin that is hydrolyzed to oxytocin and neurophysin I, and the prohormone propressophysin that is hydrolyzed to vasopressin and neurophysin II, in addition to a short glycopeptide (**Figure 1**). Studies previously done in mammals have shown that these distinct neurophysins may be essential for the implementation of hormonal activity (30). The axon terminals of these hypothalamic neurons extend to the neurohypophysis, where the secretions of these neurosecretory cells are picked up by the circulatory system and transported to target organs.

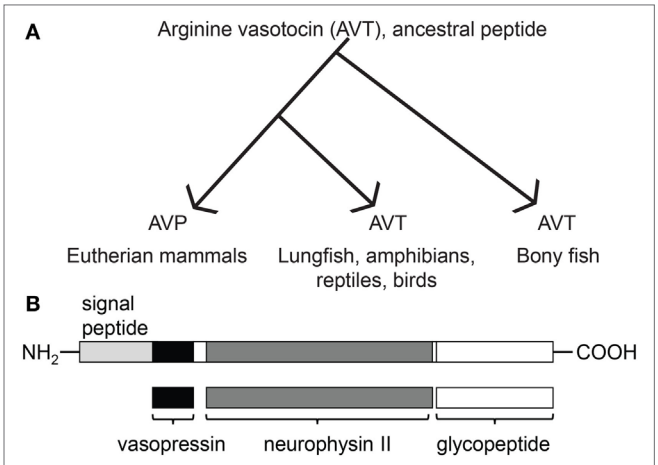

FIGURE 1 | Evolutionary relationship between arginine vasotocin (AVT) and arginine vasopressin (AVP), and the composition of AVT/AVP prohormone and its products. **(A)** Evolution of the vertebrate AVT nonapeptide family [originally modeled after Acher and Chauvet 1995 and adapted from Ref. (31)]. **(B)** Prepropressophysin undergoes post-translational modifications and yields three peptides, namely vasopressin, neurophysin II, and a glycopeptide [based on data derived from Ref. (32)].

In the brain, AVP/T exerts its effects in particular regions by binding to distinct receptors. The expression of these receptors differs across tissues and by function (33, 34). For example, the AVP/T receptor subtype, V1a, has been shown to regulate sex and species differences in many social behaviors in mammals, birds, amphibians, and fish (24, 35–37). For teleosts in particular, AVT receptors consist of one V2-type and two V1a types (V1a1 and V1a2) (38–40). The distributions of these receptors are widespread throughout the brain and are found in regions of interest for social regulation, such as the olfactory bulb (OB), telencephalic areas, POA, hypothalamus, midbrain sensory regions, and hindbrain regions important for social approach responses (41, 42).

AVP/T cell bodies are found in the preoptic area-anterior hypothalamus (POA-AH) complex, an integration center that also regulates numerous physiological and hormonal processes through the pituitary gland (16, 23, 43–48). AVP/T peptides are produced by populations of magnocellular and parvocellular neurons within this POA-AH complex. In amniotes, these magnocellular neurons are found in the supraoptic nucleus (SON) of the hypothalamus, while parvocellular neuron populations are found in the paraventricular nucleus (PVN) of the hypothalamus (3, 47). In fish and amphibians, AVT in these magnocellular and parvocellular neuronal populations are found in the POA and AH. These cell groups project to the neurohypophysis, where AVP/T exerts a wide range of peripheral effects (31). Previous studies have used immunohistochemical (IHC) techniques to label immunoreactivity of AVP/T protein product, or *in situ* hybridization (ISH) to label AVP/T preprohormone mRNA across the brain. **Table 1** provides a summary of the brain regions where AVP/T has been

Abbreviations: AC, anterior commissure; An, anterior thalamic nucleus; aTn, anterior tuberal nucleus; Cn, central nucleus of the inferior lobe; CP, central posterior thalamic nucleus; CV, cerebellar valvula; D, dorsal (pallial) part of the telencephalon; Dc, central part of D; Dc-2, subdivision of Dc; Dd, dorsal part of D; DH, dorsal hypothalamus; Dl, lateral part of D; Dld, dorsal region of Dl; Dlv, ventral region of Dl; Dlvv, ventral zone of Dlv; Dm, medial part of D; Dm-1,2,3, subdivisions of Dm; Dm2c, caudal part of Dm-2; Dn, diffuse nucleus of the inferior lobe; Dp, posterior part of D; Dx, unassigned part of D; E, entopeduncular nucleus; GR, corpus glomerulosum pars rotunda; H, habenula; HC, horizontal commissure; IL, inferior lobe; LHn, lateral hypothalamic nucleus; LPGn, lateral preglomerular nucleus; LR, lateral recess; LT, longitudinal torus; LZ, zona limitans of the diencephalon; MB, mammillary body; mPGn, medial preglomerular nucleus; nLT, nucleus of the lateral torus; nMLF, nucleus of the medial longitudinal fascicle; OB, olfactory bulb; OPT, optic tract; OT, optic tectum; P, pituitary; PAG, periaqueductal gray; PGCn, preglomerula commissural nucleus; PN, prethalamic nucleus; POA, preoptic area; PPd, dorsal periventricular pretectal nucleus; PPr, rostral periventricular pretectal nucleus; pTGN, preglomerular tertiary gustatory nucleus; pTn, posterior tuberal nucleus; PVO, paraventricular organ; ST, semicircular torus; TPp, periventricular nucleus of the posterior tuberculum; V, ventral (subpallial) division of the telencephalon; Vc, central part of V; Vd, dorsal part of V; Vdc, caudal region of Vd; Vdr, rostral region of Vd; VH, ventral hypothalamus; Vi, intermediate part of V; Vl, lateral part of V; VM, ventromedial thalamic nucleus; Vp, postcommissural nucleus of V; vPPn, ventral portion of the periventricular pretectal nucleus; Vs, supracommissural nucleus of V; Vsl, lateral region of Vs; Vsm, medial region of Vs; vTn, ventral tuberal nucleus; Vv, ventral part of V.

found, along with the technique used to map either AVP/T protein product or label AVP/T preprohormone mRNA in the respective studies. In general, amniotes have similar patterns of AVT expression throughout the forebrain. In teleosts, however, AVT-containing neurons have been shown to be localized to the POA region.

TABLE 1 | Presence of forebrain arginine vasotocin/arginine vasopressin across vertebrates.

Class	Brain regions	Species	Study	Methods
Fish	Diencephalon: Preoptic area	*Anguilla anguilla*	Olivereau et al. (49)	IHC
		Astatotilapia burtoni	Greenwood et al. (21)	*In situ* hybridization (ISH)
		Carassius auratus	Reaves and Hayward (50)	
		Halichoeres trimaculatus	Hur et al. (51)	qPCR
		Oncorhynchus keta	Ota et al. (52)	ISH, IHC
		Oncorhynchus masou	Ota et al. (28, 53)	ISH, IHC
		Oncorynchus mykiss	Gilchriest et al. (54)	ISH
		Poecilia latipinna	Batten et al. (55)	IHC
		Protopterus aethiopicus	Goossens et al. (56)	IHC
		Prochthys notatus	Goodson and Bass (22, 23)	IHC
		Salmo gairdneri	van den Dungen et al. (57)	IHC
		Scyliorhinus caniculus	Vallarino et al. (58)	IHC
		Thalassoma bifasciatum	Godwin et al. (59)	ISH
		Xiphophorus maculatus	Schreibman and Halpern (60)	IHC
Amphibians	Pallial telencephalon	*Pleurodeles waltlii*	Gonzalez and Smeets (61, 62)	IHC
	Subpallial telencephalon	*Rana catesbeiana*	Boyd et al. (63); Gonzalez and Smeets (61, 62); Mathieson (64)	IHC
		Rana ridibunda	Gonzalez and Smeets (61, 62)	IHC
		Rana sylvatica	Mathieson (64)	IHC
		Taricha granulosa	Lowry et al. (65); Lowry et al. (45)	ISH, IHC
		Xenopus laevis	Gonzalez and Smeets (61, 62)	IHC
	Diencephalon: BNST and POA	*Bufo japonicus*	Jokura and Urano (66)	IHC
		Pseudemys scripta	Smeets et al. (67)	IHC
		Rana catesbeiana	Boyd et al. (63)	IHC
		Taricha granulosa	Lowry et al. (45)	ISH, IHC
		Typhlonectes compressicauda	Gonzales and Smeets (68)	IHC
		Typhlonectes natans	Hilscher-Conklin et al. (69)	IHC
		Xenopus laevis	Gonzalez and Smeets (61, 62)	IHC
Reptiles	Subpallial telencephalon	*Anolis carolinensis*	Propper et al. (70)	IHC
		Pseudemys scripta elegans	Smeets et al. (71)	IHC
		Python regius	Smeets et al. (71)	IHC
		Gekko gecko	Stoll and Voorn (72); Thepen et al. (73)	IHC
	Diencephalon: POA, thalamic regions	*Anolis carolinensis*	Propper et al. (70)	IHC
		Gekko gecko	Stoll and Voorn (72); Thepen et al. (73)	IHC
		Lacerta muralis	Bons (74)	IHC
		Mauremys caspica	Fernandez-Llebrez et al. (75)	IHC
		Natrix maura	Fernandez-Llebrez et al. (75)	IHC
		Pseudemys scripta elegans	Smeets et al. (71)	IHC
		Python regius	Smeets et al. (71); Smeets et al. (67)	IHC
Birds	Subpallial telencephalon	*Coturnix japonica*	Aste et al. (76)	ISH
		Gallus domesticus	Aste et al. (76); Jurkevich et al. (77)	ISH, IHC
		Junco hyemalis	Panzica et al. (78)	IHC
		Serinus canaria	Kiss et al. (79)	IHC
		Taeniopygia guttata	Voorhuis and de Kloet (80)	IHC
	Diencephalon: POA, thalamic regions	*Columba livia*	Berk et al. (81)	IHC
		Coturnix japonica	Bons (82); Panzica et al. (83)	IHC
		Serinus canaria	Kiss et al. (79)	IHC
		Taeniopygia guttata	Voorhuis and de Kloet (80)	IHC

(*Continued*)

TABLE 1 | Continued

Class	Brain regions	Species	Study	Methods
Mammals	Subpallial telencephalon	*Felis catus*	Caverson et al. (84)	
		Macaca fascicularis	Caffe et al. (85)	IHC
		Mesocricetus auratus	Dubois-Dauphin et al. (86)	IHC
		Mus musculus	Castel and Morris (87)	IHC
		Rattus norvegicus	Rhodes et al. (88); DeVries et al. (89); van Leeuwen et al. (90); Urban et al. (91); Wang et al. (92); Planas et al. (93)	IHC, ISH
		Sus scrofa	van Eerdenburg et al. (94)	IHC
	Diencephalon: POA, hypothalamic regions	*Cavia porcetella*	Dubois-Dauphin et al. (86)	IHC
		Felis catus	Caverson et al. (84)	
		Jaculus orientalis	Lakhdar-Ghazal et al. (95)	IHC
		Macaca fascicularis	Caffe et al. (85)	IHC
		Meriones unguiculatus	Wu and Shen (96)	IHC
		Mus musculus	Castel and Morris (87)	IHC
		Rattus norvegicus	Rhodes et al. (88); DeVries et al. (89); Dobie et al. (97); Miller et al. (98); Miller et al. (99); Brot et al. (100); Szot and Dorsa (101); Szot and Dorsa (102)	IHC, ISH

Tetrapod vertebrates exhibit additional anatomical characteristics that remain largely conserved. AVP is produced in neurons of the bed nucleus of the stria terminalis and the medial amygdala, and projections extend to the lateral septum, nucleus accumbens, amygdala, and periaqueductal gray (PAG) (47, 103, 104). These circuits are particularly important for social behavior, such as mate affiliation, nest defense, and parental care of offspring (92, 105–107). Putative teleost homologs of these regions also contain AVT fiber innervation, though these fibers are generally thought to originate in the POA (22, 55). AVP/T fibers are located throughout the brain in jawed vertebrates, likely conserved for at least 500 million years, including the POA, anterior and lateral hypothalamic areas, midbrain tegmentum, PAG, isthmal structures (i.e., locus coeruleus), and viscerosensory areas of the caudal medulla (3).

In the teleost POA, the magnocellular and gigantocellular AVT neuron populations are hypothesized to be homologous to the supraoptic nucleus in tetrapods based on colocalization with corticotropin-releasing hormone-producing neurons and expression of the Nurr1 receptor, while the parvocellular cell group is the putative homolog of the PVN of the mammalian POA (47, 49, 108, 109). AVT appears to be limited to the POA (1). Weaker expression also appears in the anterior tuberal nucleus of the hypothalamus [aTn; (21, 23)], the putative teleost homolog of the mammalian ventromedial hypothalamus [VMH; (110, 111)]. As in tetrapods, AVT is found in the parvocellular, magnocellular, and gigantocellular neuron groups, which are distinguished by soma size and location, with gigantocellular populations being found most caudally. These AVT neurons have been shown to project to the posterior pituitary through the preoptico-hypophysial tract as well as various regions in the ventral telencephalon and ventral thalamus (23, 112). Overall, the expression of AVT preprohormone mRNA and peptide seems to be fairly conserved across vertebrates. There might be an ascertainment bias as most studies only report on the POA and/or used IHC methods to map AVT-positive neurons, which may not be sensitive enough to detect low levels of peptide expression in other brain regions [but see Ref. (21, 51, 59, 113)].

Importantly, AVP/T has been shown to be socially regulated [see Ref. (3, 20) for reviews]. For example, non-monogamous male Montane voles have fewer V1a receptors in the ventral pallidum compared to monogamous Prairie voles, and the induction of these receptors in the Montane voles *via* viral vector gene transfer yields pair bonding behavior similar to Prairie voles (114). White-throated male sparrows (*Zonotrichia albicollis*) have more AVT expression in the medial portion of the BNST and in a subdivision of the caudal lateral septum compared to tan-striped male sparrows. This neural AVT expression is associated with aggression, since white-striped males defend their territories more vigorously and intrude into other territories more often than their tan-striped male counterparts (115). Research in teleosts suggests that AVT preprohormone mRNA levels might be more reliable indicators of social status than the number or size of AVT-positive neurons (as determined by immunohistochemistry). In Burton's Mouthbrooder cichlid, *Astatotilapia burtoni*, socially dominant males exhibit higher levels of AVT expression than subordinate males in gigantocellular nucleus of the preoptic area, whereas the inverse was found in the parvocellular preoptic nucleus (21). The number or size of AVT-immune-reactive (ir) neurons was, however, not correlated with behavior (126). Similarly, in the sex-changing Bluehead wrasse, *Thalassoma bifasciatum*, preoptic AVT mRNA levels predicts male behavior robustly, while AVT-ir neuron size does not (59). These examples illustrate the role AVP/T plays in modulating social behavior across species, and how these effects are not just sex- and context-specific but also brain region-specific.

The majority of studies that examine the expression and distribution of either AVT preprohormone mRNA or the AVT peptide in teleost fish have primarily focused on the POA. These studies utilize quantitative real-time polymerase chain reaction (qPCR), immunohistochemistry, immunocytochemistry, or radioactive ISH to quantify mRNA and/or protein expression (for more

information regarding these methods see **Table 2**). In the present study, we revisit the neural distribution of AVT nonapeptide expression, in particular expanding on the existing knowledge of its mRNA distribution within the forebrain of a highly social cichlid fish. We first used ISH to examine whether the AVT preprohormone mRNA is expressed in pallial and subpallial regions of the telencephalon of *A. burtoni*. In a second experiment, we used qPCR to ask whether AVT preprohormone mRNA expression in pallial area Dm, the putative homolog of the mammalian basolateral amygdala, is modulated by social context. We provide evidence of AVT preprohormone mRNA expression in forebrain regions never previously reported to contain nonapeptides in teleost fish. Furthermore, our results suggest that AVT preprohormone mRNA expression in the putative homolog of the mammalian basolateral amygdala can be regulated by social context.

MATERIALS AND METHODS

Study 1: AVT Distribution in the Cichlid Forebrain

Animals

The African cichlid fish, *Astatotilapia burtoni* (Burton's Mouthbrooder), has become an important model system for the study of social neuroscience. Males of this species can be one of two phenotypes—dominant or subordinate—and this reversible phenotype depends on the immediate social context. Dominant males are highly territorial, aggressive, and reproductively active while subordinate males are non-reproductive and non-territorial. *A. burtoni* descended from a wild-caught stock population were kept in aquaria under naturalistic environmental conditions and stable naturalistic communities as previously described (116). The animals used for mapping the distribution of AVT with ISH were the same as those used in a previous study (42). All work was carried out in compliance with the Institutional Animal Care and Use Committee at the University of Texas at Austin.

In Situ Hybridization

Brains from dominant and subordinate males and females were rapidly dissected and fresh frozen in OCT compound (Tissue-Tek, USA) on dry ice, and stored at −80°C. Brains were subsequently sectioned and stored until processing for ISH as previously described (116). Due to regions of high sequence similarity in the coding regions between neuropeptides and receptors used in the original study (42), the probe for AVT was designed to identify the 3′ untranslated region. The template used to make the AVT probe was 378 bp in length (21). Experimental slides were exposed to anti-sense fluorescein-labeled probe, whereas control slides were incubated with sense fluorescein-labeled probe (**Figure 2**). After the overnight hybridization, slides were processed for detection of mRNA by non-radioactive, non-fluorescent detection. Sections were washed in a series of 0.2x SSC washes at 65°C and equilibrated in 150 mM NaCl/100 mM Tris (pH 7.5) at room temperature before incubation in 1:1,000 anti-fluorescein-alkaline phosphatase Fab fragments (Roche) in 0.05% Tween 20/PBS for 2 h at room temperature. Sections were then washed in 150 mM NaCl/100 mM Tris (pH 7.5). Chromogenic product was formed using BM Purple (Roche) at room temperature until desired darkness was achieved and was terminated simultaneously for all slides within a gene group. Slides were then washed, dehydrated in an ethanol series ending in xylene, and cover-slipped with Permount (Fisher Scientific). These slides were previously used in Ref. (42) to examine the distribution of AVT and isotocin receptor in *A. burtoni*.

Microscopy

Micrographs were captured and processed as previously detailed (42). Brightfield optics were used to visualize staining throughout the brain at low (5×) and high magnification (10×). Photographs were taken with a digital camera (AxioCam MRc, Zeiss) attached to a Zeiss AxioImager.A1 AX10 microscope using the AxioVision (Zeiss) image acquisition and processing software. Images were compiled and brightness-enhanced in Adobe Photoshop.

Study 2: AVT Expression Variation in Dm in Socially Relevant Contexts

Animals

A. burtoni descended from a wild-caught stock population were kept in stable naturalistic communities, as described (117) until they were transferred into the experimental conditions. These animals were the same as those used in a previous study (118). All work was carried out in compliance with the Institutional Animal Care and Use Committee at the University of Texas at Austin.

Behavior

Animals were placed in experimental tanks which had one territorial male and two non-reproductive females [as described in

TABLE 2 | Differences between methodological techniques.

Technique	How does it work?	What is measured and visualized?	Advantages of each method
Quantitative real-time polymerase chain reaction (qPCR)	Binds cDNA (complementary DNA, after reverse transcription of mRNA) with a light-emitting molecule	Amplified cDNA	Quantitative
In situ hybridization (ISH)	Binds nucleic acid strands complementary to the mRNA of interest which is labeled with a chromophore or radioisotope	mRNA, fluorophore, or silver grains	Spatial resolution
Immunohistochemistry (IHC)	Uses an antibody that specifically binds a protein of interest for visualization in sectioned tissues, these antibodies are visible under fluorescence or brightfield microscopy when bound to a fluorophore or chromophore	Protein, cells or fibers	Spatial resolution

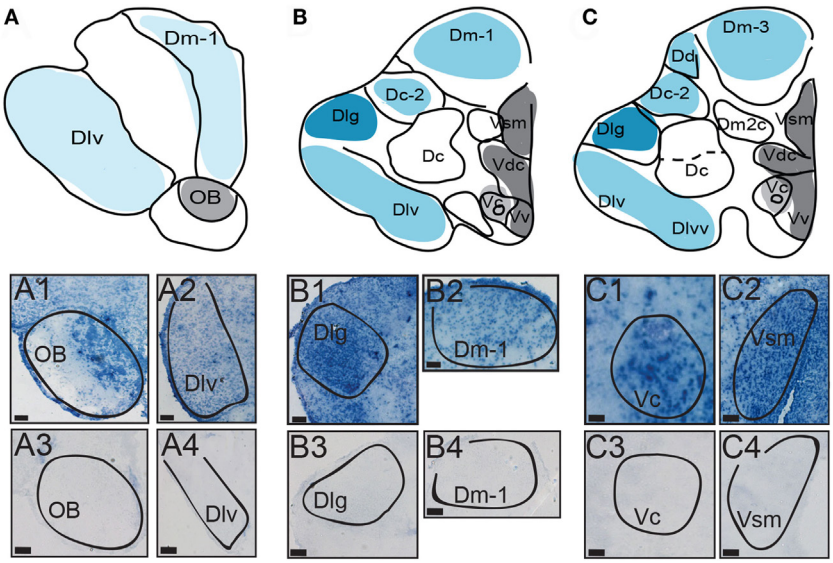

FIGURE 2 | Distribution of AVT preprohormone mRNA in the telencephalon. **(A–C)** The first row represents a template marked with the distribution of AVT preprohormone mRNA. mRNA is shown as shading on the representative template, and the degree of shading corresponds to the qualitative density of expression. Micrographs show AVT preprohormone mRNA in the olfactory bulb (OB; A1), in the ventrolateral part of D (Dlv; A2), the granular region of D (Dlg; B1), a subregion of the medial part of D (Dm-1; B2), the central part of V (Vc; C1), and in the medial part of Vs (Vsm, C2). The sense controls show a lack of AVT preprohormone mRNA signal in the OB (A3), Dlv (A4), Dlg (B3), Dm-1 (B4), Vc (C3), and Vsm (C4). All scale bars are shown at 20 μm.

Ref. (118)]. Focal males were tested in one of three social contexts; namely (1) a Reproductive Context, in which an adjacent tank contained one gravid and two non-reproductive females, (2) a Familiar Neighbor context, in which the adjacent tank contained one size-matched territorial male and two non-reproductive females, and (3) a Neutral Stimulus context that contained three non-reproductive females. Non-reproductive females were stripped of their brood immediately before placement in each tank, ensuring that they would remain non-reproductive for the duration of the study (119). Males were killed by rapid cervical transection and brains were flash frozen in O.C.T. (Tissue-Tek; Fisher Scientific Co., Pittsburgh, PA, USA) and stored at −80°C.

Quantitative Real-time Polymerase Chain Reaction

Brains were sectioned on a cryostat in the transverse plane at 300 μm. A 300 μm diameter sample corer tool (Fine Science Tools, Foster City, CA) was used to micro-dissect the Dm-1. Two micro-dissected punches (left and right hemisphere) were taken from a single brain slice and stored in DNA/RNA Shield (Zymo Research, Irvine, CA, USA) at −80°C until processing. ZR BashingBeads (Zymo Research) were added to samples suspended in DNA/RNA Shield for tissue homogenization before RNA extraction. Proteinase K digestion was done for 2 h at 55°C to lyse tissue. Total RNA was then extracted in accordance with the protocol for the Quick-RNA MicroPrep kit (Zymo Research, Irvine, CA, USA). RNA samples were treated with DNase (Zymo) during isolation procedure to prevent DNA contamination. The GoScript Reverse Transcription System (Promega Corporation, Madison, WI, USA) was used to reverse transcribe RNA to cDNA.

Quantitative real-time polymerase chain reaction was used to measure the mRNA levels of AVT preprohormone and the primers were designed to flank exon-exon boundaries (AVT forward: 5′-AGGCAGGAGGGAGATCCTGT; AVT reverse: 5′-CAGGCAGTCAGAGTCCACCAT. 18S forward: 5′-CCCTT CAAACCCTCTTACCC; 18S reverse: 5′-CCACCGCTAAGAGT CGTATT). Target gene expression was measured in triplicate in the ViiA™ 7 Real-time PCR System (Applied Biosystems, Foster City, CA, USA) using GoTaq qPCR Master Mix (Promega). Amplification efficiency for the primer pair was determined using standard curves made from serial dilutions of cDNA.

Statistical Analyses

Statistical tests were performed using R v. 3.1.0. We used the R package mcmc.qpcr to determine relative gene expression for each sample. 18S was used as a control gene, and other target genes measured within the same region were included in the normalization analysis. This package analyzes qPCR data using generalized linear mixed models based on lognormal Poisson error distribution, fitted using Markov chain Monte Carlo statistical methods (120).

RESULTS

In Situ Hybridization of AVT Preprohormone mRNA across the Pallium and Subpallium

We first describe the distribution of AVT preprohormone mRNA throughout the *A. burtoni* pallium and subpallium using ISH.

In **Figures 3** and **4**, we present a distribution maps along with photomicrographs of representative brain areas for AVT expression in the *A. burtoni* brain. For each representative section of the map, the teleost nomenclature is displayed along with the preprohormone distribution. The degree of shading represents the approximate density of mRNA expression in that brain region. Pallial regions are colored in shades of blue while subpallial regions are colored in shades of gray. The general patterns are qualitatively independent of reproductive or social status and similar in males and females. Control slides hybridized with sense probes showed no specific signal (**Figure 2**).

Robust expression of AVT preprohormone mRNA is seen throughout the *A. burtoni* pallium. AVT preprohormone mRNA is present in the central, medial and lateral parts of the pallium (Dc, Dm, and Dl, respectively, **Figure 2**). The ventral subregion of Dl (Dlv) has mild staining of AVT preprohormone mRNA (**Figure 2**, A2), while the granular part of Dl (Dlg) has darker staining (**Figure 2**, B1). AVT preprohormone mRNA is present across all subdivision of the Dm (Dm-1,2,3) but has lighter stain in the Dm-1 subdivision (**Figure 2**, B2). The Dc-2 subdivision of the Dc telencephalon also shows light staining of AVT preprohormone mRNA, which is absent from the Dc (**Figure 2B**). In general, AVT expression becomes more robust in more caudal sections of these pallial regions.

There is robust AVT expression within the OB and subpallium as well as in the granule cell layer of the OB (**Figure 2**, A1), while preprohormone mRNA is predominantly absent from the glomeruli region. Ventral, central, and supracommissural parts (Vv, Vc, Vs; **Figure 2C**) of the subpallium also show robust AVT expression. This is also present in Vv, Vd, and the subregions of the Vs (Vsm and Vsl). There is AVT expression in the Vc (**Figure 2**, C1), and expression is more robust in more caudal regions of the Vs (Vsm, **Figure 2**, C2). AVT preprohormone mRNA is widely expressed throughout the POA (**Figure 3**). There is robust expression in parvocellular populations of the POA (**Figure 3B**), as well as in the magnocellular population (**Figure 3C**). AVT preprohormone mRNA expression is also present in the gigantocellular population (**Figure 3D**).

AVT Expression in the Medial Dorsal Telencephalon

Next, we use qPCR to examine whether AVT preprohormone mRNA expression in the medial dorsal telencephalon is modulated by social context. We find significant variation in AVT expression in the Dm region of the *A. burtoni* telencephalon across social contexts (**Figure 4**). Specifically, AVT expression is higher in the Familiar Neighbor context as compared to a context with a Neutral Social Stimulus ($p = 0.003$). There is no difference in AVT expression between Reproductive Opportunity context and either Familiar Neighbor or Neutral Social Control contexts.

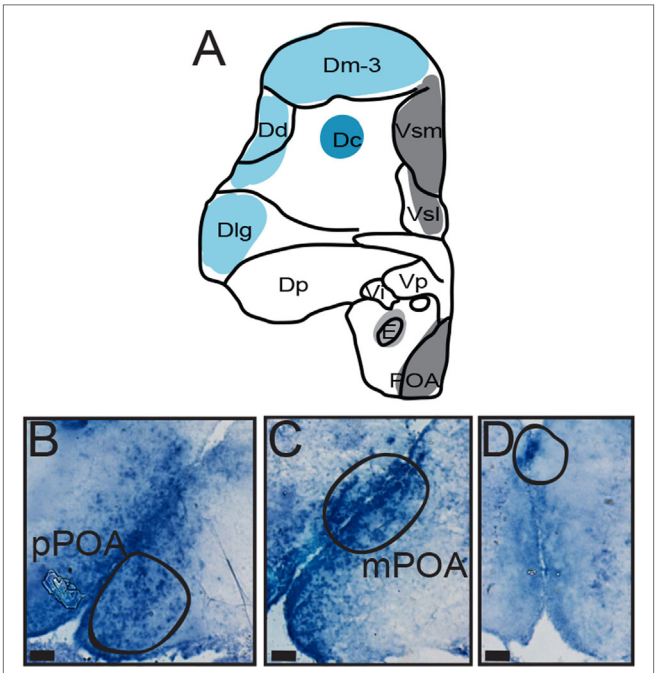

FIGURE 3 | Distribution of AVT preprohormone mRNA in the preoptic area (POA) of *A. burtoni*. **(A)** A template marked with the distribution of AVT preprohormone mRNA (shading). The degree of shading corresponds to the qualitative density of expression. **(B)** Micrograph shows AVT preprohormone mRNA in the parvocellular population of the POA. **(C)** Micrograph shows AVT preprohormone mRNA in the magnocellular population of the POA. **(D)** Micrograph shows AVT preprohormone mRNA in the gigantocellular population of the POA. All scale bars are shown at 20 μm.

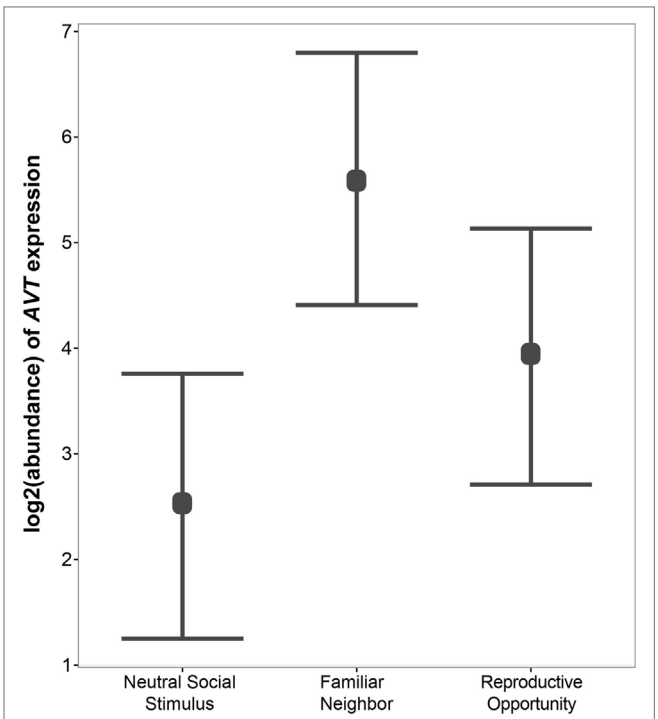

FIGURE 4 | Relative gene expression of AVT preprohormone mRNA across social contexts. AVT expression was highest in individuals engaged in Familiar Neighbor social context, and is generally higher in contexts in which the social stimulus is not neutral, such as Familiar Neighbor and Reproductive Opportunity contexts, as compared to a context with a Neutral Social Stimulus.

DISCUSSION

In the present study, we have shown that expression of AVT preprohormone mRNA in the cichlid fish *A. burtoni* is not limited to preoptic nuclei and the anterior tuberal nucleus. Rather, AVT preprohormone mRNA is expressed widely throughout pallial and subpallial regions not previously associated with the expression of the AVT nonapeptide. We have also found evidence for the social regulation of AVT expression within area Dm-1, the putative homolog of the mammalian basolateral amygdala. These surprising findings provide an important addition to our understanding of the distribution of AVT in the teleost brain and how nonapeptides modulate social behavior in cichlids.

Previous studies in teleost fish have reported the presence of AVT preprohormone and AVT peptide primarily in the POA and the aTn of the hypothalamus (21, 59, 121). Several studies also mapped AVT-immunoreactive fibers and found that they project extensively throughout the teleost brain, although where these fibers originate is not always obvious (55, 121). Our data expand on these studies to show the expression of AVT preprohormone mRNA in multiple regions of the dorsal, medial, central, and ventral pallium. Specifically, subpallial regions, such as the medial and lateral divisions of area Vs [putative homolog of the medial amygdala and the bed nucleus of the stria terminalis (109)] along with area Vv (putative septum homolog) and the central part of area Vd (putative striatum homolog) showed robust expression of AVT preprohormone mRNA, while pallial regions, including basolateral amygdala (area Dm) and hippocampus (area Dl), showed less but still reliably detectable abundance. Our qPCR results confirm expression of AVT in area Dm, and we show this expression to be modulated by the social context. These results suggest that AVT expression in teleosts may be more similar to AVP/T expression in birds and mammals.

If there are indeed AVT expressing neurons in the teleost pallium, why did previous authors fail to detect them? First, methodological limitations may provide an answer: all studies examining the expression and distribution of either AVT preprohormone mRNA or the AVT peptide in teleost fish to date utilize IHC, qPCR, or radioactive ISH to detect peptide and/or mRNA expression (see **Table 1**). Most do not provide information on telencephalic brain regions, instead focusing exclusively on the preoptic AVT cell populations. The few studies that investigate whether AVT preprohormone or peptide is present in the teleost telencephalon and other areas outside the preoptic nuclei and hypothalamus (21, 51), rely on either radioactive ISH or qPCR of the entire forebrain. Importantly, it is well understood that the former requires short exposure times so as to not overdevelop the signal in preoptic AVT neurons, where the preprohormone is expressed at very high levels [see, e.g., Ref. (21, 59)].

Second, it is also conceivable that AVT transcripts are transported from preoptic cell bodies to fibers (putative axons) in various telencephalic regions for local synthesis (possibly near varicosities or putative release sites). Using both ISH and PCR (122), found oxytocin preprohormone mRNA in axons and Herring bodies in the lateral and ventral hypothalamus, the median eminence, and the posterior lobe of the pituitary in rats. While it is unclear whether this can also occur in axons projecting into the telencephalon, these results nevertheless indicate that at least in the rodent oxytocin preprohormone mRNA can be transported axonally. Given that (a) oxytocin and AVP/T genes as paralogs may share a similar molecular and cellular machinery, and (b) teleosts have brain regions putatively homologous to these rodent regions (109, 117), the signal we detect in pallial regions may indeed be the consequence of axonal transport of AVP/T mRNA. Given the ISH methods used in this study, we cannot conclusively deduce if the mRNA signal resembles varicosities or puncta. Detailed tract tracing studies in combination with sensitive assays such as ISH will allow us to test this hypothesis.

Finally, another possible explanation for the distribution of AVT mRNA expression throughout the teleost telencephalon could be that we are observing preprohormone mRNA that never is translated and processed into the mature peptides AVT and/or neurophysin II. Although the enzymes processing preprohormones could be present in putative pallial AVT neurons for processing peptides others than AVT and neurophysin, any future analysis (e.g., by ISH) demonstrating that these enzymes do not co-localize in these neurons would support this idea. Alternatively, only neurophysin might be produced, for a yet to be discovered function, which can be tested once a specific antibody is available. These possible explanations notwithstanding, our results should be seen as an encouragement to examine telencephalic AVT expression in a range of teleost species.

Is telencephalic AVT of functional importance in *A. burtoni*? Interestingly, we did find significant variation in AVT preprohormone mRNA levels, albeit lowly abundant, depending on social context in area Dm-1, the putative homolog of the mammalian basolateral amygdala (109). This region is known to be important for fear conditioning in mammals, as well as being a sensory integration center that mediates emotional behavior (123, 124). Here, AVT shows increased relative expression in dominant males in the presence of a familiar neighbor, which has important implications for territory defense (125). A possible explanation for this result is that AVT expression in the Dm-1 may be modulating an individual's behavioral response to a familiar neighbor, possibly facilitating social habituation. It is important to note that we do not know baseline AVT levels in the Dm-1, and the data only represent expression in response to an intruder in a joint defense paradigm (118). Further support for a functional role of AVT expression in the basolateral amygdala homolog is provided by the finding that other candidate genes followed the same expression pattern across experimental groups that we observed with AVT expression, possibly regulated by testosterone (118).

CONCLUSION

Nonapeptides are important mediators of social behavior, such as aggression, reproduction, and paternal care, across vertebrates. Their effects are mediated by the presence of receptors and neuronal fibers found throughout the brain. AVP/AVT expression, in particular, has previously been examined across species, and it is canonically held that expression patterns in telencephalic

regions of the brain are different between tetrapods and other vertebrates. Previous work has suggested that teleost fish only express AVT cell bodies within the POA-AH complex, and send projections to other telencephalic regions. However, here we find evidence for the presence of AVT preprohormone mRNA in regions previously not associated with AVT expression, such as the dorsomedial, ventral, and central regions of the *A. burtoni* telencephalon. Based on these results, it is worthwhile to reconsider the similarity in AVT/P expression patterns between teleosts and other vertebrates.

ETHICS STATEMENT

The original research reported here was performed under guidelines established and was reviewed and approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin and in compliance with all local, state, and federal regulations.

AUTHOR CONTRIBUTIONS

CW, HH, and LH designed the studies; LH conducted the *in situ* hybridization study; CW and JN performed the qPCR experiments; LH, MR-S, and CW performed the data analysis; MR-S and HH wrote the manuscript.

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Early Intranasal Vasopressin Administration Impairs Partner Preference in Adult Male Prairie Voles (*Microtus ochrogaster*)

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Research supports a modulatory role for arginine vasopressin (AVP) in the expression of socially motivated behaviors in mammals. The acute effects of AVP administration are demonstrably pro-social across species, providing the justification for an ever-increasing measure of clinical interest over the last decade. Combining these results with non-invasive intranasal delivery results in an attractive system for offering intranasal AVP (IN-AVP) as a therapeutic for the social impairments of children with autism spectrum disorder. But, very little is known about the long-term effects of IN-AVP during early development. In this experiment, we explored whether a single week of early juvenile administration of IN-AVP (low = 0.05 IU/kg, medium = 0.5 IU/kg, high = 5.0 IU/kg) could impact behavior across life in prairie voles. We found increases in fecal boli production during open field and novel object recognition testing for the medium dose in both males and females. Medium-dose females also had significantly more play bouts than control when exposed to novel conspecifics during the juvenile period. Following sexual maturity, the medium and high doses of IN-AVP blocked partner preference formation in males, while no such impairment was found for any of the experimental groups in females. Finally, the high-dose selectively increased adult male aggression with novel conspecifics, but only after extended cohabitation with a mate. Our findings confirm that a single week of early IN-AVP treatment can have organizational effects on behavior across life in prairie voles. Specifically, the impairments in pair-bonding behavior experienced by male prairie voles should raise caution when the prosocial effects of acute IN-AVP demonstrated in other studies are extrapolated to long-term treatment.

Keywords: pair-bond, fecal boli, play, anxiety, social, aggression

INTRODUCTION

Arginine vasopressin (AVP) is a neuropeptide, which exerts its effects in both the brain and periphery. Within the brain, the AVP system acts to influence socially motivated behaviors (1) utilizing several different neurocircuits (2), including social recognition, communication, and aggression. The AVP system is widespread throughout the central nervous system well before birth (3), suggesting an organizational role in development (4).

Early manipulations of the AVP system have been shown to alter behavior across life. In rats, prenatal AVP injections impact fetal suckling behavior (5) while juvenile injections of AVP receptor 1a antagonists disrupt play behavior (6). The effects of early postnatal injections of AVP can stretch into adulthood, increasing male aggression in prairie voles (7) and affiliative attachment in zebra finches (8). Pharmacological manipulations of the AVP system help elucidate its various functions while confirming the presence of critical periods for the organizational impact of AVP signaling.

More recently, studies have found associations between disruption of the AVP system and the expression of certain neurodevelopmental disorders in humans, like autism spectrum disorder (ASD). For example, plasma AVP levels [Ref. (9–11); but see Ref. (12)] and certain single-nucleotide polymorphisms of the genes for AVP and its receptor (13) have been correlated with social functioning in individuals with ASD. Given the frequently pro-social effects of acute intranasal AVP (IN-AVP) administration in humans (14–16) and animal models (17, 18), IN-AVP has been suggested as a treatment for the social deficits in children with ASD (19, 20).

However, acute studies have left several important questions unanswered. Specifically, do the potentially beneficial aspects of acute administration extend to chronic administration? Or, could prolonged exposure cause unforeseen long-term effects? Thus, the purpose of our study was to explore the long-term effects of early IN-AVP administration on behavior across life in prairie voles (*Microtus ochrogaster*). We administered three doses of AVP (low = 0.05 IU/kg, medium = 0.5 IU/kg, high = 5.0 IU/kg) or saline twice daily to male and female prairie voles from age 15 to 21 days. This age range falls within the early juvenile period in prairie voles, approximating the developmental stage at which children are being treated with IN-AVP in at least one clinical trial (https://ClinicalTrials.gov Identifier: NCT01962870). The medium dose reflects the dose used in these trials but controlled for weight. As voles are typically weaned around day 20, we explored whether parental behavior changed because of pup treatment, and then, each animal postweaning was tested in several experimental paradigms. From tests of anxiety, exploration, and sociality in the juvenile period to tests of partner preference formation and aggression in adulthood, we explored whether a single week of IN-AVP exposure could perpetuate behavioral changes across life.

We hypothesized that the effects of IN-AVP would vary by dose, possibly representing differential activation of multiple AVP sub-circuits within the brain, or activation of oxytocin receptors at high doses. Male prairie voles have higher AVP immunoreactivity in several brain regions, including the lateral septum, lateral habenular nucleus, and bed nucleus of the stria terminalis [Ref. (21, 22); but see Ref. (23)]. As such, we expected IN-AVP to have the most profound effects in males. Finally, we predicted that the effects of IN-AVP would be context-specific, increasing sociality during non-threatening encounters (e.g., juvenile affiliation) and increasing aggression during competitive encounters (e.g., adult affiliation following pair-bond formation).

MATERIALS AND METHODS

Subjects

We recruited 103 prairie vole subjects (52 males, 51 females) from our breeding colony located in the Department of Psychology at the University of California, Davis. We maintained the animals on a 14:10 h light cycle at approximately 21°C and provided food (Purina High Fiber Rabbit Chow, PMI Nutrition International, Brentwood, MO, USA) and water *ad libitum*. Animals were housed in large polycarbonate cages (44 cm × 22 cm × 16 cm) with their parents and marked with non-toxic Nyanzol D dye (American Color and Chemical Corporation, Charlotte, NC, USA) for identification purposes until weaning at postnatal day (P) 20. We then separated all subjects from their parents, gave them ear clip markings, and placed them with a same-sex sibling in smaller cages (27 cm × 16 cm × 13 cm) until sacrifice. Subjects that were treated with IN-AVP were housed with untreated siblings. To help control for potential litter effects, each litter had at least one AVP-treated animal and one saline-treated animal within sex.

Intranasal Treatments

Each test subject was randomly assigned to one out of four treatment groups, including saline control, low-dose AVP (0.05 IU/kg), medium-dose AVP (0.5 IU/kg), and high-dose AVP (5.0 IU/kg). The medium dose was specifically calculated to represent the same dose given in some clinical trials (https://ClinicalTrials.gov Identifier: NCT01962870), only controlled for weight. AVP solutions were purchased from Sigma-Aldrich (V0377 SIGMA), already mixed in NaCl. The solution was then diluted to provide the necessary concentrations for the treatment groups and aliquoted into 200 μL test tubes. The tubes were stored in a refrigerator at 4°C until use.

From P15 to 21 (early juvenile period), voles were given intranasal treatments twice daily. Each day, the first treatment was given between 0900 and 1100 hours, while the second treatment was given between 1500 and 1800 hours. Treatments were administered through cannula tubing, which was attached to a blunt cannula needle (33 gauge, 2.8 mm length; Plastics One, Roanoke, VA, USA) secured to an airtight Hamilton syringe (Fisher Scientific, Pittsburgh, PA, USA). The animal was held still while 25 μL of solution was expelled slowly through the cannula system and allowed to absorb into the nasal mucosa (divided evenly between the two nostrils). Following administration, the animal was returned to its home cage while the Hamilton syringes and cannula system were cleaned with 70% isopropyl alcohol solution and de-ionized water. Treatment order was randomized each day and administration was rapid (less than 30 s) making handling consistent across treatment groups.

Observations and Behavioral Testing

All animals were subjected to a series of testing paradigms from weaning to adulthood. These tests were digitally recorded and manually scored using Behavior Tracker 1.5 (www.behaviortracker.com). Each scorer was blind to subject group assignment. See **Figure 1** for a summary of all experimental procedures.

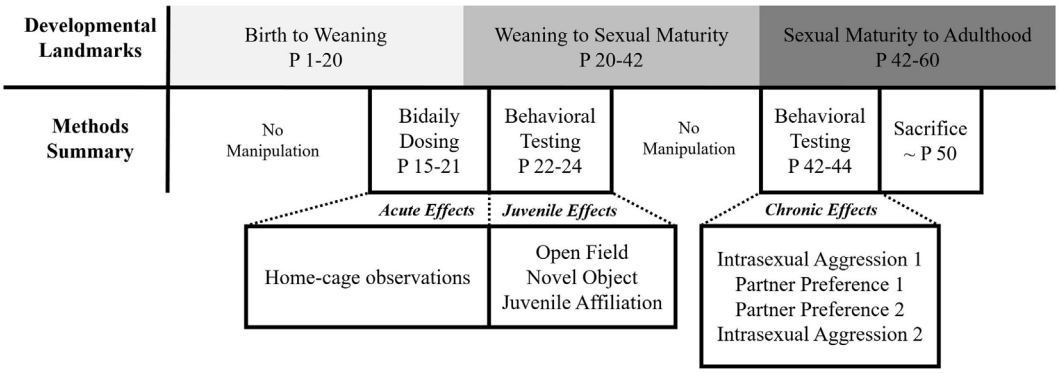

FIGURE 1 | Summary of experimental procedures.

Acute Observations

Following the first treatment on the first (P15) and sixth day (P20) of dosing, each animal was observed in their home-cage approximately 15 min posttreatment for a total of 5 min. We measured the duration of contact, nursing, and licking/grooming behaviors directed toward the subjects.

Open Field Test

All subjects received an open field test on P22. The open field consisted of a 40 cm × 40 cm × 40 cm Plexiglas box with a 5 × 5 grid marked on the floor. At the beginning of the test, the vole was placed in the center of the arena while their behavior was digitally recorded for the following 10 min using a camera. Observers recorded the frequency of line crosses, fecal droppings, and rearing and the duration of autogrooming, thigmotaxis, and freezing behavior.

Novel Object Recognition Test

We split the test into two phases, which included a familiarization phase (NOF) and a testing phase (NOT) occurring on separate, consecutive days following open field testing (P23 and 24, respectively). Both phases were conducted in the open field test arena to help habituate the animals to the environment. Thus, subtle differences in object interactions would not be overshadowed by the environment's novelty.

During the familiarization phase, two identical objects were placed in opposite corners of the open field arena. Like open field testing, the subject was placed in the center of the arena and their behavior was recorded for 10 min. The following day, the animal was reintroduced to the arena for the testing phase for 10 more minutes. During this phase, a familiar object from the day before was placed into the arena with a novel object; object placement was consistent between the tests. We measured the same behaviors in this paradigm as open field while including a measure for the duration of time spent interacting with the objects.

Juvenile Affiliation Test

At P25, each test subject and novel conspecific was placed into a neutral cage (27 cm × 16 cm × 13 cm) where interactions were digitally recorded for 10 min. Observers recorded affiliative behaviors (sniffing, contact, allogrooming, play), anxiety-related behaviors (digging, rearing, autogrooming, defensive rearing), and aggressive behaviors (i.e., lunging, chasing, wrestling). Play behavior was recorded as a sum of the different behaviors described by Chau et al. (24). In addition to play and rearing, all aggressive behaviors were recorded as frequencies. All other behaviors were recorded as durations.

Intrasexual Aggression Tests

The intrasexual aggression test was similar to the juvenile affiliation test, with two significant differences: (1) the stimulus animal was collared for identification purposes, (2) the test was done twice during adulthood. The first test occurred the day before partner preference testing (P42) and the second took place the day after (P45). The first test provided a baseline for adult sociality and aggressiveness while the second was meant to test for post-pair-bonding behaviors like mate-guarding. Each test was digitally recorded for 10 min and observers scored all the same behaviors as in juvenile affiliation.

Partner Preference Tests

Following the first intrasexual aggression test, all subjects underwent two partner preference tests over two consecutive days (P43 and 44, respectively). For partner preference testing, each test subject was given a cohabitation period with a sexually naïve partner of the opposite sex (25). For the first partner preference test, male subjects underwent a cohabitation of 2 h, while females were given 30 min. The discrepancy in cohabitation times between the sexes reflect differences in the time it takes for males and females to form a pair-bond naturally. While females can form a pair-bond after only 6 h of cohabitation, males generally require at least 24 h (26). Thus, the deficient cohabitation periods employed on the first day were meant to test for IN-AVP-stimulated facilitations of pair bonding.

Following the cohabitation period, the partner and an additional mate choice ("stranger") were loosely tethered within distinct testing chambers. Tethers consisted of a cable tie around the neck of the vole (employed carefully while animals are monitored) attached to fishing line, which is then secured firmly to the

side of the cage. The testing apparatus consisted of three identical polycarbonate cages (27 cm × 16 cm × 13 cm) attached by Plexiglas tubes (8.5 cm × 16 cm). The test animal was free to move throughout the apparatus while the two stimulus animals were confined to their separate chambers. The three-chambered paradigm provided the subjects with a choice of a familiar partner, novel stranger, or an empty cage for 3 h. Food and water was readily available in all chambers throughout the testing period.

Following the first partner preference test, the test animal and familiar partner were housed together overnight. A second partner preference test was then done the following day (approximately 24 h of cohabitation between tests) after a sufficient cohabitation period was provided to normally establish a pair-bond in both males and females. Thus, the second partner preference test was used to detect potential IN-AVP-stimulated deficits in pair bonding. A different stranger vole was used for this second test. For both tests, we measured the duration of cage location and side-to-side contact while recording the frequency of aggression.

Weight

To determine whether the potential effects of IN-AVP administration could be explained by weight changes, we measured all subjects on the first day of treatment, last day of treatment, and once after all testing had been completed.

Statistical Analyses

As direct treatment comparisons across the sexes were confounded by the difference in behavioral baselines, we decided to analyze males and females separately. Thus, we examined the effects of developmental AVP exposure in both sexes but not between sexes. We also controlled for the potentially confounding effect of litter on our results by assigning a unique identifier to all pups from the same litter and including this variable in our analyses.

All analyses were conducted using R version 3.3.3 (27). We began by fitting two models for each dependent variable, one including the litter variable as a random effect and one without it; both models included treatment group as a fixed effect. These two models were compared using an exact likelihood ratio test from the RLRsim package (28) to determine whether the presence of the variance component provided a better fit for the model. The test statistic from this likelihood ratio test is based on simulated values from the exact sample distribution as derived by Crainiceanu and Ruppert (29). When the statistic of the observed likelihood ratio was significant ($\alpha = 0.05$), we chose the mixed model over the linear model, having found evidence for a significant effect of litter on the dependent variable. Each model that included the random effect was fit using the lme4 package (30), while all other models were fit using base R functionality.

After selecting the best model, we conducted a series of follow-up tests to confirm that our model met the assumptions for ANOVA testing. For the normality assumption, we prioritized visual inspection of Q–Q plots (31), but confirmed our observations using a combination of the Shapiro–Wilk test and measures of skewness and kurtosis. Despite the Shapiro–Wilk test having the best power for a given significance when compared to other normality tests (32), it is biased by sample size (33). Therefore, normality was assumed when the Shapiro–Wilk test was statistically insignificant ($\alpha = 0.05$), the Shapiro–Wilk test statistic was high ($W > 0.95$), or when values for skewness and kurtosis fell between −2 and +2 (34, 35). We also utilized Levene's test to determine whether group variances were homogenous. When models contained outliers or heteroscedastic data, we refit the model using robust techniques (36, 37). Robust linear models were fit using the MASS package (38), while robust mixed linear models were fit using the robustlmm package (39).

After selecting the best model for each dependent variable and satisfying the assumptions for one-way ANOVA testing, we passed the models to the car package (40) to produce the ANOVA tables. For mixed ANOVA models, *F*-test statistics were calculated using Kenward–Roger's approximation for degrees of freedom. Using the lsmeans package (41), we conducted *post hoc* analyses on all models that contained a statistically significant effect of treatment ($\alpha = 0.05$). We only considered direct comparisons between each treatment group and control, warranting the use of Dunnett's test to control for Type I errors (42).

To determine whether overall parental handling differed between the groups, we combined the data from the dam with the sire and then compared total parental handling on individual observation days. Preliminary analyses confirmed no treatment differences within each observation day, so we then summed all parental handling behaviors across both days and reanalyzed the data. For partner preference data, we standardized the contact scores by subtracting the time spent with the stranger from the time spent with the partner, depicting the magnitude of the preference for the partner over the stranger. We tested whether our difference scores were significantly greater than 0, indicating a preference for the partner over the stranger. Then, we compared these scores across treatment groups to see if the magnitude of partner preference was affected by AVP treatment.

RESULTS

Early Effects

Intranasal AVP administration had no effect on acute parental handling; **Table 1**. For the open field test, IN-AVP altered fecal

TABLE 1 | Parental handling and weight change statistics.

		Acute observations	Weight
Sex	**Group**	**Parental handling**	**Weight change**
Males	Control	446.0 ± 47.7	25.3 ± 1.0
	Low	408.8 ± 82.8	29.1 ± 1.5
	Medium	528.4 ± 98.7	27.0 ± 1.7
	High	434.4 ± 84.3	24.9 ± 1.1
Females	Control	423.5 ± 37.6	19.5 ± 0.8
	Low	425.2 ± 48.7	21.0 ± 1.2
	Medium	373.5 ± 52.0	19.9 ± 1.7
	High	290.5 ± 56.0	20.6 ± 1.8

Values represent empirical means ± SEM. Parental handling represents the total amount of parental contact received (e.g., licking, nursing) across two individual observations periods (seconds). Weight change represents the weight gain from day 1 of treatment to sacrifice (grams).

TABLE 2 | Arena test statistics.

	Sex	Group	Line crosses	Autogrooming	Rearing	Exploration	Fecal boli	Freezing
Open field	Males	Control	475.3 ± 58.8	26.0 ± 5.7	41.7 ± 6.0	64.7 ± 9.3	1.7 ± 0.6	31.1 ± 4.2
		Low	370.7 ± 67.4	34.7 ± 8.8	40.7 ± 7.0	96.8 ± 28.2	2.4 ± 1.0	40.7 ± 13.4
		Medium	374.5 ± 51.2	21.8 ± 4.7	41.3 ± 9.5	60.7 ± 14.6	4.5 ± 1.4	88.6 ± 36.1
		High	392.9 ± 87.2	26.9 ± 10.4	46.5 ± 8.7	61.8 ± 15.8	1.7 ± 0.8	24.5 ± 9.3
	Females	Control	362.6 ± 54.9	37.9 ± 7.5	34.5 ± 5.4	59.1 ± 12.1	1.1 ± 0.3	71.9 ± 25.1
		Low	291.6 ± 37.9	25.5 ± 6.3	31.1 ± 6.2	63.5 ± 11.5	3.3 ± 1.4	57.8 ± 16.6
		Medium	326.8 ± 46.9	44.6 ± 10.9	28.4 ± 5.5	59.7 ± 10.8	3.7 ± 1.3	76.0 ± 74.1
		High	351.9 ± 116.4	26.4 ± 10.3	35.1 ± 13.3	46.9 ± 13.2	4.2 ± 1.1	58.3 ± 38.0
Novel object 1	Males	Control	497.3 ± 62.7	26.7 ± 5.1	42.4 ± 5.6	250.8 ± 17.4	1.5 ± 0.4	27.3 ± 5.1
		Low	381.6 ± 96.9	37.1 ± 9.9	41.4 ± 9.4	191.6 ± 34.8	2.2 ± 1.1	33.4 ± 13.8
		Medium	548.7 ± 102.3	16.7 ± 7.0	39.9 ± 6.3	237.0 ± 21.6	3.1 ± 1.3	18.1 ± 4.9
		High	456.0 ± 97.7	16.1 ± 5.8	45.1 ± 13.0	223.6 ± 37.9	3.3 ± 0.7	16.9 ± 7.5
	Females	Control	391.0 ± 55.6	36.4 ± 8.5	37.6 ± 5.8	225.5 ± 16.1	1.0 ± 0.5	31.5 ± 5.9
		Low	277.6 ± 47.4	45.1 ± 7.8	29.3 ± 7.9	205.5 ± 40.0	1.6 ± 0.9	56.7 ± 11.3
		Medium	280.9 ± 43.5	30.9 ± 9.4	33.2 ± 7.8	196.2 ± 32.7	3.1 ± 1.6	84.5 ± 37.8
		High	369.2 ± 126.8	32.3 ± 9.9	48.7 ± 26.1	179.8 ± 23.2	2.6 ± 1.4	33.0 ± 10.8
Novel object 2	Males	Control	381.3 ± 51.9	33.3 ± 7.6	37.7 ± 5.2	−14.8 ± 35.3	1.8 ± 0.6	51.9 ± 15.7
		Low	349.6 ± 108.7	46.8 ± 16.7	34.7 ± 10.4	−8.2 ± 41.7	0.8 ± 0.3	31.6 ± 13.7
		Medium	525.3 ± 96.7	23.3 ± 5.7	44.8 ± 6.5	23.0 ± 36.1	4.1 ± 1.3	38.0 ± 18.2
		High	433.2 ± 106.2	22.6 ± 5.1	47.0 ± 13.4	58.7 ± 22.3	2.8 ± 0.7	27.1 ± 13.2
	Females	Control	295.7 ± 65.4	33.0 ± 7.4	47.4 ± 8.7	−6.5 ± 20.9	1.4 ± 0.5	28.5 ± 7.1
		Low	417.9 ± 48.0	23.1 ± 4.5	38.6 ± 9.4	53.6 ± 43.8	1.3 ± 0.6	23.9 ± 4.6
		Medium	290.7 ± 70.4	47.3 ± 11.8	30.0 ± 8.1	−49.9 ± 50.8	2.7 ± 1.0	50.0 ± 18.6
		High	371.3 ± 132.1	13.9 ± 2.3	50.2 ± 23.4	64.2 ± 45.8	2.7 ± 1.2	22.0 ± 4.0

Values represent empirical means ± SEM. Line crosses, rearing, and fecal boli represent count data while autogrooming, exploration, and freezing are measured in seconds. The exploration variable is measured differently across the three paradigms. In the open field test, exploration represents the time spent in the center of the arena. During novel object 1, exploration is the total time spent in object zones. For novel object 2, exploration is the difference in time spent with the novel relative to the familiar object.

boli production in males, $F\,(3, 51) = 2.839$, $p < 0.05$. Specifically, males treated with the medium dose produced more fecal boli than control, $z = 2.801$, $p < 0.05$. IN-AVP also altered fecal boli production in females, $F\,(3, 50) = 4.497$, $p < 0.01$; high-dose exposure increased fecal boli production relative to control, $z = 3.650$, $p < 0.001$. We did not find effects of IN-AVP on any other recorded behavior in either sex; **Table 2**.

During both phases of the novel object recognition test, the time spent in each object's interaction zone was similar across treatment groups regardless of sex. In addition, none of the treatment groups, including control, preferentially maintained proximity with the novel object over the familiar object. Like with open field testing, we found no treatment group differences in anxiety or exploratory measures across both phases of recognition testing; **Table 2**. We decided to combine fecal boli production across the three paradigms to confirm an overall effect of treatment. For males, IN-AVP altered the total fecal boli production across testing days [$F\,(3, 51) = 3.656$, $p < 0.05$], confirming an increase for medium-dose males (M = 11.70, SEM = 3.13) compared to control (M = 5.00, SEM = 0.99), $z = 3.036$, $p < 0.01$; **Figure 2**. AVP also impacted the total fecal boli produced across testing days in females, $F\,(3, 50) = 3.069$, $p < 0.05$; **Figure 2**. While the high dose (M = 9.44, SEM = 2.71) tended to increase [$t = 2.39$, $p = 0.056$], the medium dose (M = 9.50, SEM = 2.49) significantly increased fecal boli production relative to control (M = 3.54, SEM = 0.95), $t = 2.502$, $p < 0.05$.

For juvenile affiliation, we found no effect of IN-AVP on social- or anxiety-related behaviors in males. But, IN-AVP did impact play behavior in females [$F\,(3, 50) = 2.750$; $p = 0.05$]; the medium dose increased bouts of play compared to control, $t = 2.729$, $p < 0.05$; **Figure 2**. No other behaviors were altered by juvenile IN-AVP treatment; **Table 3**.

Adult Effects

As with juvenile affiliation, we found no effect of IN-AVP across all recorded behaviors during the first intrasexual aggression test in males; **Table 3**. However, IN-AVP did have an effect on male aggression during the second intrasexual aggression test, $F\,(3, 45) = 4.735$, $p < 0.01$. Males treated with the high dose engaged in more bouts of aggressive behavior than control males, $z = 3.031$, $p < 0.01$; **Figure 3**. For intrasexual aggression testing in females, we found no effect for IN-AVP in females across all recorded behaviors regardless of testing day.

For the first partner preference test, we found no evidence of mate preference for any of the treatment groups; **Table 4**. However, IN-AVP did have an effect on partner preference in males during the second partner preference test, $F\,(3, 50) = 5.847$, $p < 0.01$ (**Figure 4**; Figure S1 in Supplementary Material). Further analyses revealed that while the control and low dose groups significantly preferred their partners over strangers ($t = 6.096$, $p < 0.00001$ and $t = 4.329$, $p < 0.0001$, respectively), such preference was not seen in both the medium- and high-dose groups. In addition, male medium- and high-dose groups spent significantly less time in preferential contact with their partner than control, $t = 2.856$, $p < 0.05$ and $t = 3.055$, $p < 0.05$, respectively. For these two groups, the reduction in time spent in contact with the partner could not be explained by increases in time spent in the neutral compartment, $F\,(3, 47) = 0.378$, $p = 0.769$.

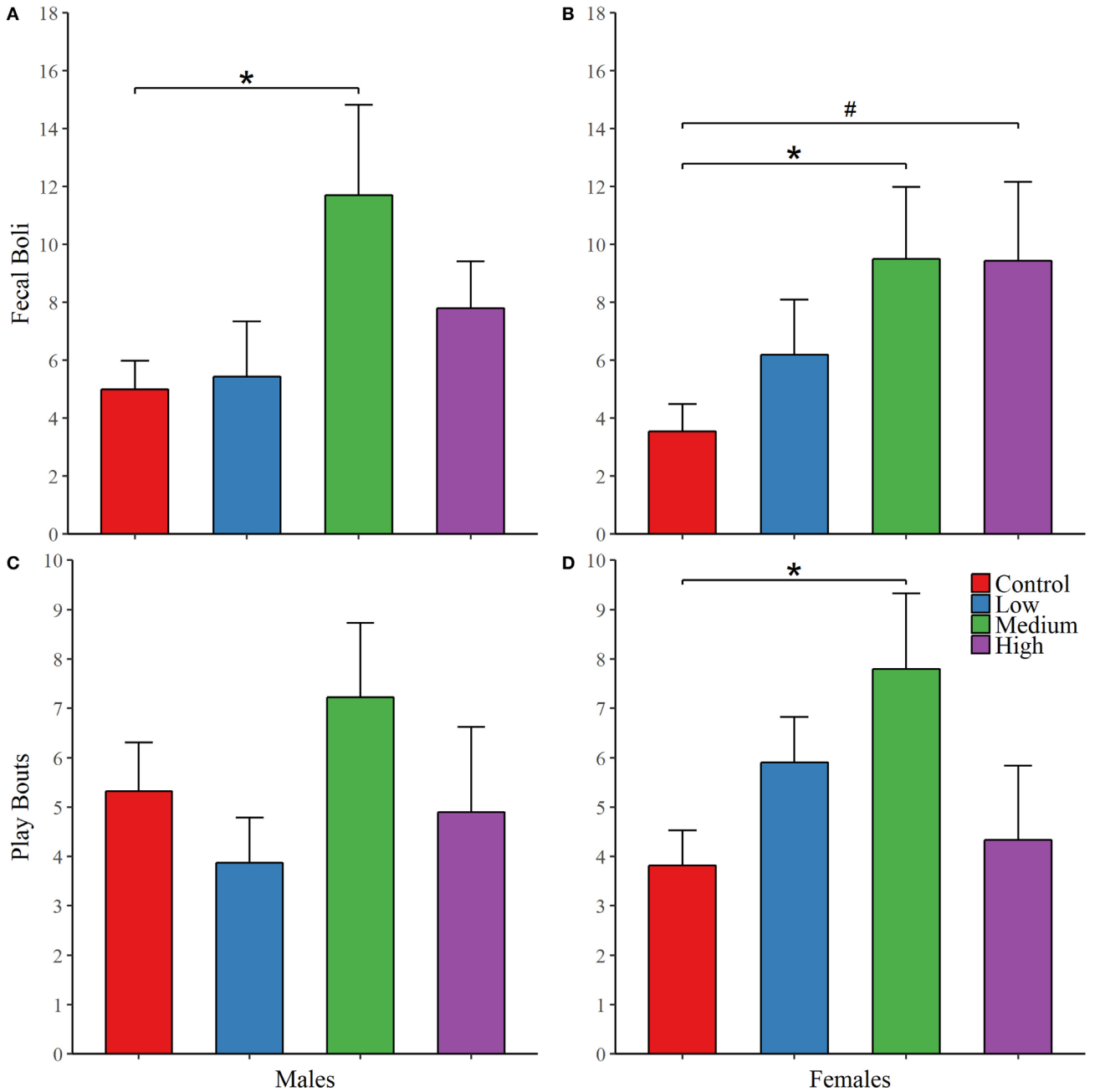

FIGURE 2 | Early exposure to intranasal AVP (IN-AVP) alters juvenile behavior. Values represent group means + SEs. Fecal boli were aggregated across open field and novel object tests (upper row). The dose–response curves appear to differ by sex for fecal boli production; male results reflect a *U*-shaped curve **(A)** and female results approximate a linear effect **(B)**, peaking at the medium dose. Only the medium dose increased fecal boli production in males while both the medium and high doses of IN-AVP increased fecal boli production in females. Bouts of play (bottom row) approximated *U*-shaped curves in both males **(C)** and females **(D)**, only the medium dose in females significantly increased play. *Statistically significant, #trend for significance.

Intranasal AVP did not affect partner contact in females during the first partner preference test and no group preferred the partner over the stranger. As for the second test, we did not find a significant effect of treatment on preferential mate choice. But while no differences existed between treatment groups, we did conduct *post hoc* analyses to confirm partner preference within each group. Unlike males in the second test, all female treatment groups did demonstrate a significant partner preference (control: $t = 7.347$, $p < 0.00001$; low: $t = 4.755$, $p < 0.0001$; med: $t = 5.914$, $p < 0.00001$, high: $t = 4.127$, $p < 0.01$) (**Figure 4**; Figure S1 in Supplementary Material). See **Table 4** for all partner preference testing descriptive statistics.

TABLE 3 | Juvenile affiliation and adult intrasexual aggression test statistics.

	Sex	Group	Sniffing	Autogrooming	Rearing	Play	Aggression
Juvenile affiliation	Males	Control	59.6 ± 6.7	44.7 ± 7.7	41.8 ± 5.2	5.3 ± 1.0	–
		Low	59.4 ± 9.7	57.9 ± 13.4	42.6 ± 7.7	3.9 ± 0.9	–
		Medium	64.1 ± 9.3	43.6 ± 14.5	40.6 ± 5.0	7.2 ± 1.5	–
		High	66.4 ± 7.0	44.0 ± 14.3	36.8 ± 5.6	4.9 ± 1.7	–
	Females	Control	49.6 ± 7.4	37.5 ± 8.0	42.8 ± 7.1	3.8 ± 0.7	–
		Low	101.8 ± 22.7	40.6 ± 11.2	41.7 ± 5.5	5.9 ± 0.9	–
		Medium	92.8 ± 20.9	32.8 ± 11.3	42.5 ± 6.6	7.8 ± 1.5	–
		High	72.7 ± 10.4	45.9 ± 11.4	50.8 ± 9.9	4.3 ± 1.5	–
Intrasexual Aggression 1	Males	Control	84.4 ± 9.9	43.1 ± 7.9	43.8 ± 8.5	–	2.8 ± 1.2
		Low	54.9 ± 11.8	61.2 ± 14.3	33.9 ± 5.2	–	7.0 ± 2.9
		Medium	80.4 ± 14.3	38.2 ± 12.3	42.9 ± 12.0	–	0.7 ± 0.4
		High	93.3 ± 17.0	28.9 ± 6.4	48.3 ± 13.5	–	1.1 ± 0.7
	Females	Control	67.0 ± 9.3	59.5 ± 11.1	47.2 ± 10.1	–	2.0 ± 1.2
		Low	85.1 ± 10.4	60.6 ± 14.9	38.6 ± 4.6	–	0.9 ± 0.5
		Medium	76.7 ± 15.6	52.9 ± 10.4	54.0 ± 10.2	–	3.4 ± 2.6
		High	48.1 ± 10.5	86.0 ± 17.7	45.8 ± 12.8	–	0.9 ± 0.6
Intrasexual Aggression 2	Males	Control	59.6 ± 8.1	76.3 ± 12.0	42.9 ± 7.1	–	9.2 ± 2.4
		Low	37.2 ± 12.5	105.0 ± 27.2	27.3 ± 8.4	–	8.2 ± 6.6
		Medium	56.3 ± 12.0	45.5 ± 23.2	43.3 ± 11.6	–	9.1 ± 6.6
		High	58.6 ± 16.8	65.1 ± 17.9	26.9 ± 5.6	–	20.6 ± 5.5
	Females	Control	49.1 ± 10.1	63.1 ± 14.4	45.9 ± 7.1	–	11.8 ± 2.4
		Low	43.3 ± 15.9	83.7 ± 17.4	44.9 ± 9.8	–	7.0 ± 2.5
		Medium	56.3 ± 19.2	69.9 ± 13.0	49.5 ± 11.3	–	7.9 ± 3.8
		High	61.9 ± 16.2	81.8 ± 26.3	64.1 ± 14.7	–	10.3 ± 4.6

Values represent empirical means ± SEM. Sniffing and autogrooming are measured in seconds while rearing, play, and aggression are counts.

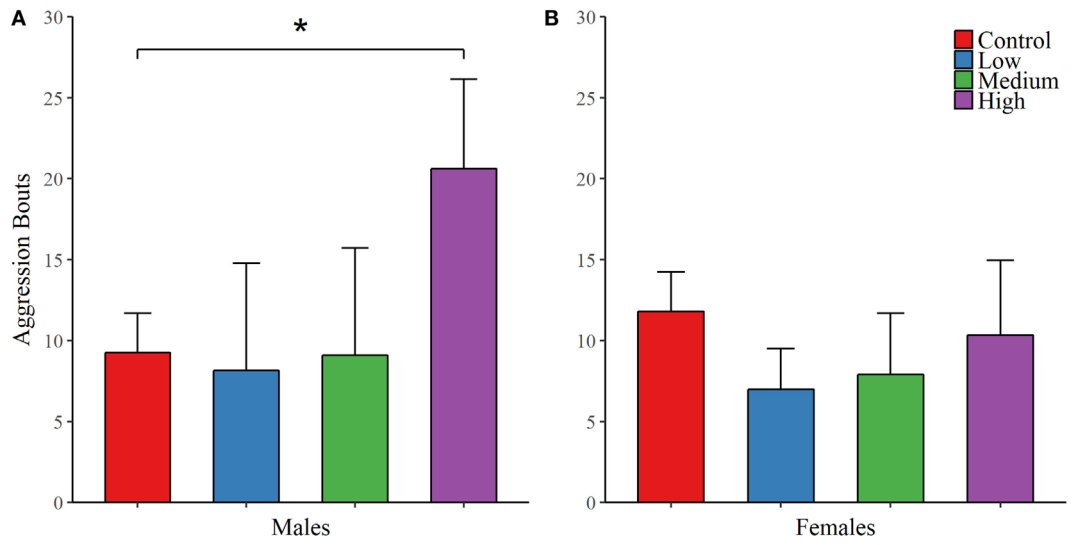

FIGURE 3 | Early exposure to intranasal AVP (IN-AVP) increases aggression in adult males. Values represent group means + SE. While bouts of aggression (e.g., lunging, wrestling) were unchanged during the first iteration of intrasexual aggression testing (not shown in figure), the high dose significantly increased aggression in males following partner preference formation **(A)**. IN-AVP had no detectable effect on female aggression **(B)**.

Finally, each animal was weighed on the first day of treatment and on the day of sacrifice. We found an effect of IN-AVP on weight change across life in males [F (3, 32.481) = 5.234, $p < 0.01$], but not in females; **Figure 5**. Further analysis revealed an increase in weight for low-dose males compared to control, $t = 3.672$, $p < 0.01$.

DISCUSSION

Effects of IN-AVP Vary by Sex, Dose, and Context

We hypothesized that the effects of IN-AVP would (1) vary by dose, (2) be more prominent in males, and (3) exhibit contrasting

TABLE 4 | Partner preference test statistics.

	Sex	Group	Partner contact	Stranger contact	Contact difference	Neutral zone
Partner preference 1	Males	Control	864.4 ± 187.6	409.1 ± 114.4	455.3 ± 265.0	2,756.5 ± 243.1
		Low	357.7 ± 235.2	545.3 ± 276.1	−187.7 ± 421.2	3,546.0 ± 428.8
		Medium	531.5 ± 264.9	887.0 ± 357.6	−355.5 ± 546.2	2,567.0 ± 290.4
		High	687.0 ± 262.3	472.2 ± 210.4	214.8 ± 422.5	2,978.6 ± 393.5
	Females	Control	704.7 ± 190.9	151.8 ± 91.5	552.9 ± 229.7	4,242.7 ± 431.7
		Low	767.5 ± 409.4	189.5 ± 118.2	578.0 ± 460.6	3,220.8 ± 526.8
		Medium	418.2 ± 179.5	472.6 ± 242.1	−54.4 ± 360.4	3,304.1 ± 392.7
		High	453.8 ± 233.8	724.7 ± 248.0	−270.9 ± 409.6	3,492.2 ± 527.7
Partner preference 2	Males	Control	1,931.8 ± 240.2	185.4 ± 79.8	1,746.3 ± 286.8	2,296.7 ± 197.1
		Low	2,107.1 ± 329.9	4.1 ± 2.7	2,103.0 ± 330.8	2,186.3 ± 383.9
		Medium	877.8 ± 339.9	617.8 ± 211.9	260.0 ± 491.5	2,445.7 ± 257.1
		High	712.0 ± 302.5	555.3 ± 233.1	156.7 ± 462.1	2750.5 ± 692.5
	Females	Control	1,921.0 ± 267.8	68.7 ± 53.4	1,855.4 ± 283.6	2,511.8 ± 266.4
		Low	2,084.6 ± 413.4	198.9 ± 198.9	1,905.6 ± 490.8	2,147.1 ± 311.2
		Medium	2,527.2 ± 270.2	0.0 ± 0.0	2,527.2 ± 270.2	1,706.9 ± 338.8
		High	1,856.8 ± 337.0	97.4 ± 97.4	1,770.2 ± 397.6	2,756.4 ± 307.9

Values represent empirical means ± SEM. All variables are measured in seconds. Contact behaviors represent total time spent in social immobility (seconds), while neutral zone is the time spent in the neutral cage.

effects depending on context. Our results confirm that IN-AVP modulates behavior in dose-specific ways. For example, the low dose increased weight gain, the medium dose increased fecal boli production, and the high dose increased aggression. We also found the most profound effects of IN-AVP administration in males, who exhibited both behavioral and physiological changes across life. Specifically, we found no effects of IN-AVP on adult female behavior, but males experienced impairments in partner preference formation and increases to aggression depending on the dose. We also detected context-specific contrasts in behavior. While IN-AVP did increase aggression during competitive encounters, we did not find increases in social behavior during non-threatening encounters. IN-AVP impaired sociability in males during partner preference testing without impacting sociability in juvenile affiliation or the first intrasexual aggression test (prior to partner preference). The changes to aggression experienced after partner preference testing also confirm that the effects of IN-AVP depend on context. This is further supported by the IN-AVP-stimulated increases in anxiety experienced by females in non-social contexts and increases to play behavior during social contexts (e.g., juvenile affiliation).

Early IN-AVP Modulates Juvenile Anxiety and Sociality

In the present study, we found increases in fecal boli production across open field and novel object recognition testing for both males and females treated with the medium dose. Context-specific increases in fecal boli production have been associated with increased anxiety (43, 44); habituated animals produce successively fewer fecal boli with repeated testing (45). But, previous studies have also demonstrated a role for AVP in the regulation of gastric motility; systemic AVP injections increase gastric motility (46, 47) while AVP microinjections within the rat vagal nerve inhibit gastric motility (48). Given the fact that we did not find increases in other anxiety-related behaviors (e.g., freezing, autogrooming) alongside, the increases to fecal boli production, we suspect that peripheral AVP receptor effects on gastric motility may provide a stronger explanation for our findings. It would have been interesting to see if a more anxiogenic paradigm would have elicited a stronger response or if our results could be blocked by antidepressants.

The effects of IN-AVP during the juvenile period also appear to differ by context. While the treatment may potentially increase anxiety in both males and females during non-social novelty, we found evidence for increases in sociality with a novel social partner. Specifically, the medium dose selectively increased play bouts in females (with no increase in males). This may reflect slight differences in the quantity of play exhibited by male and female prairie voles; male control voles engaged in marginally higher bouts of play than female control voles. Alternatively, it is also possible that females may be more susceptible to the effects of AVP in play behavior since male prairie voles have more AVP-containing neurons than females in several neural regions, such as the medial amygdala and bed nucleus of the stria terminalis (22).

In rodents, the AVP system seems to regulate social play differently between males and females. For example, intracerebroventricular (ICV) administration of AVPR1a antagonists in rats increased social play in females and decreased it in males (49). However, site-specific injections of AVPR1a into the lateral septum produced the opposite results, increasing play in males while reducing it in females (6). Without measuring the effect of chronic IN-AVP on regulation of AVP receptors and peptide throughout the brain, it is difficult to determine the neural mechanism for our findings that medium-dose IN-AVP increased play bouts in females. Repeated activation of AVPR1a has been shown to cause internalization, decreasing the membrane density of AVPR1a (50). It is possible that the administration period and frequency implemented in this study was sufficient to decrease AVPR1a densities across the

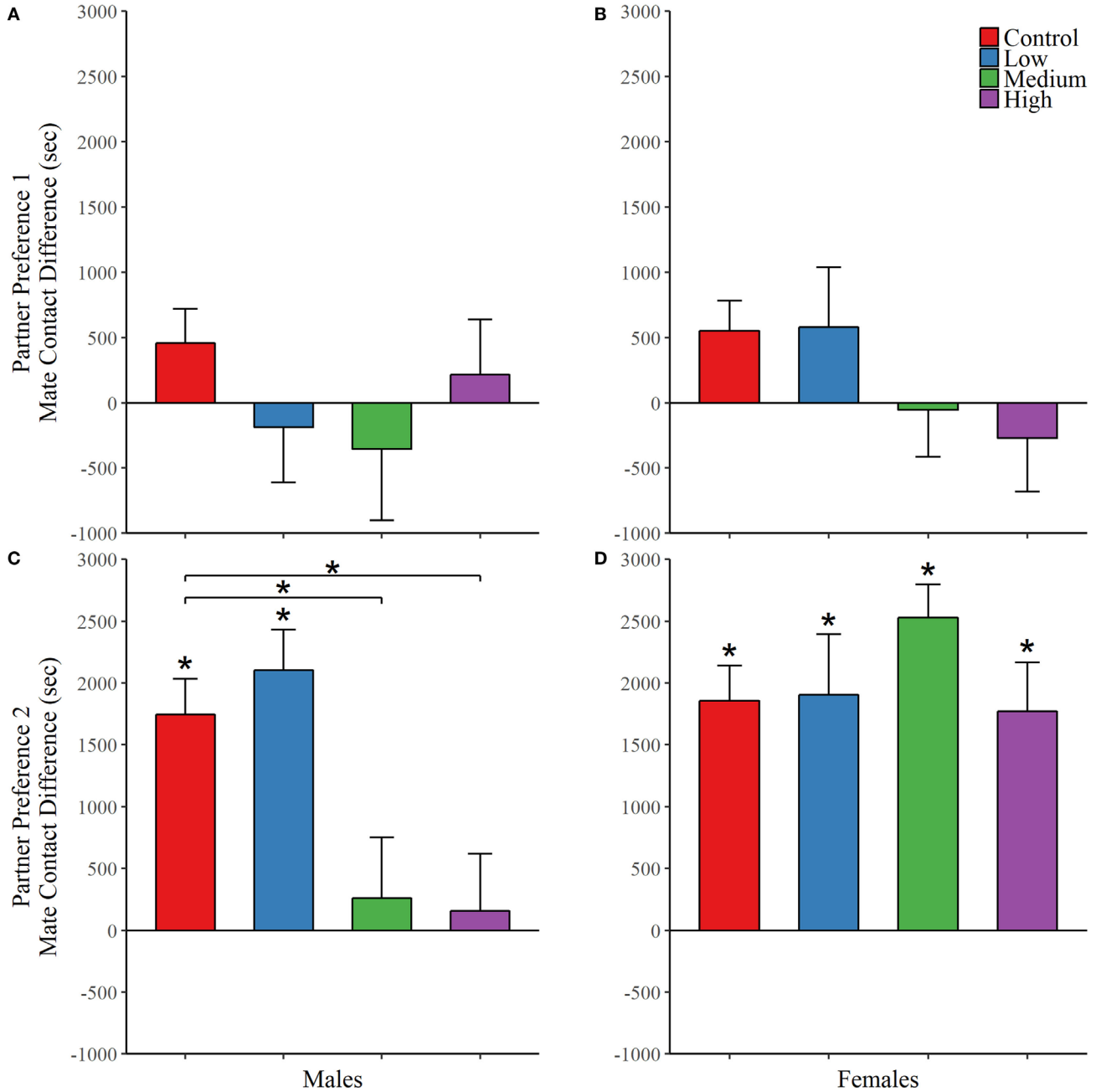

FIGURE 4 | Early exposure to intranasal AVP (IN-AVP) blocks partner preference formation in males. Values represent the mean difference in side-to-side contact between the partner and stranger + SE. Asterisks immediately above group means indicate a significant difference from zero (e.g., more contact with partner than stranger) while asterisks above comparison lines indicate significant group differences in preference. During the first partner preference test, subjects were housed with potential mates for an insufficient amount of time to form a preference (upper row). IN-AVP did not facilitate partner preference in either males **(A)** or females **(B)** during this test. The second partner preference test was completed following 24 h of cohabitation between each test subject and their respective partners from the first test (bottom row). The medium and high doses of IN-AVP shunted partner preference in males **(C)** but all female treatment groups **(D)** successfully preferred the partner over the stranger.

brain, approximating the effect of ICV AVPR1a antagonist administration as shown by Veenema et al. (49). But, if down-regulation of AVPR1a is the cause of our observed effects, it is curious that we did not see a simultaneous decrease in play activity for males treated with IN-AVP. Regardless, we might expect the effects of intranasally administered AVP in prairie voles to differ from other more direct delivery routes (e.g., microinjections) and from other rodent species, especially, since the mechanism for behavioral effects of intranasal delivery has not yet been confirmed (51).

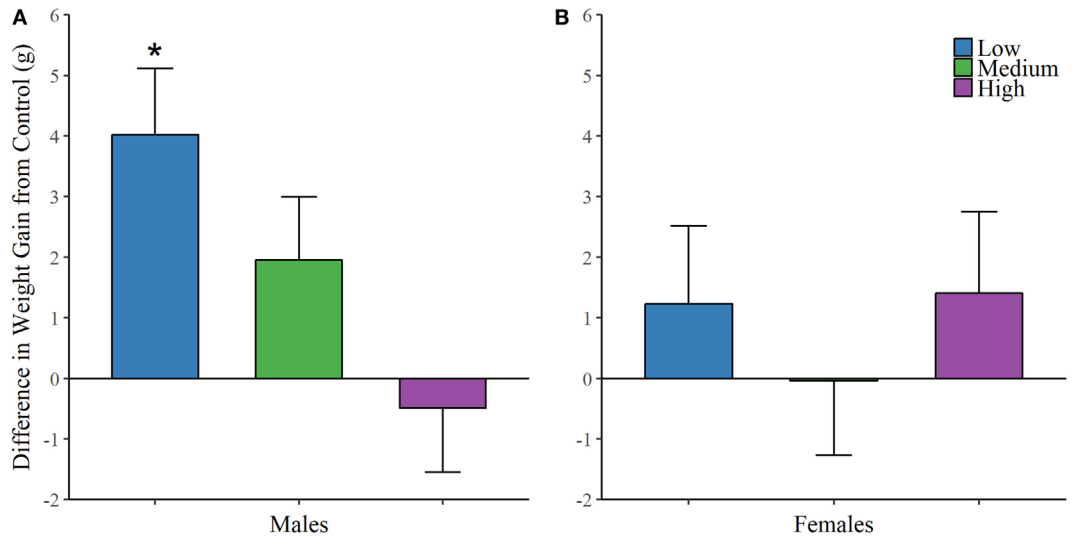

FIGURE 5 | Early exposure to intranasal AVP (IN-AVP) increases weight gain in males. Values represent the difference in total weight gain across life from control (means + SE). The low-dose of IN-AVP increased weight gain across life in males **(A)** while no detectable effect was found in females **(B)**.

Early IN-AVP Modulates Pair-Bonding Behavior Later in Life

Early studies suggested that OT activity on the OTR was more important for pair-bond formation in females, while AVP activity on the AVPR1a was more important in males (52–55). More recent studies have produced more subtlety than this strict dichotomy, suggesting instead that OT and AVP are involved in partner preference formation in both sexes (56–59). Cho et al. (57) found that ICV administration of OT or AVP could facilitate pair-bond formation in both males and females while concurrent administration of either OT receptor or AVPR1a antagonists blocked this effect. In our study, we demonstrated that a single week of twice daily exposure to IN-AVP during the early juvenile stage can disrupt pair-bond formation later in life. These effects were sex-dependent, occurring only in the male medium- and high-dose groups, and did not reflect changes in the amount of time spent in the neutral cage. On the contrary, all experimental groups in females successfully demonstrated a partner preference by the time of the second test.

Previous studies have demonstrated that pair-bond behavior in prairie voles is highly susceptible to manipulation of the AVP system. In adult prairie voles, site-specific AVPR1a antagonists within the ventral pallidum prevented pair-bond formation (60), while selectively increasing AVPR1a densities in the ventral forebrain facilitated pair-bond formation (61). Overexpression of AVPR1a within the ventral pallidum (60) and ventral forebrain (62) of the promiscuous meadow vole substantially increased partner preference behavior. As mentioned before, repeated activation of AVPR1a can lead to physiological tolerance (50). Since the observed chronic effects of IN-AVP administration in our study approximate the effects of acute AVPR1a antagonist administration in other studies, we suspect that a single week of twice daily exposure to AVP should be sufficient to decrease AVPR1a densities in specific neural regions, which are critical for pair-bond formation in males. Complementary work from our lab has shown that chronic OT exposure also impairs partner preference formation in prairie voles (63) with subsequent changes to OTR receptor and AVP peptide concentrations (unpublished data).

Given our results, we suspect that the physiological mechanisms behind the disrupted partner preference in males may differ between the medium- and high-dose groups. Specifically, the high dose also resulted in a substantial increase in aggressive behavior during the second intrasexual aggression test, which was conducted approximately 24 h following partner preference testing. These findings are similar to those found by Stribley and Carter (7); early postnatal exposure to the high dose of AVP increased aggression in sexually naïve prairie voles compared to control. In prairie voles, aggressive tendencies naturally increase following induction of the pair-bond. Gobrogge et al. (64) showed that 2 weeks of cohabitation with a female intensely increased male aggression toward both novel male and female conspecifics; these males maintained elevated levels of social affiliation with their female partners during this time. But, the increase in aggression experienced by high-dose males in our study occurred in the absence of a preference for a female partner.

Outside of the pair-bond, AVP modulates aggressive behavior in sex-specific ways. AVP injections within the anterior hypothalamus stimulate aggression in male Syrian hamsters but inhibits it in females (65, 66). Also, male prairie voles who received low amounts of parental handling early in life engaged in more aggression in adulthood (67). Given the stimulatory effects of AVP in males on aggression and the lack of pair-bond formation in high-dose males, the increases in aggression is likely unaffiliated with mate-guarding. On the contrary, there is also the potential for delayed pair-bond formation in the high-dose males. While this group may not have formed a

partner preference at 24 h, they may have formed it at some point between the end of the second partner preference and the second intrasexual aggression test (which was an additional 24 h). Thus, it is possible that high-dose males experienced a delay in partner preference formation, but had an exacerbated mate-guarding response once the pair-bond occurred. Regardless, the medium and high doses may have different effects on AVPR1a in brain regions involved in pair bonding versus aggression.

We also found that the low-dose males gained significantly more weight than control. Inappropriate AVP secretion to the periphery (68) and the use of synthetic AVP (desmopressin) has been linked to weight gain in humans (69). Though we reported only the results of weight gain across life, we also measured the difference in weight gain across the dosing period. No significant difference in weight gain was found for any of the treatment groups during this time, but there was a suggestive increase in weight gain for the low-dose group in males (Cohen's $d = 0.66$). As supported by this experiment, early life manipulations can change adult behavior (and likely physiology). Therefore, it is possible that the slight (statistically insignificant) changes in weight gain for males treated with the low dose of IN-AVP across the dosing period were subsequently exacerbated across life.

Limitations

Caveats in interpretation of these results are the lack of any animal model for autism with clear constructive validity (70, 71), and the variability of OT and AVP receptors across different taxa and species. This variability may lead to differences in responses to these neuropeptides across species. In a previous study, we found that chronic OT impaired pair bonding in male prairie voles at certain doses (63). However, the same dose did not change mouse social behavior, either in BTBR mice (a rodent model of reduced sociability) or in their strain control (72). In our quest to translate neuropeptide results from animal models, we should consider the neurobiology and natural history of the animal model, as well as the dosage, sex, context of administration, and other testing conditions. Future studies in animal models and in humans will reveal which model is most predictive.

Another limitation is the potential for the confounding effects of repeated behavioral testing on the results. Our study employs several behavioral paradigms in the early juvenile period as well as adulthood. It is possible that this combination of testing could have obscured or attenuated treatment and sex effects of IN-AVP, particularly given the density of testing and the frequency of experimental handling early in life. But as mentioned previously, the increases in aggression experienced by the high-dose group in adulthood do replicate the results of Stribley and Carter (7) who did not employ the same intensity of behavioral testing.

CONCLUSION

The results of this study confirm that the contextually pro-social effects of IN-AVP administration may differ from the long-term effects of a developmental exposure. Specifically, the impairment of partner preference displayed by male prairie voles in our study is notably opposite of the acute, facilitatory effects of AVP administration on partner preference formation (55) and social contact (73, 74) demonstrated in other studies. Further studies exploring differences in developmental timing and varied dosing schedules will contribute to our understanding of the AVP system while potentially informing clinical pursuits.

ETHICS STATEMENT

This study and protocol were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Davis, and complied with National Institutes of Health ethical guidelines as set forth in the Guide for Lab Animal Care.

AUTHOR CONTRIBUTIONS

TS planned and executed all aspects of this work under the direction of KB. JB, JD, SY, and JV helped provide intranasal treatments, behavioral testing, and video scoring. JT scored video. All authors participated in writing and editing this article.

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ORIGINAL RESEARCH
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Effects of Chronic Social Stress and Maternal Intranasal Oxytocin and Vasopressin on Offspring Interferon-γ and Behavior

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Recent studies support the hypothesis that the adverse effects of early-life adversity and transgenerational stress on neural plasticity and behavior are mediated by inflammation. The objective of the present study was to investigate the immune and behavioral programing effects of intranasal (IN) vasopressin (AVP) and oxytocin (OXT) treatment of chronic social stress (CSS)-exposed F1 dams on F2 juvenile female offspring. It was hypothesized that maternal AVP and OXT treatment would have preventative effects on social stress-induced deficits in offspring anxiety and social behavior and that these effects would be associated with changes in interferon-γ (IFNγ). Control and CSS-exposed F1 dams were administered IN saline, AVP, or OXT during lactation and the F2 juvenile female offspring were assessed for basal plasma IFNγ and perseverative, anxiety, and social behavior. CSS F2 female juvenile offspring had elevated IFNγ levels and exhibited increased repetitive/perseverative and anxiety behaviors and deficits in social behavior. These effects were modulated by AVP and OXT in a context- and behavior-dependent manner, with OXT exhibiting preventative effects on repetitive and anxiety behaviors and AVP possessing preventative effects on social behavior deficits and anxiety. Basal IFNγ levels were elevated in the F2 offspring of OXT-treated F1 dams, but IFNγ was not correlated with the behavioral effects. These results support the hypothesis that maternal AVP and OXT treatment have context- and behavior-specific effects on peripheral IFNγ levels and perseverative, anxiety, and social behaviors in the female offspring of early-life social stress-exposed dams. Both maternal AVP and OXT are effective at preventing social stress-induced increases in self-directed measures of anxiety, and AVP is particularly effective at preventing impairments in overall social contact. OXT is specifically effective at preventing repetitive/perseverative behaviors, yet is ineffective at preventing deficits in overall social behavior.

Keywords: social stress, depression, depression and anxiety disorders, interferon-γ, oxytocin, vasopressin, social behavior, inflammation

INTRODUCTION

Alterations in maternal care can mediate the developmental consequences of early-life experiences. Early maternal–infant interactions serve as a potential source of information concerning the environment to which offspring will need to adapt. For example, levels of maternal care can profoundly influence stress physiology in the infant and their developmental trajectory (1). Changes in infant glucocorticoid responses can be altered by the mother's behavior, eliciting lasting alterations in glucocorticoid responsiveness, and related behavioral changes (2). Further studies have shown that alterations in maternal care can be non-genomically inherited. Wild-type offspring born to a mother mutant for the Peg3þ/(paternally expressed gene 3) gene, which shows impairments in various aspects of maternal behavior, exhibited a reduction in their own ability to retrieve pups to a nest in a retrieval test (3). We have also demonstrated that chronic social stress (CSS) can alter levels of maternal care behavior and associated neuroendocrine changes in offspring (4, 5).

However, the mother's influence extends beyond classic neuroendocrine stress response systems. Early-life stress and maternal care is able to activate not only neuroendocrine systems but also the innate immune system, which effects behavioral responsiveness (6, 7). The presence of the mother effectively suppresses the behavioral consequences of innate immune activation (8). Recent studies have supported the hypothesis that the adverse effects of early-life adversity on neural plasticity and behavior are mediated by inflammation (9). For example, mice deficient in adaptive immunity exhibit social deficits and hyper-connectivity of fronto-cortical brain regions, which are mediated by interferon-γ (IFNγ) (10), a key regulator of immune responses (11). These findings introduce the possibility that long-term adaptive behavioral change can be mediated by the mother's influence on immune-related activity of her pups. This further raises the question on the role of maternal neuroendocrine factors in infant immune activity. Concerning the present study, it is known that vasopressin (AVP) and oxytocin (OXT) stimulate T-helper cells to produce IFNγ (12), and OXT treatment suppresses TNFα-production in LPS-stimulated microglial cells and a decrease in microglial activation *in vivo* in rats (13).

The CSS model of postpartum depression and anxiety (see **Figure 1**) depresses maternal care, impairs lactation, and increases maternal anxiety in F0 rat dams exposed to chronic male intruder stress during days 2–16 of lactation (14–16) and has similar effects in F1 and F2 dams (4, 17, 18). Juvenile and adult F2 offspring of F0 CSS dams exhibit deficits in social behavior (5). For the F1 and F2 offspring of stressed dams, the effects of CSS on social behavior may be mediated by early-life exposure to depressed F0 maternal care and/or the male intruder stressor (F1 offspring) or depressed F1 maternal care only (F2 offspring). At the neuroendocrine level, CSS F0 dams have decreased OXT gene expression in the MeA, and CSS F1 dams also have lower MeA OXT, lower AVP in the MeA and PVN, and higher OXTR gene expression in CeA (18). CSS F2 juvenile females have higher basal serum OXT levels (5) though basal OXT levels in the CSS F1 dams do not differ (4), suggesting the transgenerational accumulation of the effects of social stress. Furthermore, female F2 adult offspring of CSS dams exhibit decreases in the immune factors alpha 1 acid glycoprotein (α1AGP) and intercellular adhesion molecule 1, and α1AGP levels are correlated with allogrooming during a social behavior test (7) supporting the role of immune factors in behavioral programing by maternal care. Chronic AVP infusion into the lateral ventricles improves maternal care in CSS-exposed F0 dams (19), but treatment of the F1 or F2 generations has not been attempted.

Both animal and clinical studies have indicated that intranasal (IN) OXT has potent effects on parental behavior (20, 21). Acute OXT studies in animals stimulated clinical studies using

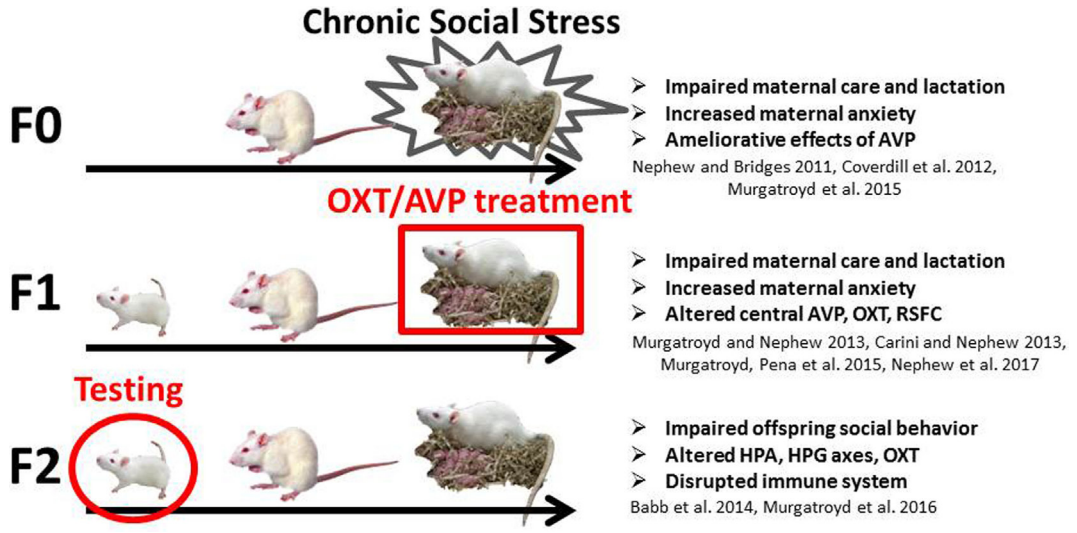

FIGURE 1 | **Diagram of the chronic social stress model**. The current study focuses on the F2 juvenile female offspring (circled) of intranasal vasopressin- and oxytocin-treated F1 dams.

acute and chronic IN dosing, despite a lack of preclinical data with chronic dosing. Recent rodent studies report that chronic IN OXT may have adverse long-term effects on social behavior (22), and while IN OXT is being tested as a potential treatment for postpartum depression and anxiety with mixed results (23, 24), the effects of maternal OXT, or closely related AVP, on offspring behavior and physiology have not been thoroughly studied. The objective of the present study was to investigate the immune and behavioral programing effects of IN AVP and OXT treatment of CSS-exposed F1 dams on F2 juvenile female offspring. It was hypothesized that both AVP and OXT would have preventative effects on social stress-induced deficits in social behavior and increased anxiety and that these effects would be associated with changes in TNFα and/or IFNγ in juvenile F2 females. It was predicted that CSS would decrease F2 IFNγ and increase TNFα, and F1 OXT and AVP treatment would prevent these changes, resulting in similar immune and behavioral levels in controls and the F2 offspring of OXT- and AVP-treated controls.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats (Charles River Inc., Kingston, NY, USA) in this study were maintained in accordance with the guidelines of the Committee of the Care and Use of Laboratory Animals Resources, National Research Council, and the research protocol was approved by the Tufts Institutional Animal Care and Use Committee. "CSS dams" refers to the adult females exposed to CSS during lactation (F0), "CSS F1 dams" refers to the maternal adult female offspring of the CSS F0 dams, which were treated with chronic saline, AVP, or OXT, and "CSS F2 juveniles" refers to female juvenile F2 offspring, which are the focus of the present study. The five experimental groups consisted of control (CON), control + intranasal saline (CON SAL), CSS + intranasal saline (CSS SAL), CSS + intranasal AVP (CSS AVP), and CSS + intranasal OXT (CSS OXT). Open-field and social behavior were tested on days 37–38, the marble burying test was done on days 38–39, and all rats were euthanized on days 39–40 (the day following the marble burying test) between 0900 and 1100 hours.

CSS Model: F0 Dams

The CSS dams were subjected to the CSS protocol from days 2 to 16 of lactation as reported (14, 15). This procedure consisted of placing a similarly sized (220–300 g) novel male intruder into a lactating female's home cage for 1 h from days 2 to 16 of lactation. CON dams were not exposed to the CSS protocol. The pups were left in the cage during the novel male intruder presentation, and the CSS exposure results in reduced maternal care (pup grooming and nursing) and increased anxiety-related behavior and maternal aggression in F0 dams (14), creating an early-life stress of depressed maternal care and social conflict for the F1 generation.

CSS Model: F1 Females

The CON and early-life CSS F1 females were the offspring of the F0 CON and CSS dams; the differences between the treatments of the CON and early-life CSS F1 females consisted of the exposure of the CSS F1 females to attenuated maternal care and conflict between their F0 mothers and the male intruders during age 2–16 days. The F1 CON and early-life CSS animals were treated identically after the age of 16 days. After weaning all F1 pups on day 23, the female offspring from the 12 CON and 12 CSS dams were housed in groups of four until 70 days of age, when 1–2 females from CON and CSS litters were mated with breeder males to obtain 12 CON and 18 CSS F1 females.

F1 Dam Intranasal Treatment Groups and Their F2 Offspring

F1 dams from a CSS background were randomly assigned to one of the three possible IN interventions: CSS SAL, CSS AVP, and CSS OXT, while F1 dams from a control background were randomly assigned to one of the two possible groups: CON and CON SAL. F1 CON dams were kept in a separate housing room because of the daily intrusion the other groups experienced for IN administrations. OXT and AVP (Sigma) or sterile saline alone were administered at a dosage of 0.8 IU/kg in 25 µl sterile saline based on previous studies (22). IN administrations occurred daily for 3 weeks of lactation. Rats were restrained using a flexible plastic cone called a DecapiCone (Braintree Scientific), which provided access to the nostrils and allowed for rapid and consistent administrations (20–30 s). IN fluids were administered using a 100 µl pipettor and rigid plastic non-puncturing pipette tips. Half doses were administered to each nostril.

Total F2 pup number and litter weights were recorded on the day of parturition, and litters were then culled to four females and four males. Other than the described IN treatments, the F2 groups of animals were treated identically throughout the study. The final F2 female juvenile sample sizes were seven for CON, six for CON SAL, eight for CSS SAL, eight for CSS AVP, and nine for CSS OXT. There were no treatment differences in F2 litter size or number or bodyweights at day 40 (all p's > 0.1).

Behavioral Tests

Open-Field and Social Behavior Testing

The experimental female rat was removed from the home cage and placed in a clean Plexiglas cage with black walls and a white floor (12″ × 20″ × 12″) for 5 min to allow for locomotor acclimation to the novel environment and video record open-field behavior. Videos were scored using Odlog (Macropod, Inc.). Open-field behaviors scored consisted of the durations and frequencies of moving along the edge (outer 2″), stationary along the edge, rearing along the edge, moving in the center, stationary in the center, rearing in the center, and self-grooming. At the end of the 5-min open-field test, one black wall was replaced with a clear barrier with a 0.5″ window 1″ from the floor. A same age novel rat from the same treatment group was in a same size cage on the other opposite side of the clear barrier. After 10 min, the clear barrier was removed to allow for direct social interaction for another 10 min. Social behaviors scored included rostral and caudal investigation, lateral contact, dorsal contact, allogrooming, self-grooming, locomotor activity, aggression, and total social contact (the sum of investigation, contact, and allogrooming).

Marble Burying Test

The marble burying test was conducted to assess repetitive and perseverative behavior (25). Juvenile female rats were placed in a clean cage with bedding where six marbles were evenly spaced on top of the bedding for 15 min. The number of marbles completely buried or at least 75% covered were counted at the end of 15 min.

Measurement of IFNγ and TNFα

All experimental animals were euthanized within 3 min of entering the animal room between 0900 and 1100 hours, the day following social behavior testing, and trunk blood was collected for the analysis of TNFα and IFNγ. These were measured by individual rat ELISAs (R&D Systems, USA). Samples were run in duplicate in an individual assay to eliminate interassay variation, and intraassay variability was 4%.

Statistical Analyses

Basal cytokine and behavior levels were analyzed with one-way ANOVA (on all five treatment groups and four groups with the two control groups combined) as well as *t*-tests of the combined control groups compared to the combined CSS groups to assess the overall effect of CSS (**Table 1**). These CSS-focused tests were followed by additional *t*-tests comparing individual treatment groups if there were significant treatment effects with ANOVA or the combined CON vs. combined CSS *t*-test. There were no differences between the CON and CON SAL groups with any of the variables, so these groups were combined to compare to individual CSS groups. All graphical results are presented as mean + SEM, significance was denoted as $p \leq 0.05$, and p values refer to two-tailed tests unless noted. The use of one-tailed tests was justified for use with IFNγ based on previous findings of its importance in neuronal connectivity and social behavior (10) and recently published data on impaired functional connectivity in the CSS model (26). The use of one-tailed tests was justified for use with behavioral data by initial effects of CSS in the present study or previous reports of increased anxiety and decreased social behavior in the F2 generation of CSS model (5). Cohen's *d* effect size tests were used to assess effect sizes, with 0.2–0.5 considered a small effect, 0.5–0.8 a medium sized effect, and values greater than 0.8 a large effect. Pearson correlations were used to test for significant IFNγ-behavior associations.

TABLE 1 | *t*-Test and Cohen's *d* values of combined control groups vs. combined chronic social stress (CSS) group comparisons.

Variable	Combined controls	Combined CSS	*p*-Value	Cohen's *d*
IFNγ (ng/ml)	7.2 ± 2.9	17.9 ± 4.0	0.04	0.7
Marbles buried	2.9 ± 0.4	3.6 ± 0.2	0.05*	0.6
Self-grooming duration (s)	4.1 ± 1.5	11.5 ± 3.9	0.03*	0.9
Social contact duration (s)	50.3 ± 4.3	37.9 ± 4.1	0.03*	0.9
Allogrooming duration (s)	10.3 ± 4.0	3.7 ± 2.9	0.04	0.6

**Indicates one-tailed t-test.*

RESULTS

IFNγ and TNFα

Basal plasma levels of TNFα were undetectable. There were no significant differences in IFNγ following one-way ANOVA ($F_{4,38} = 1.0$, $p = 0.4$, $F_{3,38} = 1.3$, $p = 0.3$, **Figure 2**). Basal plasma IFNγ levels were increased more than two-fold in combined CSS F2 offspring compared to combined CON F2 offspring (**Table 1**). This difference was driven by the CSS OXT group (22.7 ± 6.0 ng/ml, **Figure 2**), which had higher levels than the combined control groups ($t = 2.5$, $p = 0.02$, Cohen's $d = 1.1$) and when compared only with the CON SAL group (one-tailed $t = 2.0$, $p = 0.03$, Cohen's $d = 0.3$).

Marble Burying

There were no significant differences in marble burying following one-way ANOVA ($F_{4,38} = 1.5$, $p = 0.2$, $F_{3,38} = 1.5$, $p = 0.2$,

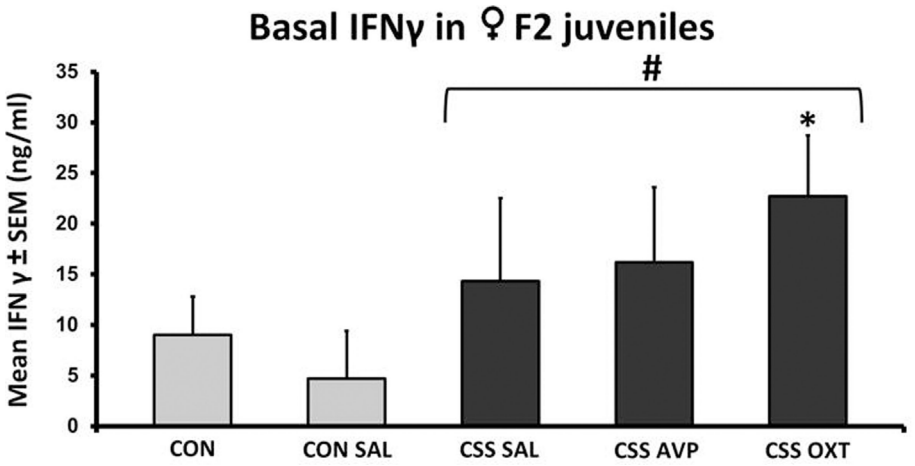

FIGURE 2 | Mean + SEM of basal IFNγ levels of chronic social stress (CSS) F2 juvenile female offspring of F1 control (CON) or CSS dams intranasally treated with saline, vasopressin, or oxytocin. # indicates overall effect of CSS treatment, * indicates significant increase compared to combined CON and control + intranasal saline (CON SAL) groups and CON SAL alone ($p < 0.05$).

Figure 3). CSS-exposed F2 female juveniles buried more marbles compared to CON animals during the 15-min test (**Table 1**). When comparing individual treatment groups, both the CSS SAL (3.9 ± 0.5 s, one-tailed $t = 1.8$, $p < 0.05$, Cohen's $d = 1.0$) and CSS AVP (3.8 ± 0.4 s, one-tailed $t = 2.0$, $p = 0.03$, Cohen's $d = 1.0$) groups buried more marbles than the CON SAL group (2.5 ± 0.6 s, **Figure 3**). CSS F1 dam treatment with OXT decreased the number of marbles buried by CSS F2 female juveniles to a level similar to controls.

Open-Field Behavior

There were no significant differences between CON and CSS-treated F2 female juvenile between any treatment groups in durations or frequencies of moving along the edge of the open field, stationary along the edge, rearing along the edge, and moving in the center of the open field (one-way ANOVA, all p's > 0.3). Durations and frequencies for stationary and rearing in the center were too low for statistical comparison. There were no significant differences in self-grooming in the open field following one-way ANOVA with all five treatment groups ($F_{4,38} = 2.6$, $p = 0.06$), but there was a significant effect of treatment when the two control groups were combined ($F_{3,38} = 3.5$, $p = 0.03$, **Figure 4**). CSS SAL juveniles expressed an almost three-fold increase in mean duration of self-grooming during open-field testing compared to the combined control groups (**Table 1**). Mean self-grooming durations in both the CSS AVP (2.3 ± 1.5 s) and CSS OXT (1.5 ± 1.0 s)

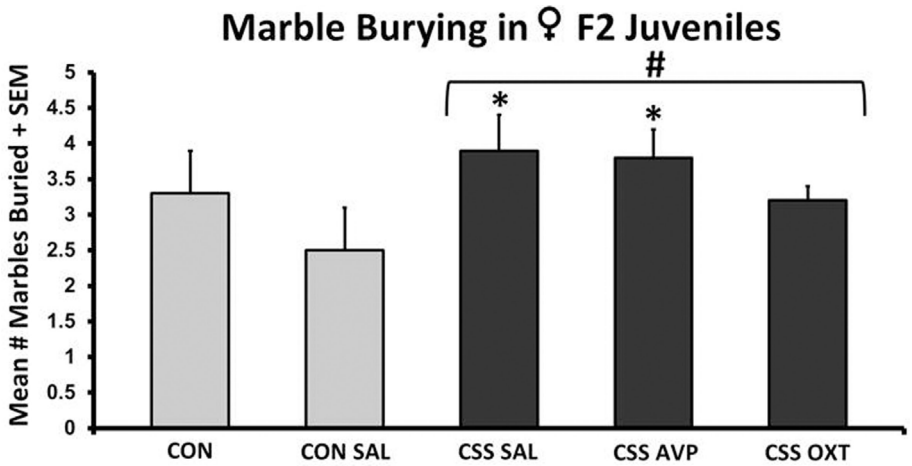

FIGURE 3 | Mean + SEM number of marbles buried by chronic social stress (CSS) F2 juvenile female offspring of F1 control or CSS dams intranasally treated with saline, vasopressin, or oxytocin. # indicates overall effect of CSS treatment, * indicates significant increase compared to control + intranasal saline group ($p < 0.05$).

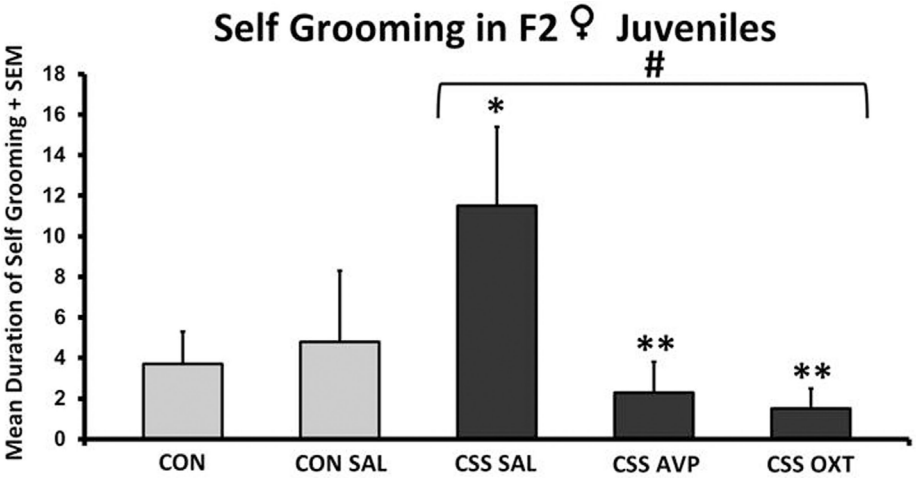

FIGURE 4 | Mean + SEM duration (seconds) of self-grooming of chronic social stress (CSS) F2 juvenile female offspring of F1 control (CON) or CSS dams intranasally treated with saline, vasopressin, or oxytocin. # indicates overall effect of CSS treatment, * indicates significant increase compared to combined CON and control + intranasal saline groups, ** indicates significant decrease compared to CSS + intranasal saline group ($p < 0.05$).

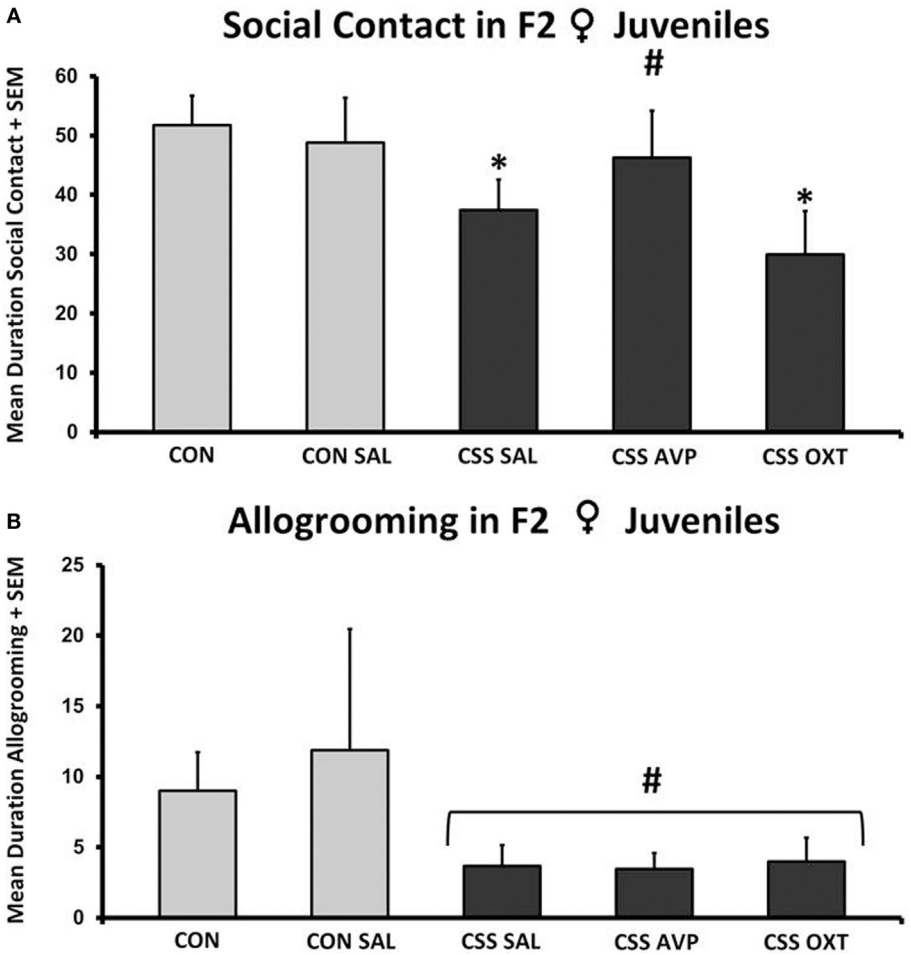

FIGURE 5 | **(A)** Mean + SEM duration (seconds) of social contact of chronic social stress (CSS) F2 juvenile female offspring of F1 control (CON) or CSS dams intranasally treated with saline, vasopressin (AVP), or oxytocin (OXT). # indicates overall effect of CSS treatment, * indicates significant increase compared to combined CON and control + intranasal saline groups ($p < 0.05$). **(B)** Mean + SEM duration (seconds) of allogrooming of CSS F2 juvenile female offspring of F1 CON or CSS dams intranasally treated with saline, AVP, or OXT. # indicates overall effect of CSS treatment ($p < 0.05$).

groups were lower than grooming in the CSS SAL group (both one-tailed t tests = 2.1, $p = 0.03$, Cohen's $d = 1.1$, **Figure 4**), and similar to control values.

Social Interaction

There were no significant differences in social interaction following one-way ANOVA ($F_{4,38} = 1.7$, $p = 0.2$, $F_{3,38} = 2.4$, $p = 0.09$, **Figure 5A**). Combined juvenile female F2 CSS rats spent 25% less time displaying social behavior during the 10-min social interaction test compared to combined CON rats (**Table 1**). This overall difference was due to significant differences between the combined controls and the CSS SAL (37.4 ± 5.2 s, one-tailed $t = 1.9$, $p = 0.04$, Cohen's $d = 0.7$) and CSS OXT (29.9 ± 7.3 s, $t = 2.6$, $p = 0.02$, Cohen's $d = 1.1$, **Figure 5A**)-treated groups. Social contact duration in CSS F2 juvenile offspring of AVP-treated F1 dams was similar to control values.

There were no significant differences in allogrooming following one-way ANOVA ($F_{4,38} = 1.2$, $p = 0.3$, $F_{3,38} = 1.5$, $p = 0.2$, **Figure 5B**). When CON and CSS groups are combined, durations of allogrooming were decreased in CSS animals compared to controls (**Table 1**).

IFNγ—Behavior Correlations

There were no significant correlations between basal peripheral IFNγ levels and durations of marble burying, self-grooming, social contact, or allogrooming (all p's > 0.1).

DISCUSSION

Recent interest in the role of the immune system and IFNγ in behavior and the pathophysiology of stress-associated psychiatric disorders stimulated the present investigation of the effects of maternal IN AVP and OXT on programing changes in peripheral immune factors and behavior in juvenile offspring. Relevant behavioral models are needed to determine the mechanisms of these interactions in the context of early-life care and its impact

on later disease. The current study reports increased peripheral IFNγ in the juvenile female offspring of social stressed dams treated with chronic IN OXT. CSS offspring also displayed increased perseverative and anxiety behavior, impaired social behavior, and behavior-specific responses to both maternal AVP and OXT treatment. The behavioral changes were not correlated with peripheral IFNγ levels, and it is postulated that maternal IFNγ indirectly mediates behavioral programing of offspring through neurodevelopmental changes (10). The data support the hypothesis that social stress and IN peptide administration in mothers alter peripheral immune measures and perseverative, anxiety, and social behavior in female offspring in a behavior- and peptide-specific manner.

F1 Maternal OXT Drives Elevated Basal IFNγ in CSS F2 Offspring

Vasopressin and OXT have substantial immune functions and modulate the immune system during its development, homeostasis, and in response to injury and stress. AVP is emerging as a critical immunoregulatory peptide, capable of maintaining immune function. This is due to the ability of AVP and OXT to stimulate both the HPA axis and prolactin release (27). The genes for OXT and OXTR are expressed in the thymus (28) and monocytes and macrophages (29). OXT is the target of immunological cytokines (e.g., prostaglandin E2, IL2, and IL6) and prolactin, which can promote its secretion into the blood (30–33). Johnson et al. (34) demonstrated that AVP and OXT were able to replace the IL2 requirement for IFNγ production by T-helper cells from mouse spleen cultures. Importantly, this did not involve cell proliferation, suggesting that these neuropeptides possess cytokine activity supporting a relationship between neuroendocrine and immune systems (34). Further work demonstrated that AVP and OXT can replace IL2 for T cell mitogen induction by IFNγ in mouse spleen cells (12), and OXT significantly increases peripheral blood mononuclear cell blastic response to phytohemagglutinin (35). Based on these findings and the current data, it is hypothesized that AVP and OXT act as regulators of immune cells.

Elevated basal IFNγ in CSS F2 juvenile females was driven by the maternal OXT treatment, suggesting a specific effect of this neuropeptide on IFNγ and general immune functioning. The developmental importance of IFNγ (10) suggests that there may be substantial neuroanatomical changes in CSS F2 offspring. Robust changes in resting-state functional connectivity have been documented in studies of CSS F1 offspring (26), and detailed neuroanatomical and neuroimmunological investigation of the CSS F2 generation and similar populations would be valuable.

F1 Maternal OXT Prevents Repetitive/Perseverative Behavior in CSS F2 Offspring

The overall increase in CSS F2 marble burying augments our previous report of social anxiety in this generation (5), supporting the hypothesis that CSS has transgenerational effects on both social anxiety and repetitive/perseverative behavior. In a natural setting, increased perseverative behavior could lead to decreased expression of critical behaviors such as foraging, sexual behaviors, and/or parental care. The CSS model is characterized by the increased expression of inappropriate behaviors, such as excessive nesting, locomotor activity, and unnecessary pup retrieval when maternal care is depressed in F0 and F1 dams. These behaviors have been referred to as maternal anxiety, but they may also reflect increased perseveration in stressed dams. A lack of a significant increase in marble burying in the CSS OXT group compared to significant increases in the CSS SAL and CSS AVP groups suggests that OXT may be specifically effective at preventing perseveration in the offspring of mothers exposed to early-life social stress. While AVP and OXT have been reported to have similar effects on anxiety and social behavior in some species (20, 36), the present results support the hypothesis that they have unique, behaviorally specific programing effects on offspring.

F1 Maternal OXT and AVP Prevent Increased Anxiety in CSS F2 Offspring

Augmented self-grooming during open-field testing indicates novelty-induced anxiety where juvenile CSS F2 females increased self-grooming when placed in a novel environment. In contrast to the nanopeptide-specific differences in burying behavior, both AVP and OXT treatments prevented the CSS-induced increase in self-grooming during open-field testing. This may suggest context- or behavior-specific effects of AVP and OXT. The lack of effect on general locomotor parameters during open-field testing indicates that the behavioral effects were not mediated by changes in activity levels, consistent with all previous studies of the CSS model.

F1 Maternal AVP Increases Social Contact in CSS F2 Offspring

In direct support of our previous work on CSS F2 female juveniles, CSS decreased social interaction. Individual group comparisons indicate that AVP, but not OXT, treatment of F1 dams has protective effects on F2 social behavior. This protective effect may be specific to investigatory activities since treatment effects of CSS on F2 allogrooming were similar. AVP is an established mediator of social recognition (37), which may explain its effectiveness in ameliorating the social stress-induced deficits in social interaction through changes in investigatory behaviors, but not allogrooming. In maternal rats, AVP is a key mediator of maternal care, maternal aggression, maternal memory, and self-grooming (38–44), and chronic central infusion of AVP enhances maternal care in CSS-exposed F0 dams (19). The preventative effects of AVP on F2 juveniles indicate that it may be an effective treatment for both depressed maternal care and the adverse effects of deficient care on offspring. Reinforcing the specificity findings in the burying and self-grooming data, AVP and OXT have differential preventative effects on the negative impact of CSS on the F2 social behavior, with AVP having protective effects on overall social contact, yet no effect of either peptide on the CSS-induced decrease in allogrooming.

Studies in voles suggest that that chronic OXT may have adverse long-term effects on social behavior (22), and the present

data could represent a similar phenomenon that is transmitted to offspring through changes in maternal care. The lack of preventative effects of F1 dam OXT on social behavior could be related to observations of elevated plasma OXT in CSS F2 juvenile females, which exhibit social deficits (5), and the present findings support related work in humans and rodents on OXT-associated disruptions in social functioning (45–47). Female rodents exposed to different social stressors exhibit long-lasting changes in the OXT system (48, 49). IN OXT is unable to ameliorate stress-induced deficits in mouse social behavior, and OXT decreased social interaction in a control population (49). It is postulated that exogenous OXT treatment of CSS-exposed F2 females may not be effective and could impair social behavior in mothers or their offspring due to context dependent adverse effects (38, 49) on F1 dam maternal care during early lactation when maternal anxiety is elevated (4). Beneficial effects of OXT on social behavior may require more positive social interactions where OXT can enhance the salience of these encounters (50–53).

In addition to the current juvenile social testing paradigm, it is possible that CSS could negatively impact several other forms of social interaction at later life history stages, such as mating, alloparental care (48, 54), parental care (55), and aggression (56, 57) in the F2 generation. The reported changes in juvenile behavior, particularly the decrease in allogrooming, may be predictive of future deficiencies in social bonding and mating behaviors. One possible mechanism supported by the allogrooming data is that depressed F1 maternal care mediates decreased F2 juvenile allogrooming, leading to general deficits in social functioning in the F2 offspring at multiple life history stages. In support of this hypothesis, similarly treated CSS F2 dams exhibit deficits in maternal care (manuscript in review), and additional CSS F2 investigations of mating, alloparental, and parental behavior are warranted.

Effects of F1 OXT and AVP on F2 Offspring Behavior through Maternal Care

The hypothesis that the effects of chronic F1 IN OXT and AVP treatment on F2 behavior are mediated through changes in F1 maternal care is supported by a wealth of literature on their roles in the establishment and expression of maternal care (20, 58). OXT's role in maternal behavior has been studied for almost 40 years (59), and there is growing evidence for its role in pathologies that adversely impact parental care. Changes in OXT and dopamine mediate the adverse effects of parental deprivation on the parental care of vole offspring (60), and OXT has been clinically implicated in depression and anxiety (46, 61–63). AVP is also a critical mediator of maternal behavior (38, 64, 65) and the rationale for the current IN AVP treatment was directly supported by increased maternal care in CSS-exposed dams following central AVP infusion (19). Recent clinical evidence supports a key role for AVP V1a receptors in maternal social cognition (66), and animal studies indicate that this role of the V1a receptor may be mediated through hypothalamic nuclei (67). Considering that the F2 females were not treated themselves, there are several possible mechanisms for the effects of the treatments, including changes in F1 maternal care and effects on neuropeptide, inflammatory, or nutritional factors in milk. While IFNγ levels were not correlated with F2 behavior, F1 dam AVP and OXT treatments may have altered IFNγ in milk and F2 offspring and induced immune-mediated neurodevelopmental effects, which mediated the behavioral changes. Key questions to address in exploring AVP- and OXT-induced changes in F1 maternal care are how do both acute and chronic treatments affect F1 maternal care and what are the behavioral effects on F2 offspring. Cross fostering and artificial grooming manipulations may be particularly valuable in these efforts, and changes occurring during early lactation are likely to be critical. Ongoing studies of the CSS model will explore the role of maternal care in the effects of IN AVP and OXT on offspring behavior and immune function.

CONCLUSION

Taken together, these results support the hypothesis that maternal AVP and OXT treatment have context- and behavior-specific effects on peripheral IFNγ levels and perseverative, anxiety, and social behaviors in female offspring of early-life social stress-exposed dams. Both maternal AVP and OXT are effective at preventing social stress-induced increases in self-directed measures of anxiety, and AVP is particularly effective at preventing impairments in overall social contact. OXT is specifically effective at preventing repetitive/perseverative behaviors, yet is ineffective at preventing deficits in overall social behavior. Neither treatment was effective in improving allogrooming, which may be suggestive of future impairments in social bonding, mating, and maternal care. A lack of significant IFNγ-behavior correlations suggests that the behavioral effects are not directly mediated by IFNγ and could be mediated by neurodevelopmental effects of this immune factor. The IFNγ data suggest a modulation in immune functioning, which could be relevant to the etiology and pathology of a vast array of stress-associated disorders not explored in the present investigation (68, 69). As the work from the Bales lab has revealed adverse long-term effects of chronic OXT treatment, the present study has revealed that chronic maternal AVP and OXT treatments can have potent effects on the offspring. Studies involving manipulations of potent behavioral mediators need to consider both long-term effects in the treated animal, as well as effects on offspring and future generations. Given the preventative effects of maternal AVP on anxiety and social behavior deficits and the relative lack of data on this neurohormone compared to studies of OXT, increased targeting of AVP for the prevention and treatment of perseverative-, anxiety-, and social behavior-associated disorders may be productive. While the clinical physiological benefits of OXT in the peripartum period are established, potential maternal and offspring effects on immune function and behavior merit further study.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the guidelines of the Committee on the Care and Use of Laboratory Animals, National Research Council. The protocol

was approved by the Tufts University Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

CM was involved in the design of the experiment and assisted with data interpretation and completion of the manuscript. AH-N assisted with protocol design, data collection and analysis, and completion of the manuscript. AF assisted with data collection, interpretation, and completion of the manuscript. GB assisted with methodological design, data collection, and interpretation of the results. KG and FE assisted with data collection, interpretation of results, and manuscript completion. FP assisted with interpretation of results and manuscript completion and revision. BN designed and supervised the study, assisted with data collection, organized and integrated the data, and wrote and revised the manuscript.

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REVIEW
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Sensitive Periods, Vasotocin-Family Peptides, and the Evolution and Development of Social Behavior

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Nonapeptides, by modulating the activity of neural circuits in specific social contexts, provide an important mechanism underlying the evolution of diverse behavioral phenotypes across vertebrate taxa. Vasotocin-family nonapeptides, in particular, have been found to be involved in behavioral plasticity and diversity in social behavior, including seasonal variation, sexual dimorphism, and species differences. Although nonapeptides have been the focus of a great deal of research over the last several decades, the vast majority of this work has focused on adults. However, behavioral diversity may also be explained by the ways in which these peptides shape neural circuits and influence social processes during development. In this review, I synthesize comparative work on vasotocin-family peptides during development and classic work on early forms of social learning in developmental psychobiology. I also summarize recent work demonstrating that early life manipulations of the nonapeptide system alter attachment, affiliation, and vocal learning in zebra finches. I thus hypothesize that vasotocin-family peptides are involved in the evolution of social behaviors through their influence on learning during sensitive periods in social development.

Keywords: evo-devo, vasopressin, nonapeptides, sensitive periods, social behavior, vocal learning, developmental psychobiology

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INTRODUCTION

Both extrinsic and intrinsic experience broadly shape the functional organization of the brain. Functional maturation critically depends on input at particular points in time to ensure that nervous systems are organized to enable the organism to survive and reproduce in maturity. The basic plan of the brain is, of course, governed by genetic expression. However, its development is influenced at every point along the way by the "environment," broadly construed (1–5).

The development of the brain is shaped by experience that comes from outside the organism, but also by chemical signals that are generated by the organism itself. These signals include chemical gradients that guide the growth of neuronal projections to their targets, or longer distance chemical messengers that modulate the activity of complex neural circuits. Importantly, chemical messengers, such as steroid hormones or neuropeptides, provide a signal to coordinate the development of multiple tissues. Thus, there are important parallels between the organizational role that hormones play during development (intrinsic experience) and the organizational effects of experience that comes from outside of the organism (extrinsic experience).

There is, however, little research investigating the mechanisms that govern how organisms are so exquisitely sensitive to particular kinds of sensory input at specific points in development. In particular, there is a significant gap in our understanding of the neural and neuroendocrine mechanisms that modulate sensitive periods in development, even for potent forms of early learning, such as imprinting or vocal learning. What mechanisms shape the timing, existence, and nature of these sensitive periods in development, particularly given the significant impact that early social learning has on adult phenotype?

The nonapeptides, which provide an evolvable mechanism for modulating the activity of whole neural circuits in specific social contexts, may play an important role in the evolution and development of social phenotypes across vertebrate taxa. The majority of nonapeptide research in the last several decades has focused on adult organisms. Fortunately, there is a re-emerging interest in the effects of the nonapeptides during development (6, 7). The hypothesis that the nonapeptides may exert organizational effects on the brain by producing long term or permanent changes in neural structure was first proposed during the 1980s [arginine vasopressin (AVP) (8); oxytocin (OT) (9)]. Despite several intriguing findings which suggested that the nonapeptides are important during development, there was relatively little interest in this area until recently. This resurgence was largely driven by the realization that exogenous administration of synthetic OT to induce labor may have unknown side effects at a critical point in brain development (10, 11). Furthermore, it was realized that the nonapeptides may also have potential relevance to understanding social deficit and neurodevelopmental disorders (12).

Here, I argue that the nonapeptides, particularly in the vasotocin family, play a critical role during sensitive periods for social learning. Nonapeptides are known to influence many brain regions involved in multiple social behaviors. I present some speculative evidence that they may also do so during development in ways that have profound consequences for adult social behavior. Similar to discoveries in the newly emerging field of evolutionary developmental biology (evo-devo), this evidence suggests that subtle variations in nonapeptide circuitry during development may underlie species differences in social behaviors through their influence on social learning processes. To frame this argument, I attempt to link findings from several previously unconnected fields, including classical work in developmental psychobiology on sensitive periods, the evolution and development of the nonapeptide system, and several theories about how nonapeptides affect social processes. Finally, I will summarize recent work in the zebra finch (*Taeniopygia guttata*) supporting this claim which demonstrates that early life manipulations of the nonapeptide system alter attachment to parents, affiliative behavior in adult males, and vocal learning.

SENSITIVE PERIODS IN DEVELOPMENT

What Is a Sensitive Period?

There is extensive evidence of early sensitive periods in development, in which an organism demonstrates a marked susceptibility or vulnerability to particular stimuli during a limited time window early in life (13, 14). This phenomenon reflects a developmental phase of built-in competence for exchange between an organism and its environment. Sensitive periods are most often observed in the context of what is known as experience-expectant learning, where an organism depends on certain types of experience in order to develop normally. By experimentally removing or altering the stimulation, we can reveal the extent to which an organism requires the species-typical input for species-typical development. For example, visual deprivation early in life in a number of species can cause disorganization of the cortical columns necessary to process visual stimuli (15). In addition, certain cues in an organism's environment may provide information about which phenotypes will be most successful given the environment the organism is likely to encounter. In this case, developing organisms "sample" their environment during sensitive periods for cues which direct development in an adaptive direction. For example, early nutritional stress can serve to program physiological function in ways that would enhance postnatal survival under conditions of intermittent or poor nutrition (16).

Some of the most striking examples of sensitive periods in development occur in the social domain. One particularly potent form of an early sensitive period is imprinting. Imprinting, such as filial or sexual imprinting, is defined as a form of learning that (1) can only take place during a restricted window of time in an individual's life, (2) is irreversible, (3) involves the learning of species specific or individual-specific characters, and (4) may occur at a time when the appropriate behavior itself is not yet performed (17). Visual imprinting phenomena have been best studied in birds (18–23), where the circuit underlying filial imprinting has been well characterized (24–26). Precocial birds, such as domestic chickens and ducks, will approach and follow any object which they were exposed to immediately after hatching, typically the mother. In addition, there is work from a number of fish species demonstrating imprinting phenomena used for species and kin recognition, primarily in the olfactory domain (27–34).

There are also more subtle forms of sensitive periods in social development, in which experience with caregivers early in life shapes later social relationships (35–38). Across taxa, isolation from conspecifics and caregivers results in significant disruptions to social functioning later in life (39–42). Furthermore, research in rats has demonstrated that early experiences of maternal care (e.g., licking and grooming behaviors) can alter both the responsiveness to stressors and maternal behavior in adulthood (37, 43, 44). Song learning in birds and vocal learning in humans, though they occur later in development, are also examples of sensitive periods in development in which the developing organism is dependent on interactions with adult caregivers to learn species-typical vocal structures (45, 46).

What appears to be common across these different kinds of sensitive periods in development is competitive exclusion—a particular class of sensory input from the environment is particularly influential, to the exclusion of others (47). These early forms of social learning, particularly about the identity, features, and valence of caregivers, provides an important foundation for later learning. Both learning the characteristics of and maintaining the motivation to be proximal to caregivers provide developing organisms with food and protection but also an abundance of

opportunities for social learning. Indeed, these early social experiences provide the foundation upon which all future social interactions are built.

In each of these cases, we have a sense of what circuits are involved in the sensitive periods (as well as a number of ways to disrupt those circuits), but critically we do not know the mechanisms of how or why there is a particular kind of sensitivity in the first place. For example, the filial imprinting work in birds has focused on forebrain regions thought to be homologous to mammalian cortex which are involved in visual processing and multimodal association (24–26, 48). But why those particular inputs at that particular time?

Established Mechanisms Underlying Sensitive Periods

One theory about how sensitive periods emerge is based on the observation that the sensory systems do not all become functional at the same time, but rather sequentially. In both birds and mammals, the first sensory modality to become functional is the tactile/vestibular system, followed in order by the chemosensory/olfactory, auditory, and visual systems (49, 50). Based on the pioneering work of Gilbert Gottlieb in avian development, Turkewitz and Kenny proposed that the invariance in the sequential onset of sensory function results in a reduction in the complexity of the sensory experience for the developing organism and a more reliable structure to the prenatal and early postnatal experience (51). Developing organisms are not bombarded with novel stimuli in all sensory modalities upon birth, but instead encounter a drastically reduced sensory experience. Earlier-developing systems (i.e., tactile and olfactory) in fact develop under reduced competition from other sensory modalities. This allows later-developing systems (i.e., visual and auditory) to build on associations formed in earlier-developing systems.

Furthermore, given that many sensory systems begin to develop prior to birth or hatching, the *in utero* and *in ovo* environments provide learning opportunities for the developing embryo. Precocial birds, which are born with all sensory systems functional at birth, benefit from extensive learning that takes place in the egg which they use to support the "emergence, maintenance, and transformation of behavior" (52). For example, in ducks, the preference for the maternal assembly call is dependent upon the prenatal exposure of the embryo either to its own vocalizations or those of its siblings in the days prior to hatching (53). Similarly, bob white quail denied interaction with broodmates after hatching fail to develop preferences for species-specific maternal cues (54). Even altricial rodents, which are less mature at birth, also use olfactory associations formed *in utero* to perform suckling behaviors (55).

In addition, generalized physiological arousal has been identified as a critical component of a young organism's perceptual learning and development. In human infants, for example, there is a strong association between arousal levels and sensitivities to sensory stimulation (56–58). Physiological arousal can be manipulated neurochemically, or by simply making sensory stimuli more salient. For example, only rat pups receiving either tactile stimulation or injected with amphetamine while exposed to an artificial odor preferred to suckle nipples coated in the familiar odor (59). Furthermore, this process can be disrupted by a poorly timed change in arousal state. Injection of norepinephrine into quail embryos in the absence of exposure to appropriate auditory stimulation resulted in disrupted preference for the familiar maternal call (60). This work suggests that normal social development depends on physiological systems that mediate arousal and attention in the appropriate social environments early in life.

NEUROENDOCRINE SIGNALS AS A POTENTIAL MECHANISM UNDERLYING SENSITIVE PERIODS

I propose that neuroendocrine mechanisms are also prime candidates for mediating sensitive periods in development. Hormones, which are typically defined as long-distance chemical signals, act directly on the cellular processes of neurons, but they also affect more general physiological systems, such as arousal, gonadal state, and metabolic function. Hormones influence multiple tissues simultaneously and modulate physiological and developmental processes across a wide spatial and temporal distance (61). This enables organisms to simultaneously coordinate many tissues or recruit whole neural circuits for an important task (62). Indeed, hormonal signals can provide a functional link between otherwise unconnected neuronal populations (63).

Most of the developmental effects of hormones have been studied in the context of steroid hormones and sexual differentiation (64–68). The focus of this work has been on how the hormones directly affect cellular function and the connectivity of neural circuits. However, many neuroendocrine signals have the potential to play a role in the organization of the social brain specifically by altering learning processes. Glucocorticoids, sex steroids, and neuropeptides have all been shown to be involved in learning and memory, both directly and indirectly (69). Nevertheless, there remains a gap in our understanding the role that such signals play in influencing the outcome of development in the context of important social experiences. Furthermore, the diversity of social phenotypes both within and between species begs the question as to how the unique features of both the organism's early social experiences, as well as evolved differences in their neuroendocrine function, support the evolution and development of novel social phenotypes. For the purposes of this review, I focus on vasotocin-family neuropeptides, but many of the general principles of my argument may apply to other neuroendocrine signals, as well.

Overview of the Nonapeptides

Over the last several decades, much research effort has been devoted to vasotocin family of neuropeptides (i.e., nonapeptides), which includes [arginine vasotocin (AVT), found in non-mammals and likely the ancestral peptide] and its mammalian homolog AVP; and the OT-like peptides [isotocin (IT), found in fish, mesotocin (MT), found in lung fish and non-eutherian tetrapods, including birds; and OT, found in mammals] (70, 71). The nonapeptides derive from an evolutionarily ancient neuromodulator. In the earliest vertebrates, only one gene was present (AVT), but sometime after *Agnatha* (lampreys and hagfish) a

gene duplication event led to the divergence of the vasotocin (AVT/AVP) and OT (IT/MT/OT) lineages (72). Although these two lineages differ in only a single amino acid, the nonapeptides appear to have evolved quite distinct functions.

This is because the nonapeptides coevolved with their receptors, which are classic G-protein coupled receptors. There are typically four receptor subtypes for the nonapeptides within each species: V1a, V1b, V2, and OT (VT4, VT1, VT2, and VT3, respectively, in birds). The amino acid sequences of each receptor subtypes are more similar to each other across species (~90%) than they are to different subtypes within a single species (~45%) (73). When binding to their receptors, nonapeptides can have a multitude of effects on neurons, including changes to gene transcription, recruitment of intracellular calcium, neuroprotective effects, and alterations to long-term potentiation mechanisms (74). The sequencing of the vertebrate nonapeptide receptor genes suggests that the core–ligand receptor interaction sites have remained remarkably conserved, while varying the intracellular components, and thus their downstream effects (75). Receptors for nonapeptides are distributed throughout the brain, but importantly, the distribution of each of the receptor subtypes can vary widely by species, sex, age, and social context (76–78).

The primary sources of nonapeptides are the AVP/OT cell groups of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) nuclei of the hypothalamus, as well as smaller extra-hypothalamic accessory cell groups, including the medial amygdala (MeA), medial bed nucleus of the stria terminalis (BSTm), lateral septum (LS), olfactory bulb (OB), and suprachiasmatic nucleus (SCN) (79, 80). The production of AVT/AVP, particularly in from the extra-hypothalamic cell groups, is often sexually dimorphic (usually male greater than female), organized by sex steroids during development, and sensitive to changes in gonadal state (81–88).

In order to understand the modulatory role of AVT/AVP during development, we need to consider the sources of the peptide, the sites of action in the body, and the functional consequences. There are three primary physiological systems influenced by AVT/AVP. The first can be summarized as AVT/AVP's involvement in vasoconstriction and water balance. AVT/AVP, when released by magnocellular neurons in the PVN and SON of the hypothalamus, is released into the posterior pituitary. From there, AVT/AVP enters general circulation where exerts antidiuretic effects throughout the body.

The second is AVT/AVP's involvement in the stress response. AVT/AVP, when it is released from the anterior pituitary by parvocellular neurons in the PVN, is at the top of the hypothalamic–pituitary–adrenal (HPA) axis. The HPA axis regulates the physiological response to stressors, helping the body mobilize resources in response to challenges in its environment. AVT/AVP, along with corticotropic releasing factor (CRF) serves as a releasing hormone for adrenocorticotropin-releasing hormone (ACTH) from the anterior pituitary, which is the chemical signal that leads to the release of glucocorticoids from adrenal tissue (89). AVT/AVP is not itself the major releasing hormone for ACTH, but it plays a critical role by potentiating the biological activity of CRF (90). Parvocellular AVT/AVP neurons are highly responsive to stress (91). Acute stress increases the production of both CRF and AVT/AVP in the PVN (92, 93).

Finally, multiple cell groups in the brain contribute to the central pool of nonapeptides with highly diverse functional consequences. Some of the same neurons that project into the pituitary also send projections back into the brain. In addition, the extra-hypothalamic accessory cell groups, including the MeA, BSTm, LS, OB, and SCN, contribute to the central pool of peptides (79, 80). Each of the AVT/AVP cell groups has a different pattern of activity and neural release, which is ultimately a function of the kinds of computation those neurons perform (87). Variation in how the nonapeptides affect the interconnected set of brain nuclei known as the social behavior network and other brain regions is thought to underlie the multiple effects of AVT/AVP across species (94, 95). Indeed, nonapeptides have been implicated in species differences in many diverse social behavioral domains (76, 77, 96–104).

Nonapeptides in Development

The vast majority of this comparative work, however, has focused on nonapeptide function in adulthood. We in fact understand remarkably little about how nonapeptides shape social behaviors during development, particularly those behaviors for which plasticity, flexibility, and learning are critical. The effect that nonapeptides have on social learning in each species is influenced by when they act relative to important social experiences and in what brain regions. Across the few vertebrate species in which it has been investigated, the sequence of nonapeptide cell group maturation appears to be conserved. The first AVT/AVP immunostaining is consistently found in the SON followed by the PVN (105–112). In tetrapods, this is followed by production in the extra-hypothalamic cell groups, such as the BSTm and MeA, which exhibit steroid hormone-mediated sexual dimorphism in AVT/AVP staining (81, 113–115).

The most detailed developmental work in the nonapeptide system comes from rodents, particularly the rat. In rats, the neurons of the SON and PVN have formed before birth by 12–14 days postfertilization (dpf), gestation is 21 days in the rat (108). In the rat brain, the first AVP staining is observed between 14–18 dpf, which steadily increases to adult levels by postnatal day 30 (108, 113). Between birth and postnatal day 21, there is a 22- to 30-fold increase in AVP production by the pituitary, suggesting that the neurons that project from the hypothalamus to the pituitary are gradually coming on-line during development. By contrast, the cell groups of the BSTm and MeA show AVP staining only after birth, with the MeA delayed relative to the BSTm. AVP mRNA was only observed in the BSTm on postnatal day 3 and in the MeA on day 5 in male rats and day 14 and day 35, respectively, in female rats (113). The levels of AVP, thus, reach adult levels by postnatal day 35 in the BSTm and day 60 in the MeA in both sexes (113).

A similar developmental trajectory is found in the domestic chicken, despite substantial evolutionary distance and its precocial development. AVT is observed early in development in the chicken embryo SON and PVN, as early as 6 dpf (109–112). AVT is detectable in the BTSm by 12 dpf, which increases until hatching at 17 dpf before dropping precipitously in days after hatching (114). AVT then increases gradually in males until 129 dpf (114).

Thus, the onset of function of all the cell groups in the SON, PVN, and BTSm occur while the chicken is still in the egg.

Very limited information on the development of these cell groups exists from other species, but the sequential development of the hypothalamic cell groups appears similar. In humans, AVP is detectable in the SON and PVN at 77 and 91 dpf, respectively (107, 116). Thus, in humans, hypothalamic production of AVP begins before birth. Even in zebra fish, two cell groups in the rostral diencephalon and hypothalamic regions show AVT mRNA expression sequentially starting at 24 h postfertilization (106). The embryonic development of the nonapeptide system has not been explored in songbirds. An early paper that explored the development of the nonapeptide circuitry in canaries found that AVT was expressed in the PVN at 4 weeks, but no staining was observed in the BSTm or LS until later at 8–12 weeks of age (117).

Taken together, these data suggest that the relative timing of the onset of function of the AVT/AVP cell groups is, in fact, remarkably conserved throughout evolution. Furthermore, the sequence of general neurodevelopmental events (from neurogenesis to eye opening) is also very predictable (118). What changes more is the timing of birth or hatching and, thus, early social experiences relative to these ontological changes. Inspired by Workman et al., **Figure 1** depicts the development of the nonapeptide system scaled according to when the brain reaches 20% total brain volume, a milestone that is highly correlated with other neurodevelopmental events (118). This is based on data from three species for which we have some information about the development of the nonapeptide system: rats, humans (SON and PVN only), and chickens. The fact that neurodevelopmental events occur in a highly stereotyped sequence allows us to make predictions about the maturation of the nonapeptide system for cell groups or other species for which we do not have data, as well. **Figure 1** shows the predicted timing of AVT synthesis in the respective cell groups in the zebra finch brain, based on general data on zebra finch neurodevelopment. Data supporting the figure can be found in **Tables 1** and **2**.

A few notes of caution are required. First, it is important to note that these predictions are still quite speculative, given the available data. The Workman et al. model has not been applied to avian systems, so the extent to which we can extrapolate the

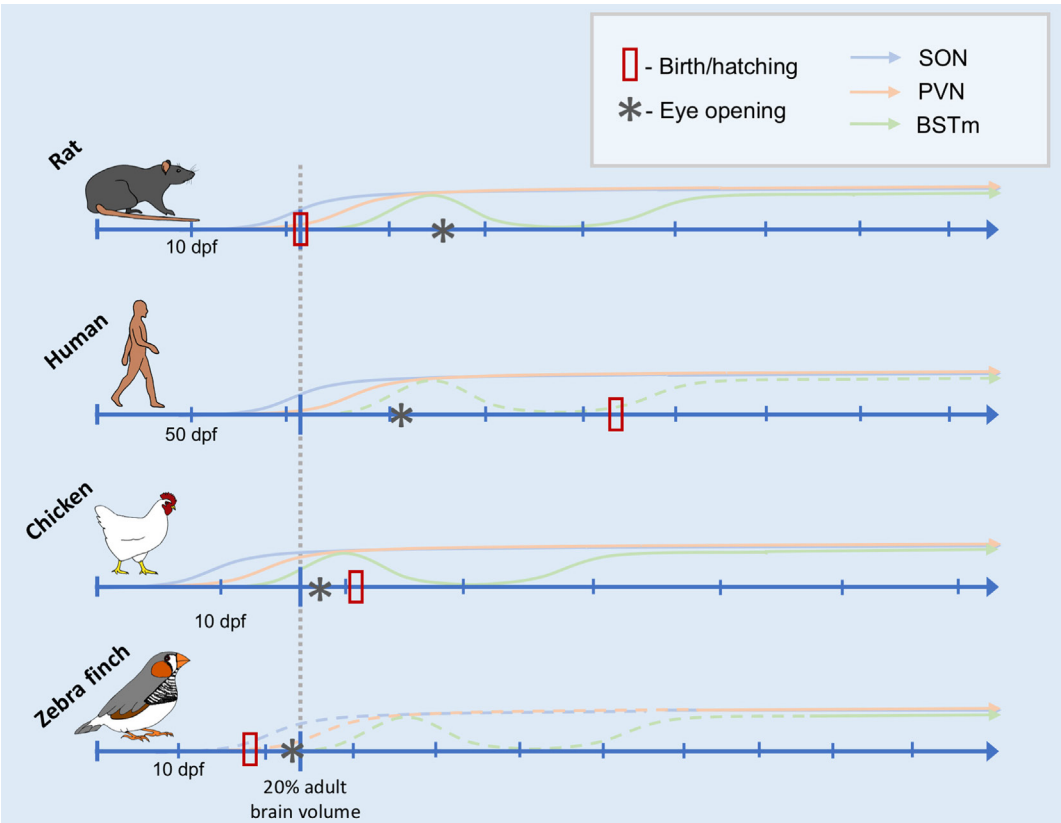

FIGURE 1 | Comparative timeline of arginine vasotocin (AVT)/arginine vasopressin (AVP) cell group development. Conceptual timeline illustrating the production of AVT/AVP across early development in rats, humans, chickens, and zebra finches in three main AVT/AVP cell groups: the supraoptic nucleus (SON, blue), paraventricular nucleus (PVN) of the hypothalamus (PVN, orange), and the medial bed nucleus of the stria terminalis (BSTm, green). The *x*-axis of the timeline is scaled to the neurodevelopmental time point of when the organism is estimated to reach 20% total adult brain volume (indicated by the gray dotted line intersecting each timeline). Days postfertilization (dpf) are indicated by hatchmarks along each timeline. Solid lines indicate solid data (see text for references), whereas dotted lines are predicted results. The data from which this illustration is created include counts of AVT/AVP immunoreactive cells, mRNA expression, and peptide concentrations (see **Table 1**). Thus, the *y*-axis does not have a scale, as it is not clear how these different data types are comparable across species, cell group, study, etc. The date of hatching or birth for each species is indicated by a red rectangle. Date of eye opening is indicated by an asterisk.

TABLE 1 | Earliest day postfertilization of arginine vasotocin (AVT)/arginine vasopressin labeling found.

Species	SON	PVN	BSTm	MeA	Reference
Rat	16 IHC, RIA	18 IHC, RIA	24♂, 35♀ ISH	25♂, 56♀ ISH	(105, 108, 113, 119, 120)
Human	77 IHC	91 IHC			(107, 116)
Chicken	6 IHC, RIA	7.5 IHC, RIA	14♂, 16♀ IHC		(109–112, 114, 115)

The type of evidence for each time point is indicated below. If available, we use data from the labeling of the AVT peptide, from either immunohistochemistry (IHC) labeling cell bodies and radioimmunoassay (RIA) from brain and pituitary. In some cases, only data from in situ hybridization (ISH) labeling AVT gene expression is available.
Sex differences are indicated, where noted. Cell group abbreviations: supraoptic nucleus (SON) of the hypothalamus, paraventricular nucleus (PVN) of the hypothalamus, medial bed nucleus of the stria terminalis (BSTm), and medial amygdala (MeA).

TABLE 2 | Data are presented for 20% brain volume, eye opening, amygdala neurogenesis peak, and the date of birth or hatching.

Species	Neurodevelopmental event	DPF	Reference
Rat	20% brain volume	19*	(118)
	Eye opening	36	(121)
	Amygdala neurogenesis peak	15	(122)
	Birth/hatching	21	
Human	20% brain volume	118*	(118)
	Eye opening	157.5	(123)
	Amygdala neurogenesis peak	46*	(118)
	Birth/hatching	270	
Chicken	20% brain volume	17	(124)
	Eye opening	18	(125)
	Amygdala neurogenesis peak		
	Birth/hatching	21	
Zebra finch	20% brain volume	24	(126)
	Eye opening	23	(127)
	Amygdala neurogenesis peak	30	(126)
	Birth/hatching	18	

Asterisks indicate that the date is predicted based on the model in Ref. (118).
DPF, days postfertilization.

findings to chickens and zebra finches is still unknown. More information about when key neurodevelopmental events occur in the zebra finch brain may also help to determine whether the maturation of the AVT system would be more similar to the altricial but distantly related rat or the precocial but more closely related chicken.

Second, **Figure 1** was created using available data, which is a mix of immunohistochemistry (IHC) labeling of AVT or AVP protein and *in situ* hybridization (ISH) labeling AVT/AVP gene expression (see **Table 1**). It is also based on labeling of these in the cell bodies within the respective brain regions. AVT/AVP is first synthesized as a large protein precursor molecule, which is enzymatically cleaved into the active hormone (80, 128). The active hormone must then be packaged into specialized neurosecretory vesicles and transported to the nerve terminals where it is released. Thus, the presence of either ISH or IHC labeling in the cell bodies is not a definitive indicator that the hormone is being released, particularly during development (105).

Third, we have limited information about where nonapeptides are acting during development. Binding sites have been found in the developing mouse and rat brain in both the amygdala and septum between postnatal days 0 and 8, as well as several brain regions where AVP receptors are not expressed in adulthood, including the hippocampus, dentate gyrus, and caudate nucleus (129, 130). In rats, many brain regions also show significant differences in between juveniles and adult (131). The consequences of these brain-region specific changes in receptor expression across development are almost certainly important, but have proven difficult to explore experimentally.

Finally, sex differences in both AVT/AVP production and receptor expression also likely influence developmental processes. For example, there is a delay in AVT/AVP synthesis in females relative to males in the BSTm and MeA (113, 115). Given these differences, we might predict that males would be more affected by AVT/AVP manipulations or by manipulations earlier in development, as compared to females. Some sex differences in receptor expression have also been found, but we have a poor understanding of the functional consequences of these differences.

Nevertheless, manipulations of nonapeptides in rodents very early in life provide evidence that nonapeptides matter in development (6, 7). For example, vasopressin–deficient Brattleboro rat pups show hyperactivity, reduced huddling, and reduced proximity to other pups in the nest compared to wild-type rats (132). Wild-type rat pups treated with a nine-day exposure to AVP showed increased emotionality, activity levels, and grooming in an open field test as juveniles, as well as smaller overall brain size (133). Acute central administration of AVP in wild-type neonatal rat pups was found to decrease the number of ultrasonic vocalizations and reduced locomotor activity in a maternal isolation test (134). In juvenile male rats, both targeted infusion of AVP into the LS and intracerebroventricular infusion increased preference for investigating novel individuals, whereas a V1aR antagonist increased the preference for investigating familiar individuals (135). In addition, V1aR blockade in the LS increased social play behavior in males and decreased it in females, but only when it tested in a familiar environment (136, 137). Neonatal manipulation of AVT or OT in the socially monogamous prairie vole, leads to sex-specific changes in nonapeptide binding in several brain regions in adults and alterations to social behaviors (138–141). For example, sexually naïve males who were treated with AVP early in life were more aggressive than control males but females were less responsive to AVP treatment (140). It is unclear whether the developmental effects of these nonapeptide manipulations are mediated through physiological effects on the body versus binding within the brain. Nevertheless, these studies provide intriguing evidence that the nonapeptides are involved in the development of social behavior.

CONSERVATION AND NOVELTY

Several researchers have proposed that one way to understand the outsize role of neuropeptides such as AVT/AVP in the evolution of behavioral diversity is through studying the differential expression of the peptides and their receptors (142, 143). The evolution of neuropeptide signaling systems may be highly

constrained within the nervous system because mutations altering the proteins themselves would have deleterious effects on the receptor–ligand interactions. However, tweaks to where and in what quantities the peptides and their receptors are expressed via changes to gene regulation provide opportunities to modify the activity of neural circuits (142). Indeed, a number of examples have been identified. For example, variation in the production of AVT has been associated with species differences in social behaviors in birds (144). The number of IT-producing cells in the POA has been associated with cooperative breeding in cichlid fish (103). And the expression of V1aR is associated with variation in mating behaviors in voles (76, 145, 146). However, perhaps an important mechanism underlying this behavioral diversity across species is not just where but also *when* nonapeptides are acting during development.

Sensorimotor Processing Hypothesis

Much of the research for the role of nonapeptides in social behavior has focused on brain regions with more generalized roles in social processing. However, the "compartmentalization" of AVT/AVP function can extend to include the modulation of species-specific behavioral circuits throughout the central nervous system (142, 147). In their sensorimotor hypothesis, Rose and Moore posit that AVT/AVP can act on sensory pathways to modulate the responsiveness of neurons to particular kinds of sensory stimuli as well as act on motor pathways to modulate behavioral output (147). In this case, the nonapeptide signal is directly modulating the specific circuits that are necessary for the production of the behavior. This theory emerged from comparative work which suggests that AVT modulates the activity of neurons in each step of a sensorimotor processing circuit which controls a complex courtship behavior in male newts (*Taricha granulosa*) (147). In these newts, AVT enhances the highly stereotyped sexual behavior, in which the male embraces the female with all four limbs to induce receptivity (148). AVT enhances this behavior by modulating sensory processing in the visual and olfactory domains as well as motor output at the level of the spinal cord (149–151).

A parallel story may also be true in the case of complex learned behaviors, such as bird song. Interestingly, there is limited evidence that anterior forebrain song learning pathway is sensitive to AVT, at least in adults AVT (88, 152–154). However, each step in the circuit controlling the expression of vocal behavior in birds appears to be partially modulated by AVT (152, 154, 155). Several auditory structures in the forebrain, including the caudomedial mesopallium and the caudomedial nidopallium, highly express V1aR in zebra finches (154). The robust nucleus of the arcopallium (RA, homologous to laryngeal motor cortex) exhibits limited receptor expression, but two nuclei involved in the motor pathway of song production contain AVT receptors. There is AVT immunoreactivity and binding in the intercollicular nucleus (ICo, a region implicated in vocal control) in several songbird species (152, 153, 156, 157). In addition, the key motor nucleus, nXIIts, which innervates the syrinx and is considered part of the song system, contains high levels of mRNA for all three subtypes of AVT receptor (154). However, a sensorimotor account of the role played by these regions during song learning remains to be tested.

Social Gating Hypothesis

Evolutionary novelty in behavior may also arise when new structures or circuits are modulated in new ways starting early in development. Syal and Finlay claim that what is necessary for the evolution of novel behaviors is changes to how the sensory and motor circuits are attached to the socio-motivational circuitry during early social interactions with caregivers, family, and conspecifics (158). In fact, they view the reciprocally connected network of brain nuclei known as the social behavior network (which includes the major nonapeptide cell groups), as the conserved neural structure that assembles the relevant sensory dimensions of a representation of other individuals (i.e., caregivers, mating partners, rivals) and attaches that representation to motivations and actions appropriate to their social context (158). The social behavior network is highly connected to the mesolimbic reward system via the BSTm, MeA, and LS, which all contain nonapeptide cell groups. Other structures commonly associated with reward and motivation, such as the ventral tegmental area, ventral pallidum, and nucleus accumbens, also densely express receptors for nonapeptides (159).

In this context, the modulatory signal produced by the nonapeptide cell groups, by acting on receptors throughout the brain, can be used to bias attention toward certain kinds of sensory stimuli or to reward the performance of certain behaviors. Consequently, it is easy to imagine how even tiny tweaks to the system, such as gene mutations that change the regulation of a receptor gene or slightly alter its downstream functions, might have large effects on whole neural circuits. Thus, the nonapeptide system may provide a mechanism whereby evolution generates novel social behavior using an otherwise highly conserved brain.

The effect of changes to the nonapeptide system would, thus, be expected to be even more consequential in development, particularly when coupled with salient social experiences. If indeed nonapeptides are gating social learning, then the nonapeptides may function by biasing a young organisms' attention toward the behaviors exhibited by their family or other socially relevant conspecifics. For example, a primary reason why the development of the nonapeptide system may underlie important social development is because of its central role in olfactory processing. Early social experiences are often highly olfactory and thermotactile in nature. For example, suckling behavior in rat pups is dependent upon odor processing in the accessory olfactory system and MeA, which allows them to learn the odor of their mother's amniotic fluid and of the saliva of their broodmates to guide nipple attachment (160). Even zebra finches, which do not have an accessory olfactory system and which are thought to be more responsive to auditory and visual stimuli, show olfactory preferences for their natal nest (161). Thus, early olfactory experiences provide some of the first forms of social learning about conspecifics at the same time that the relevant behavioral circuits begin responding to nonapeptides.

Increased attention to relevant social stimuli would provide opportunities for social learning, which could also be reinforced by socio-motivational circuits. Early sensitivity to social stimuli would support future social learning, leading to accumulating effects. On the other hand, genetic mutations that reduce social approach or attention during development might reduce the

probability that predictable aspects of the social environment are learned at all. Of course, these kinds of effects depend critically on the kinds of input that an organism receives from its environment during these social interactions. It is possible to think of the organizational effects of nonapeptide circuitry independent of its social environment, but more likely, the kinds of social experiences an organism has—and their sensitivity to those social experience—coevolved with each other. Neuropeptide systems during development may have, thus, evolved to allow organisms to plastically respond to their environment as they mature. However, by allowing for variable outcomes in adulthood, these evolvable systems also provide the raw material for evolution to act.

DEVELOPMENTAL EFFECTS OF AVT ON AFFILIATION AND SONG LEARNING IN THE ZEBRA FINCH

Recent experimental evidence from manipulating the nonapeptide system early in life in zebra finch provides support for the idea nonapeptides play an organizational role in on a broad suite of social behaviors (162–164). Intracranial injections of either AVT or [Manning Compound (MC), a V1aR antagonist] in hatchlings (days 2–8 post-hatching) altered social interest in the parents and conspecifics after fledging, suggesting that the nonapeptides are serving to gate a number of social approach behaviors in juvenile zebra finches (162). In addition, early life nonapeptide treatment also altered affiliative behaviors and courtship song in adult male, but not female, zebra finches. Both AVT and Control males showed an increased affiliative interest in females as they reached reproductive maturity (162). However, AVT-treated males showed less sexually motivated courting of females compared to Controls and instead formed highly affiliative pair bonds with their female partner (163). By contrast, MC males did not show the normal increase in affiliative interest in females as they reached maturity and showed only modest levels of both courtship and affiliation in their interactions with females (162, 163). Furthermore, nonapeptide treatment also altered neural activity and the expression of V1aR in the BSTm and MeA (163). Taken together, these findings suggest that AVT-injected males may have had more experience attending to social cues or a stronger association between affiliative interactions and reward compared to both MC and Control males, resulting in different approaches to reproduction.

This change in the affiliative interest in parents and conspecifics also had functional consequences for social learning. Male zebra finches injected with MC as hatchlings both showed decreased interest in their parents during development and ultimately sang a song that was a worse acoustic match to their father's song in adulthood compared to Controls (162, 164). By contrast, AVT males showed increased affiliative interest in their parents and family and more effectively copy their father's song (162, 164). Interestingly, affiliation with parents at 30 days post-hatch was correlated with song quality in adulthood. These data suggest that the nonapeptides may bias the motivation of developing zebra finches to attend to the behaviors of the father during development, which ultimately allows them to more accurately learn courtship song from their father. This is ultimately consistent with Syal and Finlay's hypothesis that the nonapeptides gate complex vocal learning in song birds by altering social motivation, supporting their suggestion that the nonapeptides may play an equally critical role in language learning in humans (158).

Thus, social phenotypes may evolve via relatively simple alterations to the actions of a single nonapeptide during development. In zebra finches, AVT altered early social behaviors, potentially affecting the opportunities for social learning. However, it also affected the organization of the neural substrate underlying these social behaviors. It will likely prove impossible to disentangle the direct effects of nonapeptides on the brain during development from their indirect effects resulting from how they alter the trajectory of learning from early social experiences. Indeed, this conceptual challenge is at the heart of the nature "versus" nurture debate (4).

CONCLUSION

Nevertheless, these results provide support for the idea that the actions of nonapeptides in development may play an important role in the evolution of novel social behavior. The field of evolutionary developmental biology (evo-devo) has long been concerned with how evolution shapes developmental processes to generate phenotypic novelty. However, the insights from evo-devo have rarely expanded into the social domain (165–167). Neuropeptides and hormonal systems are well-situated to play that role, given that they alter the activities of whole neural circuits. However, we are just scratching the surface in our understanding of the diversity of mechanisms which may facilitate the evolution and development of social behaviors.

Indeed, the nonapeptides are almost certainly not the only chemicals that play a role in the evolution of diverse social phenotypes. We now know of more than 100 different peptides and other signaling molecules, each of which is expressed in only a small population of neurons, and all of which signal to neurons throughout the brain via specific receptors. The endless forms of neural systems and behavior appear to be result of evolutionary changes to compartmentalization of neuropeptide signaling systems (142). However, the complex nature of diverse signaling systems suggests that they can only be fully understood by integrating research at all levels of analysis—investigating both their molecular and developmental mechanisms, as well as their adaptive significance in the life of an organism.

AUTHOR CONTRIBUTIONS

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Triarchic Psychopathy Dimensions in Chimpanzees (*Pan troglodytes*): Investigating Associations with Genetic Variation in the Vasopressin Receptor 1A Gene

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Vasopressin is a neuropeptide known to be associated with the development and evolution of complex socio-emotional behaviors including those relevant to psychopathic personality. In both humans and chimpanzees, recent research suggests a strong genetic contribution to individual variation in psychopathic traits. To date, however, little is known concerning specific genes that might explain the observed heritability of psychopathy. In a relatively large sample of captive chimpanzees ($N = 164$), the current study thus sought to investigate gene-environment associations between triarchic psychopathy dimensions (i.e., disinhibition, meanness, and boldness) and (1) early social rearing experiences and (2) polymorphisms in the promoter region of the V1A receptor gene (AVPR1A). Among chimpanzees raised by their biological conspecific mothers, AVPR1A was found to uniquely explain variability in disinhibition and in sex-specific ways for boldness and a total psychopathy score; however, in contrast, no significant associations were found between AVPR1A and any of the triarchic psychopathy dimensions in chimpanzees raised the first 3 years of life in a human nursery. Thus, when considered in its entirety, results suggest an important contributory influence of V1A receptor genotype variation in the explanation of the development of psychopathy under some but not all early rearing conditions. Results of the current study provide additional support for the assertion that psychopathic tendencies are rooted in basic, evolutionarily-meaningful dispositions, and provide support for a primate-translational operationalization of key neurobehavioral constructs relevant both to psychopathy and to broader forms of psychopathology.

Keywords: vasopressin, AVPR1A, psychopathy, chimpanzees, nonhuman primate models

INTRODUCTION

Psychopathic personality (psychopathy) is a condition that involves severe disturbance in behavioral control, social relations, and emotional experiences concealed by an outward appearance of normalcy. Although historically studied predominantly in adult forensic samples, it has become clear that psychopathy is a multi-faceted condition that includes tendencies grounded in basic

biobehavioral dispositions that vary continuously within the human population (Patrick et al., 2009; Lilienfeld et al., 2015). That is, individuals vary on psychopathic traits in degree, rather than kind. Viewed in this way, understanding of psychopathy can be advanced through study of psychopathy-related trait dimensions in a range of populations, including both clinical and non-clinical samples (Lilienfeld, 1994; Hall and Benning, 2006; Salekin, 2006). Consistent with this conceptualization, recent work has sought to more accurately capture the dimensions of the construct, through the explication of its component dispositional trait dimensions (e.g., Patrick et al., 2009; Marcus et al., 2011; Poythress and Hall, 2011). Developed for this purpose, the triarchic model (Patrick et al., 2009) characterizes psychopathy as a configuration of three dimensional traits explicitly linked to underlying biological systems: *boldness, meanness,* and *disinhibition*.

Within this framework, investigations of these biobehavioral dimensions have been extended to our closest living relatives, chimpanzees (Latzman et al., 2016a), providing a basis for comparative research on the evolutionary and neurobiological foundations of psychopathy. Similar to in humans (Farrington, 2006; Larsson et al., 2006; Tuvblad et al., 2016), recent quantitative genetics work in chimpanzees suggests that variability in psychopathy dimensions is heritable (Latzman et al., 2017). To date, however, little is known concerning specific genes that might explain the heritability of psychopathy. In a relatively large sample of chimpanzees, the current study thus aimed to examine one particularly promising gene, AVPR1A, a gene that underlies arginine-vasopressin (AVP), a neuropeptide known to associate with a range of psychopathy-relevant social behaviors.

The now influential triarchic model of psychopathy (Patrick et al., 2009; Patrick and Drislane, 2015) characterizes the symptomatic components of psychopathy in terms of three biobehavioral trait constructs (i.e., traits with clear referents in biology and behavior): *disinhibition, meanness, and boldness.* Disinhibition reflects an externalizing liability and phenotypic propensity toward impulse control problems. These problems include a lack of planfulness and foresight, difficulties regulating affect and urges and delaying immediate gratification, and deficient behavioral restraint. Meanness corresponds to the callous aggression subdimension of the externalizing spectrum of psychopathology and includes deficient empathy, disdain for and lack of close relationships, exploitativeness, rebelliousness, excitement seeking, and empowerment through cruelty (Krueger et al., 2007). Lastly, boldness encompasses low levels of fear/avoidance (Kramer et al., 2012), expressed as a capacity to remain calm in situations involving threat, an ability to recover quickly from stressful events, high self-assurance and social efficacy, and an easiness with unfamiliarity and danger (Lilienfeld et al., 2012, in press).

As noted above, Latzman et al. (2016a) developed a chimpanzee operationalization of psychopathic personality organized around the triarchic model. Specifically, drawing on caretaker-rated items from an existing primate personality instrument, Latzman et al. used a consensus rating approach to formulate scale measures of the three triarchic model constructs for use with chimpanzees. These Chimpanzee Triarchic (CHMP-Tri) scales were then validated both in terms of their associations with performance on behavioral tasks and their translational relevance to humans. As noted by Latzman et al. (2016a), it is important to note that this model was not developed to derive some ways of characterizing some chimpanzees as "psychopaths" in a clinical way nor was it to imply that chimpanzees can be psychopaths. Rather, the goal was to evaluate the triarchic model from a comparative and evolutionary standpoint.

Results from this work indicate that the triarchic model of psychopathy can be operationalized effectively in chimpanzees, an animal species uniquely well-suited for neurobiological investigations of individual variation in broad, transdiagnostic biobehavioral traits (Latzman et al., 2016b). Such an approach is particularly opportune given the National Institute of Mental Health's (NIMH) research domain criteria (RDoC; Insel et al., 2010; Kozak and Cuthbert, 2016) initiative, encouraging investigators to consider psychopathology in terms of neurobehavioral dispositions. Indeed, as described previously (i.e., Latzman et al., 2016a) the dimensional constructs of the triarchic model can be viewed as trait-dispositional counterparts to RDoC constructs (Yancey et al., 2016).

It has long been theorized that psychopathy has heritable biological foundations (e.g., Karpman, 1946; Lykken, 1995), and an accumulating empirical literature supports the idea that genetic influences contribute to variance in psychopathic personality tendencies (Waldman and Rhee, 2006). Indeed, a replicable human literature has reported appreciable heritabilities for psychopathic tendencies (e.g., Blonigen et al., 2005, 2006; Viding et al., 2005; Brook et al., 2010; Bezdjian et al., 2011; Tuvblad et al., 2016). Taken together, the available research literature with humans clearly indicates an important contribution of genes to psychopathic tendencies.

Using the CHMP-Tri model, the finding of significant heritabilities of psychopathic tendencies has recently been confirmed in chimpanzees (i.e., Latzman et al., 2017). Consistent with findings in humans, results indicate significant genetic contributions to individual variability in psychopathic tendencies. Further, within the population of apes included in this study, some were raised by their biological mothers, whereas others were raised by humans for the first 3 years of life in a nursery. As described in more detail below, this quasi-experimental manipulation allowed for the explicit consideration of early social rearing experiences on estimates of heritability. When examined separately by early rearing background, consistent with previous findings for general personality dimensions (Latzman et al., 2015), the heritability of psychopathy dimensions varied by early social learning experiences: Whereas all three triarchic dimensions showed significant heritability among mother-reared participants, heritability was not evident for any dimension in the nursery-reared subsample (Latzman et al., 2017). All told, the existing literature, for both human and chimpanzee samples, provides clear evidence of a genetic contribution to psychopathy. To date, however, little is known concerning the specific genes associated with psychopathy.

Genes that underlie arginine-vasopressin (AVP), a phylogenetically conserved neuropeptide, constitute a particularly promising candidate, given the role of AVP in a range of complex social behaviors in both humans and nonhuman animals (Donaldson and Young, 2008). For example, within a sample of patients meeting diagnostic criteria for various personality disorders, cerebrospinal fluid AVP levels were found to correlate with a history of aggression (Coccaro et al., 1998). In addition to associations with direct measures of AVP, converging findings suggest an association between AVP receptor polymorphisms and a range of psychopathy-relevant social behaviors. For example, AVPR1A, the vasopressin V1A receptor gene, has been shown to be related to several social behaviors including pair bonding, territoriality, and aggression among voles, particularly males (e.g., Young and Wang, 2004; Hammock and Young, 2005, 2006). With regard to primates specifically, studies with humans have suggested an association between a similar repetitive element in the AVPR1A promoter and relevant social behaviors including altruism (Wassink et al., 2004) and pair bonding relationships (Walum et al., 2008). Further, with regard to personality traits in humans, AVPR1A promoter polymorphisms have been found to be associated with variability in a number of psychopathy-relevant traits (Patrick and Drislane, 2015) including increased novelty seeking, decreased harm avoidance (Walum et al., 2008) and increased reward dependence (Bachner-Melman et al., 2005).

Many of the association studies reviewed above have focused on the RS3 polymorphic repetitive element. In humans, the RS3 repeat region is housed within a larger, ∼350 bp tandem duplicated region. The first of these duplicated regions, DupA, spans −3730 to −4074 bp relative to the transcription start site and contains a GT_{20-26} microsatellite, known as STR1. The second duplicated region, DupB, spans −3382 to −3729 bp and contains the complex microsatellite, RS3 ($(CT)_{6-14}(GT)_{8-24}$). In chimpanzees, approximately 65% of the AVPR1A alleles have a complete deletion of the DupB region, resulting in a 357 bp difference between the $DupB^{+}$ and $DupB^{-}$ alleles (Donaldson et al., 2008). As a result of the complete deletion of RS3 in some individuals, chimpanzees are a uniquely valuable animal species for assessing the potential effect of the RS3 polymorphic repetitive element on individual variability in neurobehavioral dispositional dimensions such as those aspects described in the triarchic model.

In chimpanzees, a small but generally consistent literature suggests an association between AVPR1A and personality. For example, Hopkins et al. (2012) found that traits of Dominance and Conscientiousness were associated with polymorphic variation in *AVPR1A*, particularly among males. Latzman et al. (2014) have reported similar findings with AVPR1A associated with factor-analytically derived Disinhibition and Dominance constructs. Similar to Hopkins et al., these associations were found to vary by participant sex. Staes et al. (2015) also reported converging results. Specifically, Staes et al. found sex-specific AVPR1A associations with behavioral observations of sociability. Further, findings of associations between AVPR1A and chimpanzee personality have also been reported by Wilson et al. (2017) who found AVPR1A to be associated with Conscientiousness and Extraversion, although not in sex-specific ways. All told, AVPR1A appears to be a promising candidate gene for research investigating the genetic basis of interpersonal dispositional traits, such as those described by the triarchic model of psychopathy.

In addition to unique AVPR1A polymorphisms, as described in more detail below, chimpanzees in the current study were raised in different rearing environments early in life. In both human and nonhuman animals, the genetic contribution to particular traits likely depends on distinct factors in the environment, resulting in the relevance of genes in some environments but not in others (Charmantier and Garant, 2005; Rutter et al., 2006). That is, the genetic contributions to various outcomes may differ depending on the environment. Across human (i.e., Moffitt et al., 2006), monkey (i.e., Suomi, 2011), and chimpanzee (i.e., Latzman et al., 2015, 2017) samples, research has suggested an interactive contribution of early adversity and genetic variation to a broad range of outcomes. Research in nonhuman animals (i.e., Charmantier and Garant, 2005), including chimpanzees (Latzman et al., 2015, 2017), has revealed similar variability in heritability estimates as a function of differences in early adversity. Indeed, as described above, recent CHMP-Tri findings suggest that the heritability of psychopathy dimensions varies by early social rearing experiences (Latzman et al., 2017). Given these existing lines of evidence, the role of early social rearing experiences is important to consider.

The overarching aims of the current study were to investigate the effects of DupB genotype on triarchic psychopathy dimensions and whether these effects appear sex-specific. Although no studies to date have investigated AVPR1A RS3 polymorphisms and psychopathy specifically, molecular genetic studies on the association between other specific candidate genes and psychopathy-related behaviors and traits suggest an important moderating role for early adversity in moderating associations (e.g., Capitanio et al., 2006; Kim-Cohen et al., 2006; Buckholtz and Meyer-Lindenberg, 2008; Karere et al., 2009). Similar findings have been shown among chimpanzees with regard to heritability estimates of psychopathy traits (Latzman et al., 2017). Specifically, whereas all psychopathy dimensions were found to be heritable among mother-reared apes, none of the heritability estimates were significant among nursery-reared apes. We thus decided to examine associations separately by early rearing experience.

Given previous findings of significant associations between AVPR1A and Conscientiousness, Dominance and Disinhibition (e.g., Hopkins et al., 2012; Latzman et al., 2014), we expected genotypic variability to relate as well to the CHMP-Tri dimensions, in distinct ways. Specifically, given previous chimpanzee findings with regard to Disinhibition (Latzman et al., 2014) and Conscientiousness (Hopkins et al., 2012), and clear links between triarchic disinhibition and each of these two constructs, we expected significant associations to emerge between AVPR1A genotype and CHMP-Tri Disinhibition, particularly for males. Specifically, we predicted that $DupB^{+/-}$ males would show lower scores on this triarchic trait dimension. Further, given previous findings of lower levels of Dominance (Latzman et al., 2014), along with higher levels of anxiety-related

behaviors (i.e., scratching, Latzman et al., 2016b; Mahovetz et al., 2016) for DupB$^{+/-}$ males, we predicted that DupB$^{+/-}$ males would score lower on CHMP-Tri Boldness, a triarchic disposititon associated with low threat-sensitivity. We did not advance any *a priori* hypotheses regarding CHMP-Tri Meanness. Whereas, previous human findings of an association between AVPR1A genotype and Dictator game performance (Knafo et al., 2008) are suggestive of an association for meanness, prior work with chimpanzees has reported no association between AVPR1A variability and scores on Agreeableness, the personality trait most strongly associated with triarchic meanness (Patrick and Drislane, 2015). Finally, given previous findings of a genetic foundation for a higher-order psychopathic personality dimension in both humans (e.g., Larsson et al., 2006) and chimpanzees (i.e., Latzman et al., 2017), we investigated the association between DupB genotype and a total CHMP-Tri score. Importantly, given previous findings of significant genetic contributions to CHMP-Tri scales among mother- but not nursery-reared chimpanzees, we expected to find significant AVPR1A effects only in the mother-reared sample.

MATERIALS AND METHODS

Participants

Chimpanzees were members of two genetically distinct colonies of apes housed at the Yerkes National Primate Research Center (YNPRC) in Atlanta, Georgia and at the National Center for Chimpanzee Care (NCCC) at The University of Texas MD Anderson Cancer Center in Bastrop, Texas. Participants for the current study included 82 adult and sub-adult chimpanzees at YNPRC, including 57 females and 25 males ranging in age from 9 to 53 years ($M_{age} = 22.15$, $SD = 8.96$) and 96 adult and sub-adult chimpanzees at NCCC, including 46 females and 50 males ranging in age from 8 to 41 years ($M_{age} = 22.88$, $SD = 6.12$). After removing 14 chimpanzees for whom AVPR1A data were not available (e.g., blood sample were not available, DNA yield was not sufficient) participants from both colonies were combined for analyses resulting in a final sample of 164 chimpanzees.

Early rearing experiences varied among individuals, with 119 being mother-reared and 59 human nursery-reared. Mother-reared chimpanzees remained under the care of their mothers for at least 2.5 years of life and were raised in "nuclear" family groups of chimpanzees, with group sizes ranging from 4 to 20 individuals. Nursery-reared chimpanzees were separated from their mothers within the first 30 days of life, due to unresponsive care, injury, or illness. These chimpanzees were placed in incubators, fed standard human infant formula, and cared for by humans until they could care adequately for themselves, at which time they were placed with other infants of the same age until they were 3 years old (Bard et al., 1992; Bard, 1994). At 3 years of age, the nursery-reared chimpanzees were integrated into larger social groups of adult and sub-adult chimpanzees.

It should be noted that all of the nursery-reared chimpanzees were raised in this manner to protect the infants' well-being. That is, the chimpanzees in this study were not nursery-reared by design, with the goal of subsequently determining the effects of early life experiences on development. The data for these subjects are therefore *ex post facto* and opportunistic; indeed, we capitalized on the fact that some of the chimpanzees received different rearing experiences in order to evaluate whether this might have long-term consequences on personality development. Importantly, as described previously (Bogart et al., 2014; Latzman et al., 2017), based on the composition of the rearing groups, potential rearing differences are not conflated with familial environment. That is, group membership reflects early experiences, rather than familial aggregation of group placement decisions. As reported previously by Latzman et al. (2017) with regard to relatedness within each rearing group, 52 different sires and 79 different dams contributed to the mother-reared group, and 34 different sires and 42 different dams contributed to the nursery-reared group. Further, as described in detail previously, the genetic diversity within each group was comparable suggesting that group membership reflects early experiences rather than familial aggregation of group placement decisions (see Latzman et al., 2017). The full pedigree structure for this sample has been described previously (see Hopkins et al., 2014b).

All aspects of the research complied with the American Psychological Association's Guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research (American Psychological Association, 2012), followed the Institute of Medicine (US) and National Research Council (US) Committee on the Use of Chimpanzees in Biomedical and Behavioral Research (2011) guidelines for research with chimpanzees, and was done with the approval of the Institutional Animal Care and Use Committees of the universities at which the research was conducted. All chimpanzees are housed in social groups ranging from 2 to 16 individuals in indoor-outdoor compounds, with free access to both portions of their enclosures 24 h a day. During the winter, the indoor facilities are heated, while air conditioning or fans and misters are provided in the hotter summer months. Lighting in the outdoor facility follows the typical seasonal cyclic change in sunrise and sunset. Standard tungsten lighting is provided in the indoor facility and the lights are on a 12 h on-off cycle. The chimpanzees are fed two to five times per day with a diet that consists of fruits, vegetables, and commercially produced primate chow. In addition, they receive a number of foraging and enrichment opportunities each day. Environmental enrichment, such as simulated tool use tasks or non-nutritive substrates, is provided to the chimpanzees on a daily basis. At no time are apes food- or water-deprived.

Assessment of Triarchic Psychopathy Dimensions

Chimpanzee Triarchic (CHMP-Tri) scales previously developed through a consensus-based approach (Latzman et al., 2016a) were used in the current study. Consistent with the triarchic model of psychopathy in humans, the three CHMP-Tri scales assess Boldness (6-items), Meanness (5-items), and Disinhibition (7-items). As described by Latzman et al. (2016a), chimpanzees were rated by colony-staff members; typically two to three independent raters, who had worked with the animals for an extended period of time and reported having "enough experience for an accurate rating" (Freeman et al., 2013, p. 1044), rated each chimpanzee. Items for each scale were rated using a 7-point Likert-type format, with response options

ranging from 1 (*least descriptive of the chimpanzee*) to 7 (*most descriptive of the chimpanzee*). Internal consistencies (Cronbach's alpha) for the three scales have been shown to be acceptable, especially considering their brevity: 0.82 for Boldness, 0.77 for Disinhibition, and 0.67 for Meanness (Latzman et al., 2016a).

DNA Extraction, Genotyping and Analysis

As described previously (e.g., Donaldson et al., 2008), DNA samples were isolated from buccal swabs or blood samples using Puregene DNA purification system (Gentra, Minneapolis, MN, USA). Following extraction, stock DNA was separated into three aliquots: one for onsite storage at −80°C, one for offsite storage, and a working stock for genotyping. Samples were tracked via a secure Filemaker Pro 8 database that linked sample codes for each aliquot, demographics for each subject, DNA quantification and purity analysis results, and genotype data.

Each individual was genotyped for the *AVPR1A* DupA/B region using the primers and conditions reported in previous studies (e.g., Hopkins et al., 2012; Latzman et al., 2014). Briefly, we used forward primer 5′-GCATGGTAGCCTCTCTTTAAT and a reverse primer of 5′- CATACACATGGAAAGCACCTAA with an annealing temperature of 57°C for 30 cycles: 95°C, 5 min; 30 × (95°C, 30 s; 57°C, 30 s; 72°C, 3 min; 72°C, 10 min; 4°C, hold). Polymerase chain reaction (PCR) amplification was undertaken using the Epicentre Failsafe kit using premixH (Illumina Inc., Madison, WI, USA) according to the manufacturer's directions. Genotyping was performed in a volume of 20 μl containing 20 ng target genomic DNA. PCR products were resolved on a 2% agarose gel (SeaKem Agarose LE, Lonza, Basel, Switzerland) at 100 V for 45 min with a 100-bp DNA ladder (New England Biolabs, Ipswich, MA, USA) in tris-borate-EDTA (TBE). The DupB-containing allele resulted in a band of ∼900 bp, while the DupB minus allele was ∼570 bp long, and genotypes were visually assigned (Donaldson et al., 2008). All genotypes were run in duplicate with gel analysis and were checked before the data set was finalized. Forty-three males and 64 females were homozygous for the short allele ($DupB^{-/-}$) and 26 males and 31 females had the long allele ($DupB^{+/-}$), yielding overall genotype frequencies of 65.2 and 34.8%. These frequencies are consistent with those previously identified in wild-caught chimpanzees (Donaldson et al., 2008). Further, as reported in previous studies, the AVPR1A genotype distribution in these two colonies of apes does not deviate from Hardy-Weinberg equilibrium (Hopkins et al., 2014a).

Data Analysis

To examine associations between CHMP-Tri dimensions and AVPR1A DupB genotype, we used multivariate analysis of variance (MANOVA). Specifically, we used CHMP-Tri scores as the dependent variables and sex as a fixed factor, and, given the age range of the sample, we included age as a covariate. The potential moderating role of sex was examined for a number of reasons. In addition to vasopressin systems in the brain being (1) found to be sexually dimorphic and (2) thought to regulate social behaviors in sex-specific ways (De Vries et al., 1981), investigations in chimpanzees of associations between AVPR1A and general dispositional traits have resulted in sexually-dimorphic results (e.g., Hopkins et al., 2012; Latzman et al., 2014, 2016b). Given prior work with chimpanzees demonstrating important moderating impacts of early rearing experience on the heritability of personality broadly (i.e., Latzman et al., 2015), and psychopathy more specifically (i.e., Latzman et al., 2017), analyses were conducted separately for mother- and nursery-reared chimpanzees.

To confirm that associations between AVPR1A DupB genotype and CHMP-Tri scores were a direct reflection of AVPR1A rather than a result of all shared genes, a series of *post-hoc* analyses were performed in the Sequential Oligogenic Linkage Analysis Routines (SOLAR; Almasy and Blangero, 1998) program. Specifically, as described in detail previously (see Fears et al., 2009, 2011), and consistent with recent quantitative genetic work on CHMP-Tri scales (Latzman et al., 2017), SOLAR uses a variance components approach that relies on maximum likelihood estimation to compute a polygenic variance term for a dependent measure of interest (i.e., CHMP-Tri scores) when considering the entire pedigree. To determine the contribution of AVPR1A DupB genotype explicitly, we included AVPR1A and AVPR1A*sex as covariates in the polygenic models for each CHMP-Tri score found associated with AVPR1A in our MANOVA analyses. The contribution of genotype and genotype*sex to the explanation of the CHMP-Tri scores independent of genetic relatedness was evaluated by testing the statistical significance of their associations within the full model.

RESULTS

AVPR1A Variation and Chmp-Tri Psychopathy Dimensions

Within the mother-reared sample, a significant main effect was found for AVPR1A[$F_{(3,\ 104)} = 8.78, p < 0.001, \eta_p^2 = 0.10$] in the prediction of CHMP-Tri scores. Subsequent univariate *F*-tests revealed a significant main effect association for DupB genotype with CHMP-Tri Disinhibition [$F_{(1,\ 106)} = 6.66, p = 0.01, \eta_p^2 = 0.06$]. Mean standardized CHMP-Tri Disinhibition scores in $DupB^{-/-}$ and $DupB^{+/-}$ are shown in **Figure 1**. Specifically, across sexes, $Dup^{+/-}$ apes scored significantly higher in disinhibitory tendencies than $DupB^{-/-}$ apes. By contrast, no direct effects between the DupB genotype and either CHMP-Tri Boldness [$F_{(1,\ 106)} = 2.75, p > 0.10, \eta_p^2 = 0.03$] or Meanness emerged [$F_{(1,\ 106)} = 1.28, p > 0.25, \eta_p^2 = 0.01$].

However, a significant two-way interaction between AVPR1A and sex [$F_{(3,\ 104)} = 4.87, p < 0.01, \eta_p^2 = 0.12$] was found in predicting CHMP-Tri scores as a whole. Subsequent univariate *F*-testsrevealed that this omnibus effect was attributable mainly to the predictive effect of the AVPR1A*sex interaction for Boldness [$F_{(1,\ 106)} = 14.70, p < 0.001, \eta_p^2 = 0.12$]. Mean standardized CHMP-Tri Boldness scores in $DupB^{-/-}$ and $DupB^{+/-}$ males and females are shown in **Figure 2**. Whereas, female $Dup^{-/+}$ apes evidenced higher scores on this trait dimensions, male $Dup^{-}/+$ apes showed lower scores on CHMP-Tri Boldness. No significant effects for the AVPR1A*sex interaction term were found in predicting CHMP-Tri Meanness [$F_{(1,\ 106)} = 2.71, p > 0.10, \eta_p^2 = 0.03$] or Disinhibition [$F_{(1,\ 106)} = 0.51, p > 0.45, \eta_p^2 = 0.01$].

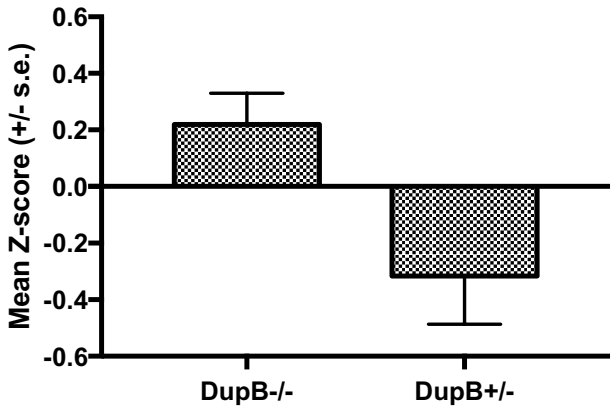

FIGURE 1 | Mean CHMP-Tri Disinhibition scores (± SE) for mother-reared chimpanzees with DupB$^{+/-}$ and DupB$^{+/-}$ GENOTYPES. $n = 77$ for DupB$^{-/-}$. $n = 34$ for DupB$^{+/-}$.

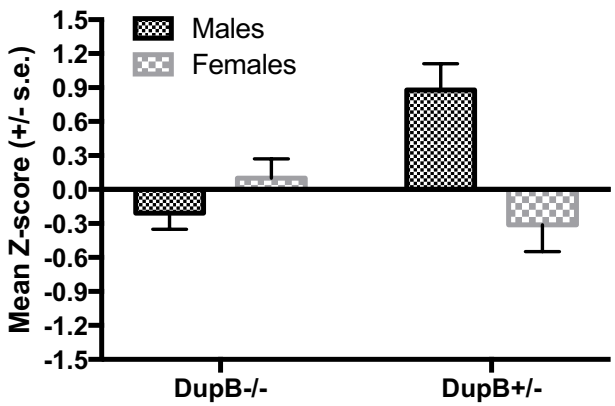

FIGURE 2 | Mean CHMP-Tri boldness scores (± SE) for mother-reared males and females with DupB$^{+/-}$ and DupB^{-} genotypes. $n = 29$ DupB$^{-/-}$ males and 48 DupB$^{-/-}$ females. $n = 16$ DupB$^{+/-}$ males and 18 DupB$^{+/-}$ females.

Within the nursery-reared sample, no significant main effects [$F_{(3, 46)} = 0.06$, $p > 0.95$, $\eta_p{}^2 = 0.004$] or interactions [$F_{(3, 46)} = 0.16$, $p > 0.90$, $\eta_p{}^2 = 0.01$] were found. No predictive effects were found for any individual CHMP-Tri scale (all Fs < 0.20, ps > 0.65).

AVPR1A Variation and Total CHMP-Tri Psychopathy

Finally, the association between DupB genotype and total CHMP-Tri psychopathy was investigated. Within the mother-reared sample, whereas no significant main effect for AVPR1A emerged [$F_{(1, 106)} = 0.83, p > 0.35, \eta_p{}^2 = 0.01$], a significant two-way interaction between AVPR1A genotype and sex [$F_{(1, 106)} = 7.13, p < 0.01, \eta_p{}^2 = 0.06$] was found in the prediction of total CHMP-Tri scores. Mean standardized CHMP-Tri total scores in DupB$^{-/-}$ and DupB$^{+/-}$ are shown in **Figure 3**. Whereas, female DupB$^{-/+}$ apes evidenced lower scores, male Dup$^{-/+}$ apes showed higher scores on CHMP-Tri total score; the opposite was true for DupB$^{-/-}$ apes. Within the nursery-reared sample, no

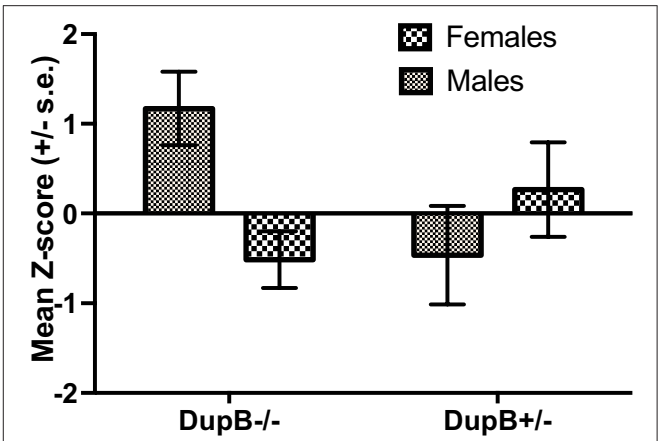

FIGURE 3 | Mean CHMP-Tri total scores (± SE) for mother-reared males and females with DupB$^{+/-}$ and DupB$^{+/-}$ genotypes. $n = 29$ DupB$^{-/-}$ males and 48 DupB$^{-/-}$ females. $n = 16$ DupB$^{+/-}$ males and 18 DupB$^{+/-}$ females.

significant main effects [$F_{(1, 53)} = 0.03, p > 0.85, \eta_p{}^2 = 0.001$] or interactions [$F_{(1, 53)} = 0.03, p > 0.85, \eta_p{}^2 = 0.001$] were found.

Contribution of AVPR1A and AVPR1A*Sex in Polygenic Models

As described above, to confirm the robustness of our MANOVA findings within the mother-reared group, tests of AVPR1A and AVPR1A*sex as potential covariates within full polygenic models were next run. Consistent with MANOVA findings, these *post-hoc* analyses suggested a significant specific effect for AVPR1A DupB genotype for CHMP-Tri Disinhibition explaining 8.56% of the proportion of variance ($p = 0.01$). Further, also consistent with MANOVA findings described above, AVPR1A*sex evidenced a significant effect for both CHMP-Tri Boldness explaining 8.32% of the variance ($p = 0.0009$) and CHMP-Tri Total score explaining 5.86% of the variance ($p = 0.005$).

DISCUSSION

The current study represents the first investigation to date of the association between variation in AVPR1A, a gene that underlies AVP, and psychopathy tendencies as a function of differences in early rearing experiences. Consistent with expectations, AVPR1A DupB genotype was found to explain variability in psychopathy dimensions uniquely and in sex-specific ways. Results underscore the translational value of a nonhuman primate model for investigating psychopathy dimensions and provide strong support for the notion of triarchic psychopathy dimensions as biologically-based and evolutionarily derived (Patrick et al., 2009; Latzman et al., 2016a).

AVPR1A and Triarchic Psychopathy

Consistent with previous findings in both humans and chimpanzees with regard to psychopathy-related behaviors and traits, results suggest a potentially important etiological pathway from AVP to triarchic psychopathy, at least for mother-reared

apes. Specifically, in line with previous findings of higher scores on the personality traits of Disinhibition/low Conscientiousness (Hopkins et al., 2012; Latzman et al., 2014), traits linked to triarchic disinhibition (Patrick and Drislane, 2015), DupB$^{+/-}$ apes evidenced lower scores on CHMP-Tri Disinhibition in the current sample. In contrast to previous findings of this association being male-specific, however, this association did not vary by sex in the current study. Contrary to both expectations and previous findings for related traits and behaviors, DupB$^{+/-}$ was found to be associated with higher CHMP-Tri Boldness scores for males. This is surprising for a number of reasons, including previous findings of higher levels of anxiety-related behaviors (i.e., scratching, Latzman et al., 2016b; Mahovetz et al., 2016) for DupB$^{+/-}$ males and the contention that triarchic boldness reflects the phenotypic expression of low threat-sensitivity. It will thus be important for future research to more explicitly investigate this finding. Finally, total CHMP-Tri psychopathy was found to associate with AVPR1A in sex-specific ways. Importantly, in a series of *post-hoc* analyses, the robustness of findings and their specificity to AVPR1A rather than all shared genes, were confirmed through a series of polygenic models in which genetic relatedness among apes were considered. All told, although not entirely consistent with expectations, the current findings in the mother-reared sample suggest an important role for AVP on variation in psychopathy dimensions.

In direct contrast to findings among mother-reared chimpanzees, and consistent with recent biometric results (Latzman et al., 2017), none of the psychopathy dimensions were found to associate with the AVPR1A RS3 polymorphism in the nursery-reared sample, providing evidence of different etiologies as a function of rearing. These findings are not only consistent with biometric results in chimpanzees, but also with accumulating evidence in the human (e.g. Moffitt et al., 2006; Rutter et al., 2006) and nonhuman animal literatures (e.g., Charmantier and Garant, 2005; Karere et al., 2009) for variations in the effects of genes as a function of environmental context. Importantly, whereas in humans it is quite difficult to disentangle etiological influences due to confounding of environmental and genetic influences, findings for our chimpanzee sample are less likely to reflect this confound. Indeed, as described previously (i.e., Bogart et al., 2014; Latzman et al., 2017), although offspring in each of the two early rearing groups were not entirely heterogeneous, the degree of genetic diversity was comparable between them.

Triarchic Model, AVP, and the NIMH Research Domain Criteria (RDoC)

A notable feature of the dispositional constructs of the triarchic model is that they are framed explicitly in neurobiological terms (Patrick et al., 2009; Patrick and Drislane, 2015). As mentioned at the outset, an analysis of psychopathy in terms of neurobehavioral dispositions is apt, given the NIMH's RDoC initiative (Insel et al., 2010; Kozak and Cuthbert, 2016), which endeavors to explicate the neurobiological bases of mental illness and reframe conceptions of psychopathology around constructs with specific brain referents. The RDoC research framework specifies biobehavioral constructs, grouped within major domains of functioning, as explanatory referents for understanding clinical problems—and encourages investigation of these constructs using measures from multiple assessment domains ("units of analysis"). Clear counterparts to the triarchic model dimensions exist within the RDoC framework. Indeed, Disinhibition fits within the RDoC construct of "response inhibition" within the Cognitive Systems domain; boldness fits within the construct of "acute threat" in the Negative Valence Systems domain; and meanness fits within the construct of "affiliation and attachment" in the Social Systems domain. The dimensional constructs of the triarchic model can thus be viewed as trait-dispositional counterparts to these RDoC constructs (Yancey et al., 2016).

The direct relevance of the current research to the RDoC initiative is further bolstered by the consideration of relations between these three biobehavioral phenotypes and AVPR1A. Indeed, the vasopressin system is explicitly referred to as a suggested unit of analysis across a variety of RDoC domains and constructs, including within the Negative Valence System, Social Processes, and Arousal and Regulatory Systems Domains. For example, vasopressin is thought to relate to the Acute Threat ("Fear") construct within the Negative Valence System, a construct with direct links to boldness in the triarchic model.

Although the current study focused specifically on psychopathy subdimensions described within the triarchic model and associations with the AVPR1A RS3 polymorphism, taken together with a growing body of research (i.e., Latzman et al., 2016b), results from the current study provide clear support for primate-translational operationalizations of specific constructs within the RDoC framework. Despite recent decisions by the National Institutes of Health (National Institutes of Health, 2011) to scale back research of some types involving captive chimpanzees, work undertaken for the current study fits clearly within the ethical framework of scientifically justifiable research with chimpanzees as outlined by the Institute of Medicine (Institute of Medicine (US) and National Research Council (US) Committee on the Use of Chimpanzees in Biomedical and Behavioral Research, 2011). In conjunction with work being conducted on other RDoC-relevant lines (e.g., Hopkins et al., 2014a; Latzman et al., 2016b), the current work highlights the importance of including a chimpanzee comparative-translational component in the NIMH RDoC research program. Along with findings from human studies, work of this kind can provide exceptionally valuable insights into core biobehavioral processes relevant to psychological illness and health (Latzman and Hopkins, 2016).

Limitations

The current study is not without limitations. First, the sample size, particularly in the case of the nursery-reared subgroup, was relatively modest. Additional research is thus needed to replicate the current findings and establish more stable estimates for contributions of AVP to psychopathy. Nonetheless, it is important to note that although potential concerns regarding sufficient power to detect effects within the nursery-reared sample are appropriate, effect sizes for these associations

approached zero (e.g., $\eta_p^2 \leq 0.01$). Additionally, while widely-used in both the human and nonhuman primate literatures, our use of scores on the CHMP-Tri scales, derived from caretaker ratings of a set of adjective descriptors with accompanying narrative definitions, is only one of a number of potential approaches to assessing the dimensions described within the triarchic model. Indeed, multi-domain operationalizations of triarchic dimensions are possible through the use of composite psychoneurometric indices of the various dimensions (e.g., Patrick et al., 2013; Yancey et al., 2016). It will be important for future research to replicate the current findings considering various domains of measurement.

Additionally, there have been a number of replicability concerns raised with regard to candidate gene studies (e.g., Munafo, 2009). Although AVPR1A has repeatedly been found to underlie variation in AVP, the current study considered a single polymorphism and did not directly assess circulating levels of AVP. Further, we are not able to determine whether the differences that emerged are due directly to gene expression caused by the presence or absence of the DupB region. Nonetheless, as described earlier, across nonhuman animal studies, AVPR1A has emerged as a reliable correlate of a variety of social behaviors and traits. One important strength of animal studies is that, as compared to humans, nonhuman animal participants are raised in homogeneous, controlled environments; indeed, as described above, chimpanzee participants in the current study were raised in a common, controlled environment. Nonetheless, it will clearly be important for future studies to replicate our findings and also include more direct measures of AVP to confirm that our findings are a result of AVPR1A expression or some other potential pathway. Further, it will be important for future studies to examine additional related neuropeptides known to be associated with social behavior, such as oxytocin and associated genes.

Finally, it is important to note that chimpanzees encounter a variety of potentially impactful early experiences, whether raised by their biological mothers or in human-managed nursery settings. Given this, as noted previously (i.e., Latzman et al., 2015), our classification of participants into subgroups based on the ostensibly topographical manner in which they were raised likely obscures important variability within each group. Notably, however, our approach of grouping chimpanzee participants in this manner likely resulted in a more conservative indication of the role of early social rearing experiences, potentially enhancing confidence in conclusions advanced from current findings. Relatedly, the nursery-rearing experience of apes is not directly parallel to experiences of early adversity in humans. As described previously (e.g., Latzman et al., 2017), nursery-reared apes are removed from their mothers as a result of caregiving needs (e.g., inadequate maternal care, injury, illness) and subsequently placed in an adequate (i.e., less adverse) condition. Nonetheless, whereas this is not the best parallel to experiences of physical adversity, this experience is likely more similar to human experiences of social adversity. Indeed, maternal deprivation at an early age is associated with a number of dysfunctional behaviors in humans (Gunnar and Quevedo, 2007). Thus, although likely not completely analogous to the early adversity encountered by many humans, it is clear that early maternal separation results in a number of behavioral sequelae indicative of adversity.

CONCLUSION

Using a powerful and unparalleled animal model, the current study points to contributions of AVP influences to psychopathic tendencies, with an important role for a specific environmental factor—early rearing experience—in affecting this contribution. Taken together, results suggest an important contributory influence of neuropeptide variation in the explanation of the development of psychopathy. Results of the current study further provide additional compelling evidence that psychopathic tendencies are rooted in basic, evolutionarily-meaningful dispositions (Fowles and Dindo, 2006; Patrick et al., 2009; Skeem et al., 2011; Patrick and Drislane, 2015; Latzman et al., 2016a, 2017), and provide support for a primate-translational operationalization of key neurobehavioral constructs relevant both to psychopathy and to broader forms of psychopathology. As such, the current work highlights the value of a chimpanzee comparative-translational component to the NIMH RDoC research framework (Latzman and Hopkins, 2016).

AUTHOR CONTRIBUTIONS

RL and WH conceived of the study. WH and SS oversaw data collection. RL performed all analyses and drafted the paper. WH and SS provided critical revisions.

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Arginine Vasopressin Effects on Subjective Judgments and Neural Responses to Same and Other-Sex Faces in Men and Women

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Arginine vasopressin (AVP) influences social and emotional behaviors across a wide range of species. In humans, intranasal AVP has been previously shown to alter physiological responses to and subjective judgments of same-sex faces in both men and women. The present study attempted to elucidate the neural mechanism for these effects by randomizing 40 healthy men and 40 healthy women to treatment with either 40 IU intranasal AVP or a saline placebo approximately 30 min before imaging their brain function with fMRI as they viewed same and other-sex faces. All subjects were also scanned a second time several days later with no treatment to evaluate the persistence of AVP effects over time. AVP acutely increased positive ratings of same-sex faces in women, with some evidence that these effects persisted until the second scan. While AVP had no acute effects on same-sex ratings in men, AVP increased positive ratings of same-sex faces several days later. On the other hand, AVP had no effect on other-sex face judgments in either sex. AVP modulation of brain function was focused on the nucleus accumbens (NAc) and the lateral septum, two reward processing areas involved in the formation of social bonds. AVP provoked acute increases in right NAc and bilateral lateral septum responses to female faces among men, with left lateral septum responses persisting over time while right NAc responses reversed over time. Finally, AVP modulated hypothalamic activation to faces in both men and women. The present study therefore indicates that intranasal AVP affects subjective ratings and neural responses to same and other-sex faces in men and women, with some effects persisting and others emerging over time. Future studies should investigate whether AVP effects are modulated by individual variables such as genotype, personality, or attachment style as previously reported for other nonapeptides.

Keywords: vasopressin, fMRI, face processing, sex differences, nucleus accumbens

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INTRODUCTION

Arginine vasopressin (AVP) is a nine amino acid peptide that is synthesized in the hypothalamus and released into the general circulation where it acts as a hormone to regulate blood pressure and water retention in the body (1, 2). In addition, AVP-producing neurons within and outside the hypothalamus release AVP into the brain where it can act *via* vasopressin receptors (and possibly related oxytocin receptors) to influence social and emotional behaviors.

Arginine vasopressin and arginine vasotocin (AVT, its non-mammalian homolog) effects on brain functions related to social behavior have been explored in numerous non-human animals. These

studies have identified both species-specific neuromodulatory circuits that have evolved in relation to unique life histories, as well as circuits that are highly conserved across species. Within these circuits, target areas have been identified in which AVT/AVP can stimulate courtship [hindbrain; rough-skin newt; (3)], promote affiliation related to pair bonding [septum and ventral pallidum, prairie voles; (4, 5)], and increase gregariousness [septum, zebra finches; (6)]. Areas in which the peptides influence aggression and aggressive communication [anterior hypothalamus, hamsters and prairie voles (7–9); amygdala, rats (10); septum, finches and sparrows (11, 12); preoptic area, plainfin midshipmen (13)], as well as social withdrawal [amygdala, rats (14); hindbrain, goldfish (15)] have also been identified. From this body of work, it has become clear that these peptides can influence a variety of social responses, promoting affiliative interactions in some species or contexts and aggressive or antisocial responses in others, *via* actions in different brain circuits [further discussed in Ref. (16)].

Arginine vasopressin effects on human social cognition and behavior have been studied using intranasal AVP administration, which is believed to cross the blood–brain barrier (17). Most studies have been done in men, where intranasal AVP has been found to facilitate cooperation (18, 19), enhance recognition of sexual cues (20), and enhance encoding of happy and angry faces (21). In addition, among both men and women, intranasal AVP increases empathic concern in those who received high levels of paternal warmth (22), as well as anxiety and skin conductance responses to angry faces (23). However, AVP can also have different effects in men and women for some social responses. In men, AVP induced agonistic facial motor patterns and decreased perception of friendliness to faces of unfamiliar men. On the other hand, in women, AVP induced affiliative facial motor patterns and increased perceptions of friendliness in faces of unfamiliar women (23). Together, these studies suggest that, in humans, AVP has sex- and perhaps context-dependent influences on a variety of social responses, as it also does in other vertebrates.

Although we know less about where within the human brain AVP acts to influence particular social responses than we do in other animals, fMRI studies have identified some regions in which AVP alters patterns of activity in parallel with its effects on social behavior and/or emotional processes. In men, intranasal AVP increases the amygdala response to emotional scenes (24), consistent with increased self-reported anxiety (23). To the contrary, it decreased both amygdala and anterior insula activation in response to negative social interactions between men in the iterated Prisoner's Dilemma (PD) Game (25). On the other hand, intranasal AVP augmented bilateral insula activation to positive social interactions in men the PD game while having the opposite effect in women (26). Thus, AVP may increase the salience of positive social interactions, while decreasing the salience of negative social interactions among men in some contexts. AVP has also been shown to decrease activation within the temporoparietal junction, a key node of the theory of mind network, when viewing unfamiliar but not familiar faces (27). Finally, AVP has been shown to modulate putative emotion regulation circuitry in the human brain. For example, AVP increased connectivity of the right amygdala with the medial prefrontal cortex (mPFC) during the processing of socially threatening scenes, which was interpreted as reflecting a reduced suppressive effect of mPFC on amygdala activity (24). In another study, it induced a relative increase in activation within the subgenual ACC, another key emotion regulation area (28), in response to emotional faces.

To further explore the neural mechanisms underlying AVP's effects on responses to social stimuli, the present study investigates whether intranasal AVP modulates the BOLD fMRI response to viewing unfamiliar same and other-sex faces in both men and women. Given known effects of AVP on learning (21, 29, 30) and long-lasting, organizational effects on social responsiveness that it and related nonapeptides can have during development (31–34), we also investigated whether any observed effects persist beyond the period of AVP exposure. Although our previous work focused on a lower dose (20 IU), preliminary data from a parallel study suggested that a higher dose (40 IU) more effectively stimulates positive assessments potentially related to affiliative responses to faces in women and may also promote positive responses in men, whereas lower doses promote negative responses (under review, this issue). Our strongest prediction was, therefore, that 40 IU AVP would increase positive responses toward the faces of other women in women. Although lower doses promote negative responses toward same-sex faces in men (23), these preliminary data also led us to predict that this higher dose (40 IU) would promote positive responses in men. We also predicted that if AVP has effects in humans that are, as in other species such as prairie voles and finches, dependent on social contexts, including the sex of the individual/stimulus with which subjects interact (35, 36), then AVP may produce different effects toward same- and other-sex stimuli, In particular, if AVP promotes affiliative responses related to pair bonding in human males (37), then we predicted that any positive effects in males would be selective for female faces. Further, we predicted that positive responses would be concurrent with increased activation in the ventral striatum, in which nonapeptides, including AVP, have been shown to promote affiliative responses in prairie voles, particularly in relation to pair bonding (see above), and in which activity is generally associated with positive social responses in humans (38, 39). In contrast, we predicted any antisocial effects might be concurrent with increased activation in regions in which AVP/AVT act to enhance aggression/antisocial behaviors in other animals, most notably the amygdala and hypothalamus (see references in second paragraph). Predictions about the lateral septum were more difficult; AVT can promote aggression *via* actions there, but also gregariousness, and AVP in the lateral septum promotes affiliative processes related to pair bonding. Further, based on previous findings in humans (28), we predicted that AVP would increase the subgenual ACC response to viewing faces.

MATERIALS AND METHODS

Subjects

Participants were 40 healthy men and 40 healthy women between the ages of 21 and 30 (mean = 23.89 ± SD = 2.19). All participants were heterosexual and not in a committed relationship. Participants were randomized to either 40 IU intranasal AVP (20 men and 20 women) on scan 1 followed by no treatment

on scan 2, or to placebo (PL) (20 men and 20 women) on scan 1 followed by no treatment at scan 2. Scans were collected within 2 weeks of each other. The mean interval between scans was 4.3 days (SD = 2.25 days). Randomization was performed by the Emory Investigational Drug Service (IDS) using Research Randomizer,[1] which randomizes each subject by using the method of randomly permuted blocks.

All potential subjects completed a full medical history questionnaire. Subjects with a history of seizures or other neurological disorders, alcoholism, or any other substance abuse, hypertension, cardiovascular disease, diabetes, and other endocrine diseases or malignancy were excluded from the study. Subjects who reported a history of asthma or migraine headaches were excluded if their symptoms were persistent, disabling, and required one or more medication adjustments within the past month. Subjects with a history of head trauma, psychiatric illness, or use of medications with known psychoactive effects over the past year were generally excluded. However, a *post hoc*, secondary review of screening forms revealed inclusion of one subject who reported mild head trauma and another who indicated seizure due to fever at the age of 2. Subjects with claustrophobia were excluded at the discretion of the Principal Investigator. Subjects were allowed to continue on their current medications if the agents in question were not reported to alter brain activity in regions of interest. Some of these medications included birth control and antihistamines for allergy.

All subjects gave written informed consent, and the study was approved by the Emory University Institutional Review Board and the U.S. Food and Drug Administration. Preparation of study medication and details of randomization were maintained by the Emory IDS and all study personnel including the PI were blind to group assignment. Administration of 40 IU vasopressin was generally safe. None of the subjects developed any major side effects of study medication, including anaphylaxis. One subject experienced a transient increase in blood pressure; however, this subject was in the PL group.

Preparation and Administration of AVP and Placebo

Intranasal AVP

Lyophilized AVP purchased from Polypeptide Group (Hillerod, Denmark) was diluted in sterile saline at concentration of 40 U/0.5 ml. The solution was immediately sterilized *via* a 0.22 μm filter before being transferred to sterile conical tube and stored at −80°C until use. On the day of the study, the drug was transferred to a nasal spray bottle after thawing from which the subjects self administered. Both prior to and after freezing, three AVP samples were tested for sterility and potency by Eagle Analytics. Samples measured 103, 112, and 90.2% of 40 IU, respectively.

Intranasal Placebo

The PL group self-administered 0.5 ml of PL spray comprised of sterile saline, pH adjusted and filtered in a similar manner as above, but not containing the neuropeptide, prepared ahead of time and stored at −80°C until use.

[1] http://www.randomizer.org.

Administration of AVP or PL

Both experimenters and subjects were blind to the treatment subjects received. All solutions were administered intranasally. The AVP group self-administered 40 IU of AVP (Polypeptide Group, Limhamn, Sweden). This required five nasal puffs to administer 0.5 ml of solution. The PL group self-administered five nasal puffs of PL. Subjects were instructed to place the nasal applicator in one nostril and depress the lever until they felt a mist of spray in the nostril, to then breathe in deeply through the nose, and afterward to place the applicator in the other nostril and repeat the process.

Monitoring Vital Signs

To monitor for unintended side-effects of AVP administration, subjects' ear temperature, heart rate, and blood pressure were measured prior to drug administration and again approximately 20 min later.

Following intranasal administration of AVP, CSF concentrations begin rising within 10 min, continue to increase for up to 80 min, and remain above those of PL-treated subjects at 100–120 min after administration (17). Thompson et al. (23) tested subjects at 15 and 50 min after intranasal vasopressin administration. Accordingly, our goal was for subjects to be fully immersed in the task at 50 min post-drug administration. We, therefore, aimed to start both the task and fMRI scan at 30 min after drug administration. In actuality, this time period averaged 31.27 min (SD = 3.82) across subjects.

Task

Nine head-shot photographs were taken from each of three Caucasian male and three Caucasian female models, displaying neutral expressions. All photos were similar, but unique in terms of dress and lighting.

For scan 1, subjects viewed pictures of two male models, two female models, and one object (a coffee mug). Three pictures of each model and object were displayed per run and there were a total of three runs. We repeated presentations of the same models to increase familiarity with the stimuli during the test to, at least in part, mimic the repeated contact with particular individuals that would typically occur during a social interaction.

Thus, subjects viewed a total of 45 pictures. A single trial involved an 8 s presentation of the stimulus, a variable fixation interval (2, 4, or 6 s) in which subjects viewed a cross in the center of the screen, a 4 s interval during which subjects were asked to rate the stimulus on approachability on a scale from −3 (threatening and unapproachable) to 3 (friendly and approachable), a 0.5 s fixation interval, another 4 s interval during which subjects rated the stimulus on attractiveness on a scale from −3 (unattractive) to 3 (attractive), and finally another variable fixation interval (2, 4, or 6 s). Pictures were presented in pseudorandom order. Total task duration was approximately 21 min.

Scan 2 stimuli were the same as for scan 1, except that we included nine pictures from one additional male model and nine pictures from one additional female model to assess whether any persisting effects of AVP treatment were specific to faces that were seen previously.

E-prime software (Psychology Software Tools, Pittsburgh) was used for stimulus presentation. Stimuli were projected onto a screen that subjects could view through a mirror mounted on the head coil in the MRI scanner. Subject responses were recorded using a response box.

Neuroimaging Data Acquisition

Anatomical Image Acquisition

Subjects lay motionless in a supine position in the scanner with padded head restraint to minimize head movement during scanning. Each scanning session began with a 15 s scout, followed by a 5 min T1-weighted MPRAGE scan (TR = 2,600 ms, TE = 3.02 ms, matrix = 256 × 256, FOV = 256 mm, slice thickness = 1.00 mm, gap = 0 mm).

fMRI Image Acquisition

Functional scans used an EPI sequence with the following parameters: TR = 2,000 ms, TE = 28 ms, matrix = 64 × 64, FOV = 224 mm, slice thickness = 2.5 mm, 34 axial slices. TE was minimally decreased from the typical value (32 ms) in order to reduce magnetic susceptibility artifact in the orbitofrontal region. The duration of each EPI scan was about 7 min (15 pictures × 8 s per picture, plus 8 s for fixation, and 8 s to rate each picture on two different adjectives).

Analysis of Subjective Ratings

Two sample *t*-tests were used to test for between-subject effects of AVP treatment (vs. PL) on approachability and attractiveness ratings of same and other-sex faces at scan 1 to test for acute effects of the drug, and again at scan 2 to test for more prolonged effects, whether acute effects were present or not.

Analysis of Neuroimaging Data

The analysis was conducted with the Oxford Center for Functional Magnetic Resonance Imaging of the Brain's software library (FSL).[2]

The preprocessing pipeline of the fMRI data involves (1) motion correction using the MCFLIRT (40), (2) non-brain tissue removal using the BET (41), (3) slice timing correction, (4) high-pass temporal filtering with a cut-off of 200 s, (5) spatially smoothing with a Gaussian kernel of full-width at half maximum (FWHM) of 4 mm, and (6) normalizing to MNI space *via* corresponding extracted T1 brain using Boundary-Based-Registration (42).

For each subject, the preprocessed fMRI data were analyzed using a general linear model (GLM). Regressors were specified for male faces, female faces, and objects seen at both scans, and also for the novel male and female faces seen at scan 2. Each task regressor was convolved with a standardized model of the hemodynamic response function. Contrasts of beta values for male faces vs. objects and female faces vs. objects were generated for use in group analyses. The individual-level GLM was implemented using FMRIB's Improved Linear Model (FILM).

Given widespread evidence for sex differences in the AVP system (43, 44), analyses were conducted separately for males and females. For group analyses, a two sample *t*-test was used to compare the contrast (face-object) for same and other-sex faces between the AVP and PL groups at scan 1. Another two-sample *t*-test was used to compare the same contrasts between the AVP and PL groups at scan 2 (i.e., carryover effects). Whole brain exploratory analyses were thresholded using clusters determined by $z > 3.1$ (voxel-wise 1-tailed $p < 0.001$), and a family wise error (FWE)-corrected cluster significance threshold of $p < 0.05$ was applied to the suprathreshold clusters. Region of interest (ROI) analyses were also conducted within bilateral nucleus accumbens (NAc), amygdala, lateral septum, and hypothalamus. NAc and amygdala were defined using the Harvard-Oxford Subcortical Structural Atlas implemented in FSL[3] with 50% probability as a threshold. The lateral septum and hypothalamus were manually defined based on the coordinates and anatomy of these ROIs and surrounding brain structures, referring to the "Atlas of the Human Brain" (45). All ROIs were defined in the MNI space (Figure S1 in Supplementary Material). Results of ROI analyses were corrected for multiple comparisons at the voxel level ($p < 0.05$) using Gaussian Random Field Theory. In addition, we imposed a minimum spatial extent threshold of three voxels. Results for each contrast were also Bonferroni corrected for the number of ROIs investigated (8), such that significance required $p < 0.006$.

Neuroimaging data from one female subject in the placebo group was unusable due to technical problems. Subjects were compensated with a total of $50 on each of the two visits.

RESULTS

Participant Demographics in the AVP and PL Groups

There was no difference in age between participants randomized to AVP vs. PL for either men [AVP group: mean = 23.05, SD = 1.99; PL group: mean = 23.50, SD = 2.46; $t(38) = -0.64$, $p = 0.53$] or women [AVP group: mean = 23.40, SD = 1.98; PL group: mean = 23.45, SD = 2.31; $t(38) = -0.07$, $p = 0.94$]. The racial distribution across groups was follows: female AVP = 5 Caucasian, 7 African American, 7 Asian, 1 mixed race; female PL = 8 Caucasian, 5 African American, 6 Asian, 1 Hispanic; Male AVP = 5 Caucasian, 3 African American, 11 Asian, 1 Hispanic; Male PL = 10 Caucasian, 3 African American, 5 Asian, 1 Mixed, 1 not available/other.

Attractiveness and Approachability Ratings

Female Participants

Arginine vasopressin treatment increased female participant's attractiveness ratings of female faces compared with PL treatment at scan 1 [$t(38) = 2.51$, $p = 0.017$]. There was a trend for this effect to persist until scan 2 when no treatment was given, although this result was only marginal [$t(38) = 1.83$, $p = 0.075$]. In the PL group, the number of days between scan 1 and scan 2 was not correlated with scan 2 attractiveness ratings

[2] http://www.fmrib.ox.ac.uk/fsl/.

[3] https://fsl.fmrib.ox.ac.uk/fsl/fslwiki.

of familiar female faces ($r = -0.03$, $p = -0.91$). However, in the AVP group, there was a significant positive correlation ($r = 0.55$, $p = 0.01$) such that female faces were rated as more attractive with increasing scan interval. Thus, AVP effects appear to become more pronounced with longer scan interval (Figure S2 in Supplementary Material). To determine if these marginal carryover effects generalized to the novel faces seen at scan 2, we also compared attractiveness ratings for novel female faces between the AVP and PL groups and found no significant difference [$t(38) = 1.46$, $p = 0.15$]. However, the effect of AVP did not significantly differ for familiar and novel faces [$F(1, 38) = 0.05$, $p = 0.83$] (**Figure 1**). Approachability ratings for female faces did not differ between the AVP and PL group on either scan 1 or scan 2. Nor was there any significant effect of AVP treatment on either attractiveness or approachability ratings of male faces, at either scan 1 or scan 2. Finally, there was no significant effect of AVP treatment on either attractiveness or approachability ratings of objects, at either scan 1 or scan 2 (Table S1 in Supplementary Material).

Male Participants

Although AVP did not increase attractiveness ratings of male faces at scan 1, it did significantly increased attractiveness ratings of male faces at scan 2 [$t(38) = 2.28$, $p = 0.03$]. In the PL group, the number of days between scan 1 and scan 2 was negatively correlated with scan 2 attractiveness ratings of familiar male faces ($r = -0.51$, $p = 0.02$). That is, men rated male faces they had seen previously as less attractive as the scan interval increased. On the other hand, in the AVP group, there was no correlation between scan interval and scan 2 attractiveness ratings of familiar male faces ($r = -0.15$, $p = 0.54$). AVP effects appear to become more pronounced with longer scan intervals (Figure S1 in Supplementary Material). To determine if these marginal carryover effects generalized to the novel faces seen at scan 2, we also compared attractiveness ratings for novel male faces between the AVP and PL groups and found no significant difference [$t(38) = 0.90$, $p = 0.38$]. However, the effect of AVP did not significantly differ for familiar and novel faces [$F(1, 38) = 1.14$, $p = 0.29$] (**Figure 2**). AVP had no significant effect on approachability ratings of male faces at either scan 1 or scan 2. There was also no effect of AVP treatment on either attractiveness or approachability ratings of female faces at either scan 1 or scan 2. Finally, there was no significant effect of AVP treatment on either attractiveness or approachability ratings of objects, at either scan 1 or scan 2 (Table S1 in Supplementary Material).

Neuroimaging Data

Whole Brain Analyses

In whole brain analyses, there was no effect of AVP treatment vs. PL treatment on the BOLD response to either same or other-sex faces in either men or women, at either scan 1 or scan 2.

ROI Analyses

Female Participants

For women viewing female faces, there was no effect of AVP treatment at scan 1. On scan 2, AVP increased the response to female faces in the left hypothalamus (4 voxels, peak activation at MNI coordinate = −2, −4, −16; peak $z = 2.68$, $p = 0.004$) (**Figure 3**).

For women viewing male faces, AVP treatment decreased the left hypothalamus response at scan 1 (6 voxels, peak activation at MNI coordinate = −4, −4, −8; peak $z = 2.65$, $p = 0.004$) (**Figure 4**). There was no effect of AVP treatment on scan 2.

Male Participants

For men viewing female faces, AVP treatment on scan 1 increased the right NAc response compared with PL (3 voxels,

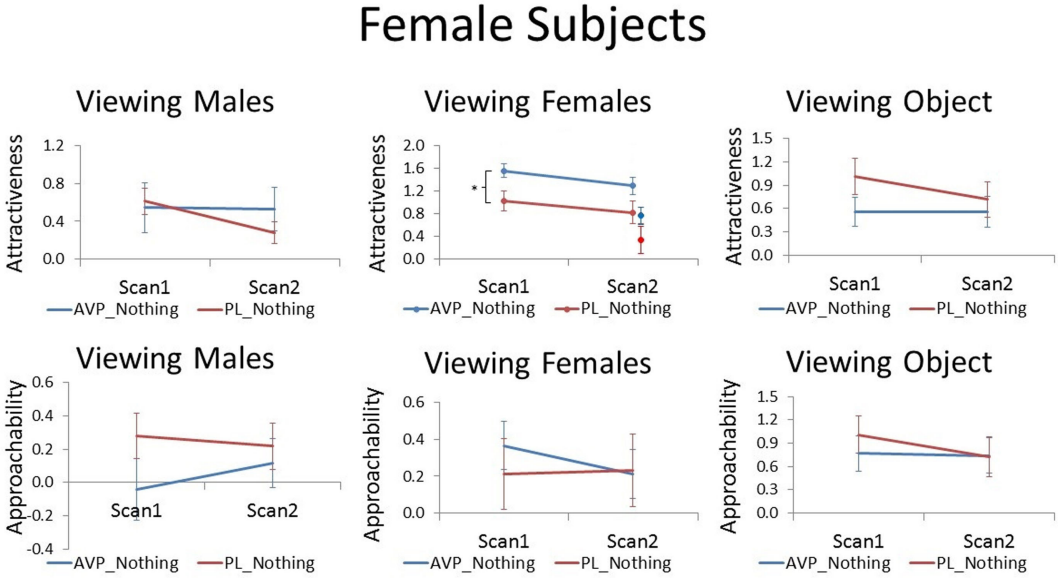

FIGURE 1 | Subjective ratings of same-sex faces, other-sex faces, and objects in women, as a function of arginine vasopressin (AVP) vs. PL treatment and first vs. second scan. For attractiveness ratings of female faces, data for novel faces at scan 2 are plotted to the right of familiar faces. Error bars = ± 1 SE. *$p < 0.05$ for AVP vs. PL at scan 1.

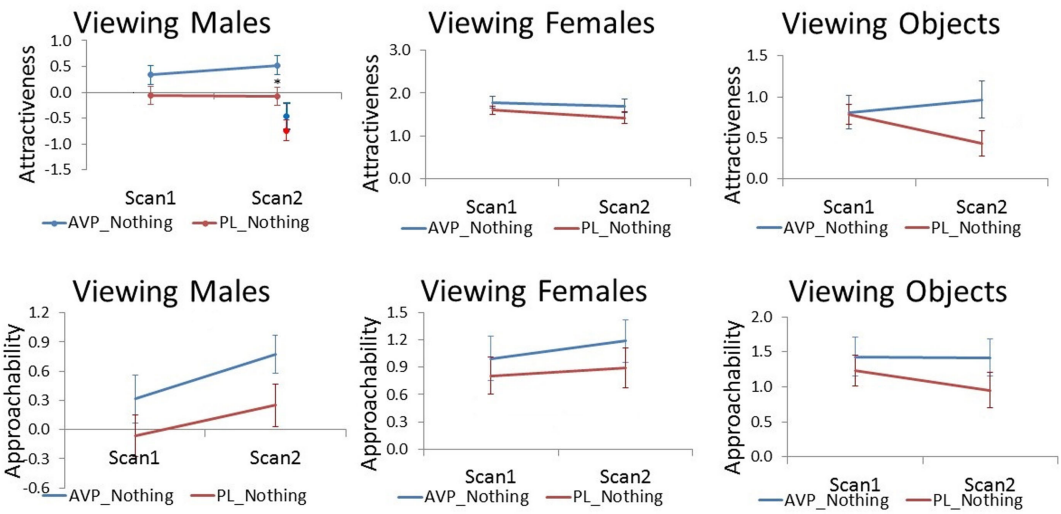

FIGURE 2 | Subjective ratings of same-sex faces, other-sex faces, and objects in men, as a function of arginine vasopressin (AVP) vs. PL treatment and first vs. second scan. Error bars = ± 1 SE. *$p < 0.05$ for AVP vs. PL at scan 2.

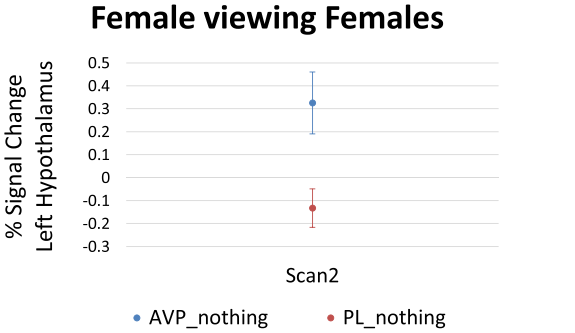

FIGURE 3 | Arginine vasopressin (AVP) effects on lateral hypothalamic activation in females viewing female faces at scan 2. Error bars = ± 1 SE.

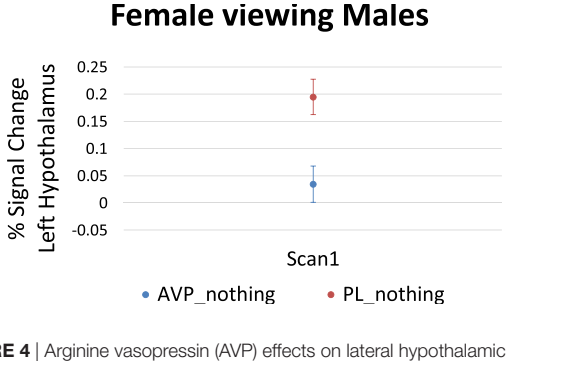

FIGURE 4 | Arginine vasopressin (AVP) effects on lateral hypothalamic activation in females viewing male faces at scan 1. Error bars = ± 1 SE.

peak activation at MNI coordinate = 6, 14, −8; peak $z = 2.94$, $p = 0.002$) (**Figure 5A**). However, the opposite effect was observed at scan 2, when AVP decreased both the right (23 voxels, peak activation at MNI coordinate = 8, 10, −6; peak $z = -2.74$, $p = 0.003$) (**Figure 5A**) and left NAc response to female faces (4 voxels, peak activation at MNI coordinate = −6, 2, −8; peak $z = -2.88$, $p = 0.002$). In addition to these effects within NAc, AVP treatment at scan 1 also increased the bilateral lateral septum response compared with PL (left septum = 6 voxels, peak activation at MNI coordinate = −2, −2, 12; peak $z = 2.67$, $p = 0.004$) (right septum = 3 voxels, peak activation at MNI coordinate = 4, −2, 14; peak $z = 2.58$, $p = 0.005$) (**Figure 5B**). AVP also increased the left, but not right, lateral septum response to female faces at scan 2 (3 voxels, peak activation at MNI coordinate = −2, 2, 10; peak $z = 2.59$, $p = 0.005$) (**Figure 5B**). While AVP had no effect on activation in the hypothalamus at scan 1, AVP decreased the left hypothalamus response to female faces at scan 2 (3 voxels, peak activation at MNI coordinate = −4, 2, −8; peak $z = 2.78$, $p = 0.003$) (**Figure 5C**). Finally, AVP had no effect on activation in the amygdala at either scan 1 or scan 2. While we could not accurately define a ventral pallidum ROI given its very small size and the lack of anatomical landmarks to guide its definition in the anatomical MRI, inspection of the uncorrected ($p < 0.05$) whole brain results showed a lack of AVP-related activation in the vicinity of the ventral pallidum.

For men viewing male faces, there was no effect of AVP treatment at either scan 1 or scan2.

DISCUSSION

Here, we show that administration of 40 IU intranasal AVP approximately 30 min before viewing faces influences both neural responses to and subjective ratings of those faces. In women, AVP treatment on the first scan day increased attractiveness ratings of female faces, and there was a trend for this effect to persist until scan 2 when no treatment was administered. AVP also increased

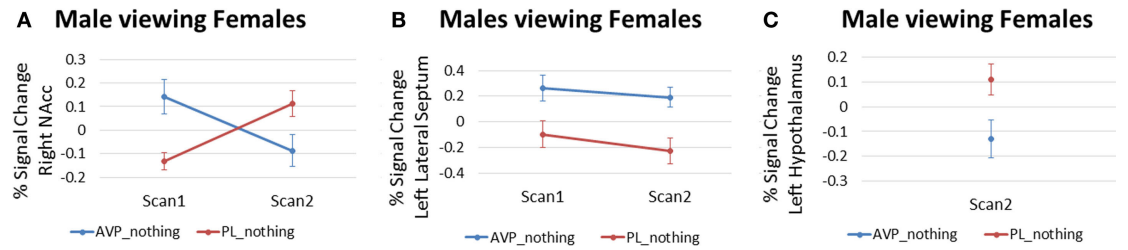

FIGURE 5 | Arginine vasopressin (AVP) effects in males viewing female faces for **(A)** right nucleus accumbens, **(B)** left lateral septum, and **(C)** left hypothalamus. Error bars = ± 1 SE.

the left hypothalamus response to female faces, but only on scan 2. Additionally, AVP decreased the left hypothalamus response to male faces on scan 1 only. In men, despite no effect on subjective ratings at scan 1, AVP increased attractiveness ratings of male faces at scan 2. AVP also increased the right NAc and bilateral lateral septum responses to female faces on scan 1. These effects persisted to scan 2 for the left lateral septum, but reversed for right NAc. Finally, AVP decreased activation to female faces within the left hypothalamus, but only for scan 2. Although these patterns are complex, three important points emerge. First, intranasal AVP can promote positive responses to same-sex faces in women and men, though on different time scales. Second, there is not always congruence between behavioral effects and influences in brain areas with functions presumably related to the behavioral responses; AVP increased attractiveness ratings in women on scan 1, but not activation in NAc, in which activity is correlated with positive assessments (46). Conversely, in men, AVP selectively increased activation in response to female faces in several areas, but did not affect behavioral responses. These patterns suggest either that some behavioral outputs may depend more on complex network or emergent processes than we currently capture, that areas in which activity was influenced are unrelated to the behaviors measured, and/or that our statistical methods are too conservative to capture such associations. Third, a single dose of intranasal AVP has the potential to induce long-lasting effects on behavioral and neural responses.

In a previous study, 20 IU intranasal AVP increased approachability ratings of women viewing female faces with neutral expressions (23). While we did not strictly replicate this effect, AVP-treated women did rate female faces as more attractive compared with PL-treated women. We believe attractiveness ratings in women are a generalized assessment of same-sex individuals and not a specific measure of sexual or romantic interest because heterosexual women in this and in our parallel studies rated the faces of other women as more attractive than the faces of men. Together, the two studies indicate that AVP selectively promotes positive assessments of the faces of other women in women. We did not measure the menstrual cycle phase of women in this study, and so cannot say whether AVP effects in women are moderated by cyclic hormones. In contrast to the effects of lower doses, which decreased social assessment of other men in our previous study, the current findings indicate that higher doses can, over time (see further discussion below), increase positive assessments of other men. Thus, different doses may produce divergent behavioral responses in men, a possibility also supported by dose differences found in our parallel study (under review, this issue). That AVP selectively enhanced assessments of same-sex faces in men and women is consistent with AVP/AVT's effects in other species being dependent on the sex of stimulus (35, 36).

Arginine vasopressin's ability to acutely increase assessments of other women in women on scan 1 or of other men in men on scan 2 was not associated with detectable alterations of brain activity, suggesting that those behavioral effects depend on modulation in areas outside the ROIs we examined, or on complex alterations in patterned activity that we did not measure. We had expected that AVP treatments that increase positive social assessments would also increase the ventral striatum response, which has been associated with increased positive ratings of other individuals (46). However, this was not the case. While these results are unexpected, NAc need not be tracking positive ratings in relation to the rewarding aspect of the faces. Indeed, if increased ratings of other female faces are part of a "tend and befriend" stress responses strategy, particularly in women (47), then AVP's behavioral influences may not be directly related to reward and/or positive affect, but rather to stress/anxiety reduction. On the other hand, there was a parallel effect on behavioral and neural responses on scan 2 in women; AVP delivered prior to scan 1 marginally increased attractiveness ratings on scan 2 and increased responses to female faces in the lateral hypothalamus. AVP enhances aggression *via* actions in the hypothalamus in male hamsters but decreases aggression in female hamsters (48), though it is not yet known if AVP's ability to inhibit aggression in females depends on activation or inhibition of hypothalamic activity. We did not measure responses directly associated with aggression, though increased social assessments of other women might be associated with decreased aggressive responses toward them. However, it is unclear why associations between increased hypothalamic activation on scan 2 would be related to increased attractiveness ratings in the absence of similar linkages on scan 1, or of linkages between the decreased activation in hypothalamic and behavioral responses to male faces on scan 1, unless AVP's acute and lasting influences on behavioral and brain responses are associated with different mechanisms (see further discussion below). It is, therefore, also possible that influences on hypothalamic responses are completely unrelated to social assessments. Perhaps most important is simply that AVP did influence hypothalamic responses to faces, as AVP influences on hypothalamic responses to social stimuli, which mediate a variety of social

behaviors in other vertebrates, have not previously been reported in humans.

In previously unpaired male prairie voles, AVP elevations in the ventral striatum that are concurrent with social contact with a novel female facilitate affiliative responses toward that individual. AVP appears to link the reward from mating with the identity of a particular female, resulting in a preference to associate with that female over others (49). Despite the independent evolution of pair bonding in prairie voles and humans and associated differences in life histories associated with pairing, males in both species are capable of forming such bonds in reproductive contexts, and AVP has been indirectly implicated in that process in human males (37). Although the design of this experiment was not identical to those used to test AVP's role in pair bonding in prairie voles, in part due to the limitations associated with manipulating human subjects, we did presumably elevate AVP in single men while they were exposed to novel female faces. We recognized that the induction of social preferences related to pair bond formation in prairie voles requires continuous AVP administration during sustained social contact with a novel female (4, 36), so we did not expect our more limited manipulation to induce selective social attachments to the briefly presented faces. Nonetheless, we predicted that intranasal AVP might, particularly in light of the sustained elevations of the peptide in the brain that follow intranasal delivery (17) (discussed further below), enhance ratings indicative of increased attractiveness toward or tendencies to interact/affiliate with novel females while decreasing similar assessments of other males in relation to potential mate competition/guarding functions. We also predicted elevations of AVP during those limited "interactions" might increase activity in areas of the brain in which AVP promotes affiliative responses related to pair bonding with females and aggression toward males in voles if convergent peptide mechanisms associated with the promotion of emotional attachments in reproductive contexts evolved in the two species. These predictions were only partially supported. Although AVP did specifically augment bilateral responses in the lateral septum and ventral striatum to female faces, the responses in the ventral striatum were in the NAc, not the ventral pallidum, where AVP promotes affiliative responses relating to pairing in male prairie voles, and it did not augment ratings of female faces in this or our parallel study, in which two doses of AVP were administered, and in which men were only exposed to a single female face, but had more exposure to that face. Nor did the dose used in this study decrease responses to other males, but rather enhanced them over time. It is possible that our rating responses do not reflect responses related to affiliative and aggressive processes associated with pair-bonding/mate-guarding, or that our tests did not stimulate sufficient, concurrent dopamine release, which is also involved in stimulating partner preferences in male prairie voles (49). Indeed, the female faces were not even smiling and thus unlikely to have represented a potential romantic interest/partner. Of course, we also must acknowledge the possibility that AVP does not, as it does in male prairie voles, facilitate affiliative processes related to pair bonding in human males, and if it does, that it does so through different neural mechanisms, i.e., through activations in the lateral septum and NAc, rather than the ventral pallidum, and perhaps also through long-term downregulation of lateral hypothalamic responses to familiar females, which were lower only on scan 2. Indeed, comparative studies have suggested that common AVT/AVP mechanisms do not underlie pair bond formation in reproductive contexts in species in which such tendencies have independently evolved (50–52).

The reversal of AVP effects on male NAc activation to female faces at scan 2 was unexpected. However, this result may be consistent with another fMRI study showing that intranasal AVP effects differ for familiar and unfamiliar faces (27), as the same female faces were used on scan 1 and scan 2 in our study. **Figure 5B** shows an increase in NAc activation from scan 1 to scan 2 in the PL group. This might reflect increased reward from the female face over time as familiarity is established. It is possible that AVP accelerates this process so that NAc activity is already augmented at scan 1 in men who received AVP. The subsequent decrease in NAc activation from scan 1 to scan 2 could reflect a social habituation effect of AVP as has been reported previously for the closely related nonapeptide OT (53).

Arginine vasopressin modulation of lateral septum and NAc activation was specific to men, and AVP had opposing effects on hypothalamic activation in men and women viewing female faces. These results are consistent with accumulating evidence for sex differences in AVP effects in both humans and other animals (43, 44). In rats, for example, treatment with a V1aR antagonist in the lateral septum significantly increased social play in males while decreasing social play in females (54). In hamsters, hypothalamic AVP injection stimulated aggression in males, while inhibiting aggression in females (48). In humans, intranasal AVP augmented bilateral insula activation to positive social interactions in men, while having the opposite effect in women (26). Finally, with a face viewing paradigm similar to that used here, we previously showed that lower doses of AVP induced agonistic facial motor patterns and decreased perception of friendliness to same-sex faces in men, while inducing affiliative facial motor patterns and increased perceptions of friendliness to same-sex faces in women (23). These sex differences in AVP effects are accompanied by and may be attributable to widespread sex differences in V1aR distribution (44). Describing sex differences in human V1aR is an important task for future research.

No effects were observed in either whole brain analyses or in ROI analyses focused on the amygdala. The amygdala plays a critical role in threat detection (55–57), and AVP has been shown to modulate amygdala response in various contexts in both humans and non-humans (25, 58–60). Given that 20 IU intranasal AVP was previously shown to stimulate agonistic facial motor patterns and to increase skin conductance responses to same-sex faces in men (23), we expected AVP to increase amygdala activation to same-sex faces in men; however, this was not observed. The current study used 40 IU intranasal AVP. It is possible that the hypothesized effects on amygdala activation in face processing contexts would emerge at the 20 IU dose, which our parallel study found yields a more negative response than the higher dose.

Arginine vasopressin did not increase the subgenual ACC response to faces as reported previously (28). Although the study by Zink et al. and our study employed the same dose of AVP (40 IU), our study included only neutral rather than emotional faces, perhaps requiring less emotion regulation. In fact, work

in other vertebrates has shown that testing contexts do influence the types of effects the peptide has (61). This seems to be true also in humans. A previous neuroimaging study showed that intranasal AVP effects on face-processing activity differed as a function of the familiarity of the face stimuli. Specifically, AVP effects were found for unfamiliar, but not familiar, faces (27). Our stimuli consisted of nine photographs of two male and two female models (for scan 1). Thus, subjects were presented with highly similar stimuli with which they were quickly familiarized, because we wanted to mimic the increased familiarity that would typically occur during an ongoing social interaction. However, it is possible that the familiarity of our stimuli may have dampened some AVP effects. Finally, we began scanning at 32 min post-AVP administration as compared with 56 min for Zink et al. These variables might help to explain the discrepant results.

There was evidence that some AVP effects persisted until the second scan day when no treatment was given. Attractiveness ratings of other female faces remained marginally higher in women given AVP on the first day than in women given PL on the first day, and responses to female faces in the left lateral septum in men were similarly increased by AVP on both scans. Additionally, some effects only appeared on scan 2, including increased attractiveness ratings of other men in men and decreased activation in the lateral hypothalamus in response to female faces, or reversed across days, most notably the increased activation in the NAc in response to female faces in men on scan 1, but decreased activation on scan 2. Thus, AVP appears to have effects on face processing that likely persist beyond the presence of drug in the system, as AVP's half-life in brains is less than 1 min, though the rate decreases over time (62) and its peptide fragment's, which can have behavioral effects, is 6.5 h in tissue (63). The exogenous drug should have thus been cleared by the second scan. To some extent, our predication that AVP would produce selective, long-lasting effects on responses to previously viewed faces when AVP levels were elevated was supported in the behavioral data, in that responses to novel faces on scan two were not elevated. AVP can enhance social recognition memory in rodents (64, 65), in some cases, through the activation of OT receptors (66, 67), and intranasal AVP has been shown to enhance the encoding of happy and angry faces in men (21). AVP influences on the encoding of the faces on the first trial could, therefore, have altered their perception on the second trial, though it is unclear how acute influences on face processing may produce prolonged influences on subjective responses of the faces or on neural responses to those faces. Importantly, behavioral effects did not appear to lessen as a function of time since AVP administration, which suggests they could be long lasting. This is consistent with lasting effects associated with different doses of AVP in our parallel study (under review; this issue).

The mechanisms through which AVP may induce lasting or delayed effects on subjective responses to faces are unclear. AVP can enhance social recognition, but most such studies involve AVP administration after interactions with an individual, and the effects, to our knowledge, have not been observed more than 24 h after administration (68). If acute VP increased how familiar the faces seemed, it could have, to the extent that increased familiarity increases subjective ratings, led to sustained enhancements in those responses, potentially through synaptic remodeling, which AVP can promote (69–71). Although it seems unlikely that a single administration of AVP would be sufficient to induce such alterations, it should be kept in mind that, despite AVP's short half-life in tissue, the elevations observed in the brain after intranasal delivery were still apparent in the original studies by Born et al. (17), with no signs of decreasing, 80 min after delivery, suggesting that intranasal AVP might, perhaps through feed-forward mechanism (72), trigger large and sustained elevations of AVP within the brain that could induce such changes.

We cannot draw any conclusions about the types of receptors that mediate these effects. It is possible that higher doses of AVP produce positive social assessments in women through the activation of related oxytocin receptors, though it should be noted that the positive effects in women, at least, are consistent with those observed for lower doses that also increased anxiety, which is not consistent with oxytocin receptor activation (23). Even if the effects observed here are, at least in part, the results of receptor cross talk, this would not negate the clinical relevance of these peptides when considering their use as therapeutics nor the potential importance of such mechanisms for normative social functioning. Some of the AVP's behavioral effects depend on activation of the OT receptor (66, 67), and in some cases, AVP's effects may depend on the simultaneous activation of both AVP and OT receptors (73). Clearly, we need to learn more about the local concentrations of peptides released within local circuits in different social contexts relative to the amounts that reach those areas through intranasal delivery. It will also be interesting to determine if different patterns of receptor activation, perhaps as a function of the amounts of peptide released endogenously or the dose applied exogenously, may produce different behavioral outcomes. Given evidence that OT and AVP receptors can heterodimerize (74), it is even possible some behavioral effects depend on interactions with complex combinations of membrane receptor proteins.

In summary, we show that treatment with 40 IU intranasal AVP increases positive ratings (attractiveness) of same-sex faces in women and that these effects may persist for several days. AVP also increased attractiveness ratings of male faces in men at scan 2 only. fMRI data show that AVP provoked acute increases in right NAc and bilateral lateral septum responses to female faces among men, with the left lateral septum response persisting until scan 2 while the right NAC response reversed at scan 2. AVP also modulated the left hypothalamus response to faces in both men and women, in some cases only on scan2. Work is ongoing to determine if AVP effects within these data sets are modulated by individual variables such as genotype, personality, or attachment style as previously reported for both vasopressin and the closely related oxytocin (75–78).

ETHICS STATEMENT

All subjects gave written informed consent, and the study was approved by the Emory University Institutional Review Board and the U.S. Food and Drug Administration.

AUTHOR CONTRIBUTIONS

JR designed the study, supervised analysis, interpreted data, and wrote the paper. TL analyzed data and edited the paper. XC analyzed data and edited the paper. PG collected and analyzed data and edited the paper. EH served as study physician and edited the paper. RT designed the study, interpreted data, and wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fendo.2017.00200/full#supplementary-material.

FIGURE S1 | Anatomically defined regions of interest in **(A)** nucleus accumbens, **(B)** amygdala, **(C)** hypothalamus, and **(D)** lateral septum.

FIGURE S2 | Scan 2 attractiveness ratings in the arginine vasopressin (AVP) and placebo (PL) groups as a function of scan interval for female (left) and male (right) participants.

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REVIEW
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Endocrine Disruption of Vasopressin Systems and Related Behaviors

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Endocrine disrupting chemicals (EDCs) are chemicals that interfere with the organizational or activational effects of hormones. Although the vast majority of the EDC literature focuses on steroid hormone signaling related impacts, growing evidence from a myriad of species reveals that the nonapeptide hormones vasopressin (AVP) and oxytocin (OT) may also be EDC targets. EDCs shown to alter pathways and behaviors coordinated by AVP and/or OT include the plastics component bisphenol A (BPA), the soy phytoestrogen genistein (GEN), and various flame retardants. Many effects are sex specific and likely involve action at nuclear estrogen receptors. Effects include the elimination or reversal of well-characterized sexually dimorphic aspects of the AVP system, including innervation of the lateral septum and other brain regions critical for social and other non-reproductive behaviors. Disruption of magnocellular AVP function has also been reported in rats, suggesting possible effects on hemodynamics and cardiovascular function.

Keywords: bisphenol, oxytocin, sex differences, EDC, genistein, soy, social, anxiety, estrogens

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Endocrine disrupting chemicals (EDCs) have garnered considerable attention over the past few decades, partly because of their omnipresence, but also because of rapidly compounding evidence that exposure, particularly during critical windows of development, is likely contributing to increasing incidence of multiple chronic diseases. Because, historically, EDC research has focused on steroid hormone disruption, especially estrogen and androgen disruption, EDCs are most often thought of in the context of reproductive disorders including infertility, genital malformations, accelerated puberty, and reproductive cancer. But the concept of endocrine disruption is far more broadly inclusive of other hormones and their targets, including the neuropeptide hormones vasopressin (AVP) and oxytocin (OT).

Broadly, AVP and OT are found only in mammals, are structurally similar, and evolutionarily derived from the pituitary hormone, vasotocin (VT). Magnocellular AVP and OT are axonally released into the periphery from the paraventricular and supraoptic nuclei (PVN and SON) *via* the neurohypophysis and coordinate a range of physiological processes, including uterine contractions, milk letdown, blood pressure, thermoregulation, and osmotic balance. The parvocellular system sends projections to the median eminence and throughout the brain and is sexually dimorphic, steroid hormone sensitive, and fundamental for the coordination of affiliative and social behaviors including courtship, pair bonding, empathy, reciprocity, trust, and context-specific aggression (1–4). In addition, populations of AVP/OT-releasing neurons have been identified in other areas of the hypothalamus and in extrahypothalamic structures such as the medial amygdala (5). OT binds to the OT receptor (OTR), and AVP binds to one of two AVP receptor (AVPR) subtypes: AVPR1A or AVPR1B, the central distribution of which can differ substantially by sex, age, and species (5). Differences in the region-specific distribution of AVPRs and/or OTRs have been linked to individual and species variation in prosocial phenotypes including social attachment, parental behavior, and

social anxiety, as have genetic polymorphisms in AVP, OT, and/or their receptors. The anatomical and functional features of AVP/OT neuronal subpopulations are beyond the scope of this review and detailed elsewhere (6, 7).

Centrally, the actions of OT and AVP largely overlap, albeit with some sexual dimorphisms, and these redundancies are not surprising given their relatively recent evolutionary divergence (summarized in **Table 1**). They can also have independent and even opposing effects. For example OT, but not AVP, appears to be critical for the extinction of social fear and promoting social interaction *via* enhancement of social preference suppression and social anxiety (8). Emerging evidence also suggests OT may suppress food intake and increase energy expenditure (9). Critically, opposing effects of AVP and OT refine the control of emotional behavior, and dysregulation of either can result in psychopathology. In general, central OT is anxiolytic, antidepressive, and prosocial, whereas AVP is anxiogenic and can heighten depressive-type behaviors (8). Consequently, there is concern that endocrine disruption of central AVP and/or OT function could adversely impact emotional control, and possibly heighten risk of psychosocial disorders. AVP and OT systems are also anatomically and functionally linked with catecholaminergic systems (10) and the mesolimbic dopamine system (1). That AVP and OT are fundamental to such a wide range of functions and behaviors indicates that disruption by EDCs could have profound and multifaceted effects throughout the neuroendocrine system. This review presents what is currently know about EDCs, AVP/OT systems, and the behaviors and physiological functions of these neuropeptides coordinate.

EVIDENCE FOR ENDOCRINE DISRUPTION OF SOCIAL TRAITS AND CHEMICAL CONTRIBUTIONS TO PSYCHOSOCIAL SOCIAL DISORDERS

Exploration and understanding of how EDCs may alter the organization and function of neuroendocrine systems outside of the hypothalamic–pituitary–gonadal (HPG) and thyroid axes is underdeveloped, particularly in mammals. This is perhaps surprising given the sensitivity of neuropeptide systems to steroid hormones, their sexually dimorphic properties, and their diverse functional roles in the brain and peripheral organs. Growing recognition that environmental factors are likely contributing to rapidly rising rates of psychosocial disorders in which neuropeptides are thought to play a central role, including autism spectrum disorders (ASDs), has ignited greater interest in understanding how EDCs might impact targets outside of the reproductive axis. For example, while disorders of the social brain clearly have a heritable component, there is growing consensus that genetics are not fully explanatory and may possibly only account for maximally half of risk. Genetic factors contribute only an estimated 30–40% of ASD heritability, with most of that attributable to common genetic variants (11). Thus, in the vast majority of instances, ASD and other disorders of the social brain undeniably result from a complex confluence of sex specific gene vulnerabilities layered with adverse, and critically timed, environmental interactions including chemical exposures. The challenge is figuring out which chemicals, and how a "perfect storm" of genetic predispositions and environmental insults manifests as clinical disease. Elucidating the specific mechanisms by which OT and AVP signaling pathways could be vulnerable to EDCs is considered critical to identifying and understanding possible linkages between chemical exposures and psychosocial disease risk.

TABLE 1 | Functions attributed to AVP/OT and thus possibly vulnerable to EDCs.

Oxytocin (OT)	Vasopressin (AVP)
Neurohypophysial actions	
↑ Parasympathetic autonomic functions	↑ Sympathetic and parasympathetic regulation
Milk letdown	↑ Vasoconstriction
Uterine contractions at parturition	↑ Blood pressure
Central actions	
↓ Aggression (♀ > ♂)	↑ Aggression and territorial behaviors
↓ Anxiety; ↑ relaxation, well-being, and trust	↑ Anxiety
↑ Initiation of social contact	↑ Attraction and partner selection
↑ Pair and social bonding	↑ Pair and social bonding (♂ > ♀)
↑ Partner preference formation (♀ > ♂)	↑ Partner preference (♂ > ♀)

There are likely upwards of 90,000 chemicals in our environment today, although a full accounting has proven to be nearly impossible, even for regulators such as the US Environmental Protection Agency (EPA) who are supposedly monitoring their potential toxicity, distribution, and use (http://cen.acs.org/articles/95/i9/chemicals-use-today.html). A subset of these is categorized as endocrine disrupting chemicals (EDCs) because of their potential to perturb endocrine systems. A consensus definition regarding what constitutes an EDC has proved elusive, and various definitions have been published each with similar but deliberately different wording (12). The precise language used for each is largely reflective of how the definition is applied and for what functional purpose it serves, particularly in a regulatory decision context. Because it was developed specifically for scientific purposes, this review will use the Endocrine Society definition, which states that an EDC is: "an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action" (13, 14). Thus, "exogenous substance" could be anthropogenic or naturally occurring [e.g., soy phytoestrogens (15)], and disruption of the organizational effects of hormones is considered most likely to result in permanent effects.

In their landmark 2006 review and call for action, environmental health scientists Philip Landrigan and Philippe Grandjean argued that industrial chemicals are undeniably contributing to the rapidly rising incidence of neurodevelopmental disorders, including ASD and attention deficit hyperactivity disorder (ADHD) (16). Although many of the chemicals identified as the most dangerous (including lead, methylmercury, and arsenic) have little to no endocrine disrupting activity, others are well known EDCs including the polychlorinated biphenols (PCBs) and many pesticides. In the subsequent decade, Landrigan and Grandjean, and numerous additional researchers have echoed and enhanced this call for greater investigation of the

non-reproductive outcomes of EDC exposure, and emphasized the pressing need to understand how EDCs might contribute to impairments in reciprocal social engagements, repetitive/stereotypic behaviors, and other hallmark features of psychosocial and behavioral disorders (13, 17–21).

While it is not difficult to find broad speculation in the scientific and general literature that chemical exposures are contributing to rapidly rising rates of ASDs and ADHD, this literature is unfortunately peppered with falsely alarmist and unsubstantiated claims backed by weak or inconclusive data. Because many of these hyper-exaggerated linkages are frequently propagated by the media, there is confusion and even distrust regarding the risks EDCs pose to human health. *Direct evidence* linking any specific EDC to a clearly defined clinical disorder involving the social brain is sparse, and no single chemical has yet been definitively implicated (18, 21–25). For example, elevated prenatal androgens have been strongly associated with ASD risk for more than a decade (26–35) leading some to hypothesize that EDCs that alter androgen action may be contributory. That is clearly, however, and at the very least, only part of the story as supporting evidence in any experimental model system is extremely limited (22).

Thus, so little is known about the mechanisms by which the social brain is vulnerable to EDCs and other chemical exposures (20, 22, 36), particularly in humans, has proven to be a formidable obstacle when trying to convince regulators and other policy makers to enact actions which reduce EDC exposure. In addition, this mechanistic information gap is a significant barrier to efficiently and proactively screening chemicals for neurodevelopmental effects, or mitigating exposures that may be contributing to psychosocial disorders. Thus, gaining clearer understanding regarding the neural underpinnings of these possible linkages is of seminal importance.

Within the EDC field, and toxicology in general, compelling phenotypes drive subsequent mechanistic inquiry. Historically, the focus of EDC research has been on the HPG axis because the first and most profound exposure-related outcomes identified were reproductive. Effects included thinning eggshells in birds, altered sex ratios in turtle clutches, distorted courtship behavior in multiple avian species, abnormal gonadal and genital morphology in alligators, and numerous instances of intersex amphibians and fish (37–39). These and other worrisome and clearly adverse reproductive phenotypes drove a multidisciplinary quest to identify the endocrine disrupting mechanisms by which such outcomes arise. Invariably, disruption of steroid hormone action was found to be causal, particularly during critical developmental windows (39–41). Similarly, as the field has matured and expanded, heightened concern over rising rates of psychosocial disorders is now driving growing interest in the impact of EDCs on non-reproductive brain regions and the hormones that coordinate social behavior, including neuropeptides.

Although no animal model can fully capture the sophisticated complexity of human social behavior, the neuroendocrine pathways coordinating numerous social traits are highly conserved (7, 42), including the coordinating roles of AVP and OT. Behaviors such as play, maternal care, aggressive or competitive acts, reciprocal grooming, investigation of novel conspecifics, pair bonding, and social recognition are frequently modeled in animals to explore the neural underpinnings of human social behaviors and, by extension, how they might be susceptible to chemical exposures (39, 43–49). Because AVP/OT and the dopaminergic pathways they feed into are heavily influenced by sex steroids across the lifespan (50–59), it is highly plausible that their sexually dimorphic ontogeny and function may be particularly vulnerable to endocrine disruption. While sparse compared to available data on reproductive endpoints, evidence from a diverse range of species has revealed that targets critical to sociality and social cognition may indeed be vulnerable to environmental exposures, including the AVP/OT system (see **Table 2** for a summary).

EARLY EVIDENCE FOR ENDOCRINE DISRUPTION OF AVP AND OT PATHWAYS

That the environment, including chemical exposures, impacts the neurohypophyseal nonapeptides was first and most comprehensively described in birds (79), particularly the Japanese quail (*Coturnix japonica*). Seminal research dating back decades has consistently and repeatedly shown that multiple aspects of the quail VT system is vulnerable to EDCs including bisphenol A (BPA), diethylstilbestrol (DES), and high doses of the phytoestrogen genistein (GEN) (69, 70). In mammals and birds, the number of AVP (VT in birds) neurons and projections in the bed nucleus of the stria terminalis (BnST) and amygdala are markedly greater in males. This is one of the most consistently observed neural sex differences across taxa, and these projections are well known to coordinate sexually dimorphic social and reproductive behaviors (80). A series of studies led by Giancarlo Panzica has elegantly demonstrated the profound sensitivity of this system to embryonic manipulation by estrogens or estrogenic EDCs. Exogenous administration of estrogen, diethylstilbestrol (DES), or an aromatase inhibitor during incubation fully blocked male copulatory behavior at puberty and induced the complete sex reversal of VT-ir in the preoptic area, BnST, and lateral septum (LS) (70, 81). Reduced male copulatory behavior was also observed following embryonic exposure to high doses of the phytoestrogen genistein (GEN) (69), which is one of the most potent EDCs on nuclear estrogen receptors (ERs), particularly ERβ (82, 83). Demasculinization of VT-ir was induced by embryonic GEN, and *p,p′*-DDE [a long-lived metabolite of dichlorodiphenyltrichloroethane (DDT)] (69). Other EDCs also shown to suppress male copulatory behavior following embryonic exposure, purportedly *via* interference with estrogen and androgen pathways, include atrazine (herbicide), methoxychlor (pesticide), and vinclozolin (fungicide) (79).

Another relatively early example of endocrine disruption in the OT/AVP system used a vole model (*Microtus*) (75). Within this genus, some species are spontaneously more prosocial (prairie and pine voles; *Microtus ochrogaster* and *Microtus pinetorum*, respectively) than their promiscuous relatives (montane and meadow voles; *Microtus montanus* and *Microtus pennsylvanicus*, respectively), and rats or mice. Social attachment is rare in mammals but a hallmark of human social interactions. Foundational

TABLE 2 | EDCs shown to impact AVP/oxytocin (OT) pathways and related behaviors.

Chemical	Category/use	Effects	Primary mode of action	Reference
Bisphenol A (BPA)	Stabilizer in hard plastics and epoxy resins	Altered AVP and OT neuron numbers and innervation of sexually dimorphic regions associated with social and aggressive behaviors in multiple species; anxiogenic in multiple species	Estrogen disruptor	(60–66)
Chlorpyrifos	Insecticide	Altered hypothalamic AVP and OT levels (mRNA and protein); sexually dimorphic impacts on social, exploratory, and anxiety-related behaviors	Acetylcholinesterase inhibitor	(67, 68)
Dichlorodiphenyltrichloroethane (DDT)	Pesticide (restricted in the USA but still in use globally)	Demasculinized vasotocin innervation in Japanese quail	Estrogen and androgen disruptor	(69)
Genistein (GEN)	Isoflavone phytoestrogen found in soy and other legumes	Altered AVP and OT neuron numbers and innervation of sexually dimorphic regions associated with social and aggressive behaviors in multiple species; anxiogenic in males of multiple species	Estrogen and thyroid hormone disruptor	(69–74)
Methoxychlor	Insecticide	Abrogated male copulatory behavior in Japanese quail; disrupted female affiliative behavior in female prairie voles	Estrogen disruptor	(75)
Polybrominated diphenyl ethers (PBDEs)	Fire retardants (currently being phased out of use but rapidly replaced with structurally similarly compounds)	Impaired AVP release from the SON in response to dehydration; disruption of nitric oxide release related to AVP function in rats	Thyroid hormone disruptor	(76–78)
Polychlorinated biphenols (PCBs)	Now banned organochlorides used in many industrial applications including in paints, hydraulic fluids, lubricants, adhesives, pesticide mixtures, and sealants	Impaired AVP release from the SON in response to dehydration in rats	Estrogen and thyroid hormone disruptor	(76, 77)
Vinclozolin	Fungicide	Suppressed male copulatory behavior in Japanese quail	Androgen disruptor	(79)

research in voles has uncovered the significance of neuropeptidergic regulation of social behaviors including paternal care, pair bonding, and maternal aggression (3, 45). In the socially monogamous pine vole, perinatal oral exposure to approximately 2,000 μg/kg bw methoxychlor produced some offspring effects, but only in females (75). A non-significant trend toward increased time spent alone in the partner preference test and less aggression toward a strange male was interpreted to indicate a reduced preference for the mate and a disruption in affiliative behavior. OTR binding was unchanged in LS but reduced in the cingulate of the exposed females compared to unexposed controls. This region is thought to play a key role in stress responses and emotional processing (84). In their conclusions, the authors advocated for wider use of the vole model because "monogamous mammals share a common reproductive pattern of long-term bond. The pine vole may prove to be a new and important model for species displaying monogamy, including humans (75)." Surprisingly, no one in the EDC community heeded this call, and no work was subsequently performed in the vole model until we began our own line of investigation with BPA and prairie voles nearly a decade after this pioneering study.

Overall, the literature on EDCs and AVP/OT pathways remains small, and work has primarily focused on a small subset of high priority chemicals, most of which are known disruptors of estrogen signaling pathways. Yet, it highlights the importance of exploring alternative modes of action when thinking about neurotoxicants and EDCs. Three representative examples are discussed in depth below.

BISPHENOL A (BPA)

There is perhaps a no more notorious or thoroughly studied EDC than BPA. Present in polycarbonate plastics, the epoxy lining of canned foods, thermal paper, and other common household products, BPA is classified as a "high volume production" compound and continuous low level (resulting in mean blood levels of 4 ng/ml or lower) exposure is virtually ubiquitous and unavoidable (85). Although frequently characterized as "weakly estrogenic" BPA effects are multi-modal. For example, we have repeatedly shown that developmental exposure to doses as low as 2.5 μg/kg bw sex specifically alters the mRNA expression of ERα and ERβ in sexually dimorphic hypothalamic and limbic subnuclei in the neonatal and older rat brain (86–90). Neurobehavioral outcomes purportedly mediated *via* androgen receptors and epigenetic effects have also been reported (91–93). The dose defined as the "no adverse effect level" for systemic toxicity and thus the dose level below which biologically meaningful effects purportedly do not occur is 5 mg/kg bw per day. The level considered "safe" for human exposure is extrapolated from this level and is 50 μg/kg bw per day according to the US EPA, and 4 μg/kg bw per day in the European Union.

Information regarding BPA-related impacts on nonapeptide pathways is limited, but we and others have generated some evidence, in a diverse range of taxa, showing that developmental exposure to BPA alters the organization and function of AVP and OT pathways. In a study done in collaboration with Andrea Gore's laboratory, we found that Wistar rats perinatally exposed to BPA

via drinking water [1 mg/L; a dosing regimen that resulted in serum levels approximately equivalent to humans (94)], displayed elevated anxiety-related behaviors as juveniles (60). Consumption of a soy-rich diet, which is hormonally active and contains estrogenic phytoestrogens including GEN, ameliorated the behavioral effects to some degree. This outcome was somewhat surprising. We had predicted that BPA and soy would have additive effects because of their similar modes of action. Interactions with diet may explain some of the inconsistencies in the BPA literature regarding effects on brain and behavior (93, 95). Nevertheless, linkages between developmental BPA exposure and an anxiogenic phenotype have repeatedly been shown in dozens of studies using a variety of animal models and human populations (representative examples include (62, 91, 93, 96–100)). Acceptance of this outcome by the risk assessment community has been tentative because the causal mechanism(s) remains unclear (94). In our Wistar rat study, exposure decreased ERβ and Mc4r expression levels in the amygdala of both sexes. These genes play crucial roles in regulating the production and release of AVP and OT in the PVN. Specifically, agonism of Mc4R in magnocellular neurons induces dendritic secretion of OT (101), an effect that is anxiolytic (102, 103). As a follow-up to our initial study, expression levels were subsequently examined in the PVN of the same Wistar rats using identical methodology. There was some evidence for downregulation of AVP mRNA in BPA-exposed females, but the effect did not quite reach statistical significance ($p \leq 0.07$), and no effect on OT, AVP1aR, OTR, or Mc4R expression was observed in either sex (*unpublished observations*). As was observed in the amygdala, BPA produced no effects on any genes of interest in rats reared on the soy-rich diet, highlighting the significance of other environmental factors, including diet, when seeking evidence of endocrine disruption. Lack of effects on transcription does not necessarily indicate lack of effects on translation, transport, or release, and it is possible that only subpopulations of PVN neurons are susceptible, necessitating an experimental approach with great anatomical resolution to assess possible effects. In addition, AVP and OT are released *via* multiple mechanisms (2, 5), sometimes simultaneously, including volume diffusion through the extracellular space following release from large dense core vesicles, widespread circulation through the ventricular system, and targeted release into specialized extrahypothalamic regions, any or all of which might be vulnerable to EDCs.

In a separate study, we showed that that neonatal exposure to BPA (50 mg/kg bw or 50 μg/kg bw) by subcutaneous injection can alter the number of PVN OT immunolabeled cells in adulthood (in female rats) (61). Internal BPA levels were not assessed for this study, but because injection bypasses first metabolism, circulating BPA levels were undoubtedly higher than the study described above and higher than typical human levels. Exposure significantly increased OT-immunoreactive (-ir) neuron numbers, but only in the anterior PVN, a result interpreted to potentially indicate sequestration of OT and reduced release from nerve terminals. Similar outcomes were observed in a subsequent study using the prairie vole model (62). Animals were orally exposed over PNDs 18-14 to 5, 50, or 50,000 μg/kg bw and tested as juveniles or adults. Females in the highest exposure group had fewer OT-ir neurons in the posterior PVN but more AVP-ir neurons in the anterior PVN. At the lower two doses, BPA eliminated the well-characterized sex difference in PVN TH-ir neuron numbers and reversed it at the highest dose. This effect was mirrored by similar alterations in social investigation. In this species, males are typically more inclined to interact with a novel animal than females. At the two lower doses, BPA eliminated this sex difference and at the highest dose, reversed it. Disruption of pBnST TH-ir neuron numbers also occurred at the lowest dose, but not the higher two making that outcome somewhat more difficult to interpret. These data are not in complete directional accord with our rat study, an outcome that could result from the different exposure window, doses used, or species differences in OT/AVP pathways. Ongoing studies are underway to try and resolve these differences.

A series of studies led by Emilie Rissman using C57BL/6J mice suggests that BPA-related effects on OT and AVP signaling pathways may be multi- and transgenerational. In this model, the dams (F0) are exposed to BPA during pregnancy so offspring (F1) exposure is gestational. The subsequent generation (F2) is exposed as developing germ cells in the ovaries of their embryonic F1 parents. Thus, this generation is also "directly" exposed. The F3 generation is the first generation regarded as unexposed and thus the first set of offspring in which truly transgenerational effects can be assessed (63). Mice reared on a diet delivering approximately 170 μg/kg bw BPA (to the dams) during gestation displayed social deficits, particularly in females, that coincided with a decrease in AVP mRNA expression levels in whole embryonic brains obtained from their ED18.5 siblings (both sexes). AVP levels were also decreased in the F4 embryos of both sexes albeit to a lesser degree (64, 65). Reduced OT mRNA expression was observed in the F4 males. Evidence of effects on social behavior, including juvenile social recognition and social investigation, was reported but directionally inconsistent across generations with the F1 generation showing heightened social investigation and the F3 generation displaying reduced responses to novel females, and the F4 mice more actively engaged with their social peers than those from an unexposed lineage (104). A subset of adult F1 and F3 mice were examined *via* immunohistochemistry to asses if BPA had altered sexually dimorphic AVP-ir levels in several brain regions. F1 males had fewer AVP-ir in the MePD than unexposed males, but females were unaffected. No evidence of disrupted AVP-ir levels was found for the MePD, PVN, LS or BnST (66).

Collectively, these findings support the hypothesis that BPA exposure may disrupt the organization of AVP/OT pathways arising in the PVN, thereby impacting related social behaviors. They also suggest, unsurprisingly, that specific outcomes likely differ between sex and species depending on the degree to which they are prosocial. Additional environmental factors, including diet, may modify outcomes and contribute to discordance across studies. Experiments with a greater emphasis on linking cause and effect are greatly needed to estimate the degree to which BPA may affect AVP/OT systems in humans.

CHLORPYRIFOS (CPF)

Organophosphate pesticides make up approximately 70% of all pesticides in the USA and are developmental neurotoxicants

related to nerve agents such as Sarin and VX. Although their primary mechanism of action is inhibition of acetylcholinesterase, they can also have endocrine disrupting properties, particularly at dose levels which are more environmentally relevant and well below those which cause systemic toxicity (16, 22, 105). CPF has been linked to ADHD and other developmental neural disorders in children, prompting the EPA to impose a ban on residential use in 2001 (22, 106, 107). A complete ban was recommended in 2016 but has not been implemented. CPF will thus remain in use for golf courses, and about 50 different types of crops, including corn, soybeans, row crops (such as broccoli), and fruit trees. Work in mice and rats has repeatedly shown that developmental exposure results in long-lasting (and sometimes sex specific) effects on emotional functions, activity, learning, serotonergic and dopaminergic transmission, neuronal differentiation, and synaptogenesis (108). Collectively, these effects implicate peptidergic pathways as a possible target for endocrine disruption.

Data in support of CPF as a peptidergic disruptor are sparse but suggestive of sex-specific vulnerability. In CD1 mice, pre- and/or postnatal exposure to CPF resulted in a dose-dependent decrease in hypothalamic AVP at 5 months of age concomitantly with increased OT (67). This outcome was more pronounced in males than females and following pre-, rather than post-, natal exposure. The same group subsequently reported effects on OT and AVP mRNA expression in the amygdala as well as the hypothalamus (68). Notably, ERβ was also found to be upregulated in the male hypothalamus. In both studies CPF-exposed mice displayed altered social and exploratory behaviors, with the specific outcomes differing by sex. Collectively, these data are consistent with a robust body of evidence showing that CPF is a developmental neurotoxin and endocrine disruptor, and a likely environmental contributor to ASD, ADHD, and other behavioral disorders. Additional work in other species is needed to provide resolution regarding the degree to which CPF can interfere with AVP and OT signaling pathways.

PHYTOESTROGENS

Not all EDCs are anthropogenic. Soy and other legumes contain isoflavone phytoestrogens, which are used to aid in the recruitment of nitrogen-fixing bacteria but are also well-characterized xenoestrogens (83, 109, 110). GEN, for example, is found in soy-based and soy-supplemented foods, including soy infant formula, and can be purchased as a dietary supplement. Evidence of the endocrine disrupting properties of GEN dates back decades, and soy is so well recognized as a hormonally active food, that it is frequently advertised and promoted as such (15, 111–115). As with BPA and other manufactured EDCs, the majority of GEN studies have focused on reproductive endpoints (although with the distinctive difference that, historically, most studies presume possible outcomes will be "beneficial" because soy is "natural" while BPA is not), but there are some data showing endocrine disruption of AVP-related pathways and systems, including behaviors (112).

Although initially thought to be anxiolytic (116), long-term consumption of soy-rich diets has been shown to enhance aggression in male cynomolgus monkeys (117) and Syrian hamsters (72), the latter of which also had lower AVP1A expression in the LS but higher AVP1A expression in lateral hypothalamus. My lab has also shown that two dietary isoflavone supplements produced anxiolytic elevated plus maze behavior in proestrus female rats, but anxiogenic responses in gonadally intact males (118). Similarly, male rats maintained on a diet containing 150 μg/g GEN and daidzein displayed increased anxiety and elevated stress-induced plasma AVP and corticosterone levels (71). Elevated hypothalamic AVP content (measured by ELISA) has also been reported in rats maintained on a diet containing 1,250 ppm GEN (73). Sexually dimorphic AVP-ir in the rodent brain can also be altered by early-life exposure to GEN, including at doses akin to the levels found in soy infant formula. For example, in CD1 mice, oral intake of 50 μg/kg GEN in the first week of life slightly, but significantly increased AVP-ir in the female BnST but did not impact the sexually dimorphic AVP innervation of the LS (74). Notably, the sexually dimorphic density of AVP-ir neurons in the medial parvicellular part of the PVN (PaMP) was eliminated by postnatal GEN, with higher numbers in females and lower numbers in males.

Dietary consumption of soy is globally increasing, meriting greater understanding of its endocrine disrupting properties, particularly in infants and young children. Although sex-specific isoflavone-related effects on the rodent AVP/OT system have been sporadically shown by multiple laboratories, including my own, work in this area remains limited and incomplete. In some regards, this is because work on "natural" EDCs is notoriously difficult to obtain funding for. There is also a problem of perception. While concerns about BPA, fire retardants, and other manufactured EDCs remains high, phytoestrogens are some of the most potent xenoestrogens humans regularly are exposed to. Arguably, they are one of the most significant but underappreciated EDCs of concern.

ENDOCRINE DISRUPTION OF AVP/OT PATHWAYS: A ROLE FOR ERs

How chemicals interact with and perturb AVP/OT pathways are undoubtedly multi-modal, but action on ERs, and disruption of ER expression, is likely a primary mechanism by which BPA, GEN, and other EDCs influence AVP/OT pathways. That non-classical EDCs like CPF can have disruptive effects on AVP/OT action, hint at alternative modes of action but most work to date has focused on estrogen-disrupting compounds because the organization and function of AVP/OT signaling pathways are exquisitely sensitive to steroid hormones. We have repeatedly shown that developmental BPA exposure can perturb ERα and ERβ gene expression throughout the rat hypothalamus and components of the mesolimbic dopamine system, including the PVN and BnST (86–90), across the lifespan. Moreover, in the transgenerational mouse model, one of the most striking findings was disrupted ERα-ir in the F3 females from the BPA-exposed lineage, with higher levels in the AVPV and lower levels in the BnST (66). The phytoestrogen coumestrol (found in clover and other pasture legumes) and GEN upregulate ERβ mRNA expression in the PVN, an effect opposite to that of 17β-estradiol

(119, 120). If and how these neural effects translate to adverse behavioral phenotypes remains to be established.

How estrogens and androgens contribute to the sexual differentiation and function of the AVP/OT system are sex and species dependent and associated with functional differences in prosociality (121, 122). Manipulation of OT/AVP levels *via* direct exposures to exogenous hormones, agonists, or alteration of the social environment can significantly modify the number of OT, AVP, and TH neurons in the PVN, thereby resulting in anxiety-like behavior and alterations of prototypical male and female sociosexual behavior (123–127). Similarly, neonatal manipulation of estradiol or testosterone alters prairie vole affiliative behaviors later in life, and estradiol administration during adulthood alters estrus and locomotor activity (57, 128, 129). Males gonadectomized on the day of birth, for example, fail to form a pair bond after AVP administration (52).

Pathways coordinating social recognition are well known to be regulated by estrogen, with both ERα and ERβ knockout mice showing social impairments (130, 131), and ERβ knockout females failing to generate OT or AVP mRNA expression in response to exogenous estrogen administration (132, 133). Although the functional role of limbic ERβ remains ambiguous, ERβ in the PVN and associated structures, including the BnST, plays a fundamental role in mediating motivational and anxiety-related behaviors (134–136). ERβ has also been identified as one component of a "four-gene micronet" regulating social recognition (7, 131) *via* the PVN, amygdala, and olfactory system. By this model, estradiol simultaneously acts through ERβ in the PVN to increase OT, and ERα in the amygdala to increase OTR expression. This view is consistent with the observation that the density of ERα markedly differs across prosocial and asocial species and is directly related to the degree to which individuals and species are prosocial (3, 137, 138). Conceptualizing and testing endocrine disruption of ER-sensitive AVP pathways as a system, such as this micronet, while simultaneously accounting for species and sex differences, would further holistic understanding of how EDCs affect non-reproductive behaviors.

Finally, a subpopulation of parvocellular PVN OT and AVP neurons, along with ERβ and coordinating input from the BnST, is thought to be involved in the stress response (135, 139, 140) supporting the possibility that EDCs may influence the hypothalamic–pituitary–adrenal axis. Surprisingly, this is a neglected area of EDC research. Given the comparatively higher promiscuity of ERβ (relative to ERα) for ligands including multiple phytoestrogens including GEN (82), and even the androgen metabolite 5α-androstane-3β,17β-diol (3β-diol) (141), this is a strikingly understudied but likely avenue for endocrine disruption.

AVP IMPACTS—BEYOND BRAIN AND BEHAVIOR

In both rats and prairie voles, we have shown that BPA alters only specific subpopulations of OT and AVP neurons. Although we surmised that the populations of OT-ir and AVP-ir neurons impacted by BPA are primarily parvocellular, the possibility that BPA alters the density and function of magnocellular neurons could not be ruled out. This would suggest an avenue for homeostatic disruptions including cardiovascular effects and hypertension associated with BPA and, by extension, other EDCs (142, 143). Intriguingly, in humans, BPA exposure has been tentatively linked with cardiovascular disease and hypertension, but the causal mechanisms remain to be characterized (144).

Long before its role in social behavior was identified, AVP was termed the "antidiuretic hormone." Magnocellular AVP neurons in the PVN and SON release AVP both centrally and systemically in response to dehydration, hemorrhage, and stress and are known to be one of the primary physiological regulators of water-electrolyte balance (145). These AVP neurons receive osmosensitive inputs from the organum vasculosum lamina terminalis *via* glutamatergic synapses and are modulated by nitric oxide (NO) signaling pathways (146–148). Work using adult male rats and SON tissue punches has yielded evidence that the PCB mixture Aroclor 1254 and the structurally related polybrominated flame retardants [polybrominated diphenyl ethers (PBDEs)], including the pentabrominated mixture DE-71, significantly reduced AVP release from the SON in response to dehydration (76, 77). Subsequent work revealed that perinatal oral exposure to 1.7 or 30.6 mg/kg/day resulted in elevated systolic blood pressure responses at 3 h post-hyperosmotic challenge, an effect the authors interpreted as possibly attributable to decreased plasma AVP levels (78). A single paper also suggests that long-term exposure to chlorobenzenes (solvents) may also disrupt neurohypophysial AVP and OT release (149). Complementary work by multiple research teams has identified NO signaling as a potent target of many EDCs including PBDEs, PCBs, and other organohalogens (representative examples include (74, 150, 151)) suggesting another possible route by which EDCs could impact AVP function. Concomitant work focused on the parvocellular aspects of AVP signaling has also found evidence of NO endocrine disruption by GEN, BPA, and other EDCs (39, 74, 151).

SUMMARY AND CONCLUSION

Although available data are sparse there is compounding evidence that EDCs can alter the ontogeny and function of AVP and OT signaling pathways critical to social behavior and, possibly, osmotic balance and other peripheral functions. Rising rates of behavioral, hypertensive, and other disorders for which AVP are known to play a coordinating role suggest a causal role for environmental factors, including chemical exposures, but which specific ones remains elusive. The complex chemical landscape we all face and inevitably invades us makes it challenging to identify the individual or subset of compounds that pose the greatest health risks to this and subsequent generations. Data from a myriad of species from birds to mammals, however, suggest that EDCs that interfere with estrogen signaling (including BPA, GEN, PBDEs, and PCBs) are plausible disruptors of AVP/OT pathways. Compared to what is known about how these EDCs alter the sex specific organization and function of reproductive neuroendocrine pathways, investigation of nonapeptide disruption is in its relative infancy, but a topic of heightening interest and exploration. Moreover, only a handful of compounds have been tested for AVP-related outcomes at all.

Going forward, there is a compelling need to understand and develop effective screening methods for EDC activity, particularly on non-steroid hormone targets, to get a better handle on what possible consequences this chemical class may pose to human health. As this paper went to press the EPA's endocrine disruptor screening program (EDSP), an effort that took more than two decades to construct, and only recently reached the capacity to effectively identify any chemical as a purported EDC, was slated for elimination. If cut, this would leave absolutely no mechanism for screening any extant or pre-market chemicals for any EDC activity of any kind. The assays within the EDSP are reasonably effective at targeting sex steroid hormone disruptors but ineffective at identifying non-steroidal endocrine disrupting activity including OT and AVP disruption (152) leaving a desperate need for improvement. Within the USA there is absolutely no federal-level mechanism in place to evaluate where EDCs are found, let alone regulate their use in common items such as cosmetics, personal care products, food containers or durable goods. Consequently, daily exposure is virtually silent and unceasingly increasing (12). Moreover, there is an ever-churning conveyer belt of worrying chemical replacements such that when EDCs, such as the PBDEs, DDT, and BPA, are finally phased out, they are rapidly replaced by structurally similar compounds. In this regard, there is a compelling need for ongoing inquiry regarding EDC activity, particularly on non-traditional targets such as AVP systems.

Future work focusing on richly estrogen sensitive limbic populations known to confer individual, sex and species differences in prosocial traits, especially the BnST, is critically needed to better understand how AVP/OT signaling pathways and, consequently, social behaviors might be vulnerable to endocrine disruption. Much EDC work, particularly on social and other non-reproductive behaviors, remains largely descriptive with limited understanding of causal relationships. Elucidating specific mechanisms of endocrine disruption is essential for establishing causality between exposure and adverse behavioral outcomes. Maximizing the probability of success will hinge on the selection of the most appropriate animal model for the specific question being addressed, and the translational value of the outcome for human neurophysiology. Neuroendocrinologists familiar with a wide range of taxa are well poised for critical discovery because classical toxicology still overly relies on dated testing strategies and traditional rat models. Incredibly, toxicology is still struggling to incorporate transgenic animals and other more modern approaches now considered basic tools of neuroscience (153, 154), leaving vast opportunity for the neuroendocrine community to make critical discoveries toward the goal of uncovering the chemical contributions to social disorders and other debilitating conditions such as cardiovascular disease and hypertension.

AUTHOR CONTRIBUTIONS

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Dose-Dependent and Lasting Influences of Intranasal Vasopressin on Face Processing in Men

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Arginine vasopressin (AVP) and related peptides have diverse effects on social behaviors in vertebrates, sometimes promoting affiliative interactions and sometimes aggressive or antisocial responses. The type of influence, in at least some species, depends on social contexts, including the sex of the individuals in the interaction and/or on the levels of peptide within brain circuits that control the behaviors. To determine if AVP promotes different responses to same- and other-sex faces in men, and if those effects are dose dependent, we measured the effects of two doses of AVP on subjective ratings of male and female faces. We also tested if any influences persist beyond the time of drug delivery. When AVP was administered intranasally on an initial test day, 20 IU was associated with decreased social assessments relative to placebo and 40 IU, and some of the effects persisted beyond the initial drug delivery and appeared to generalize to novel faces on subsequent test days. In single men, those influences were most pronounced, but not exclusive, for male faces, whereas in coupled men they were primarily associated with responses to female faces. Similar influences were not observed if AVP was delivered after placebo on a second test day. In a preliminary analysis, the differences in social assessments observed between men who received 20 and 40 IU, which we suggest primarily reflect lowered social assessments induced by the lower dose, appeared most pronounced in subjects who carry what has been identified as a risk allele for the V1a receptor gene. Together, these results suggest that AVP's effects on face processing, and possibly other social responses, differ according to dose, depend on relationship status, and may be more prolonged than previously recognized.

Keywords: social behavior, V1a receptor, face processing, intranasal, social context

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INTRODUCTION

Arginine vasopressin (AVP) and related peptides, including its ancestral, non-mammalian homolog, arginine vasotocin (AVT), act as central neuromodulators across vertebrates that regulate, among other functions, social behavior [reviewed in Ref. (1–3)]. Many of these effects are associated with influences in a conserved network of nuclei within the brain, the Social Brain Network (SBN), that are reciprocally connected and regulate a variety of social behaviors across vertebrates (4–6). The production of AVT/AVP within several of these nodes has been highly conserved, though the

projections from these nodes and the distributions of peptide receptors are highly variable across species, including numerous target sites outside of the traditional SBN. This variation likely accounts for the diversity of behavioral effects these peptides have across species [reviewed in Ref. (7)].

In addition to their species-specific influences, AVT/AVP's behavioral effects can differ between the sexes, between individuals of the same sex that display alternative phenotypes, and as a function of complex dose-dependencies. For example, in tropical damselfish, AVT's ability to stimulate aggression in males follows an inverted U function, with mid-range doses being most effective (8). This suggest that higher doses do not simply produce maximal behavioral output upon receptor saturation, but may have influences that counteract those of lower doses or induce alternative behavioral responses, perhaps by activating different patterns of receptors across the SBN. Sex/phenotype-specific influences include cases in which behavioral patterns only exhibited by one sex are affected (most often male-typical behaviors, as in the damselfish example above); cases in which the peptide induces opposite effects in the sexes (9–12), and even cases in which the peptide has different effects in individuals of the same sex that adopt alternative mating strategies (13).

Arginine vasotocin/arginine vasopressin can also produce context-dependent effects, as has been most elegantly demonstrated in birds. In the territorial estrildid violet-eared waxbill, exogenous administration of a V1a receptor antagonist reduced aggression related to mate competition in males, but did not affect resident-intruder aggression (14), and in zebra finches the antagonist reduced aggression during mate competition but increased aggression following colony establishment (15). Some context-dependent effects are a simple function of the sex of the stimulus present; knockdown of AVT production in the paraventricular nucleus of zebra finches enhances aggression toward females in males, but not toward other males (11). Similarly, AVP promotes affiliative responses toward females in male prairie voles, but promotes aggressive responses toward other males (16). Most of these context/stimulus-dependent effects likely depend on the activation of different AVT/AVP circuits that produce unique behaviors in response to particular social stimuli. For example, AVP's ability to stimulate aggression in male prairie voles depends on actions within the hypothalamus (17), whereas its ability to promote affiliative response toward females depends on actions within the ventral pallidum and septum (18, 19).

Although we know a great deal about the acute effects of AVT/AVP, we know very little about whether or not there might be long-term consequences associated with those acute effects. Work with oxytocin (OT) in mammals and AVT in birds demonstrates that nonapeptides can have long-term effects on the brain and social behavior during early developmental windows (20, 21) [reviewed in Ref. (22)], though perhaps through mechanisms that are only operative during critical periods. In adults, AVP affects social recognition/memory, though such effects are typically evident only 2–24 h after AVP manipulations (23, 24). Even exogenous AVP's effects on affiliative responses related to pair bonding in male prairie voles have only been examined immediately after manipulations of AVP that are concurrent with social interactions with females (16, 18). Intriguingly, though, recent work indicates that mating, which induces the AVP release necessary for pair bonding in this species, triggers epigenetic changes that enhance affiliative behavior (25, 26). Whether those epigenetic changes depend upon mating-induced AVP release, or how long their influences upon behavior persist, remain to be determined.

Examination of the role AVP plays in human social behaviors a has thus far been much less extensive than in other animals. However, there is emerging evidence of a diversity of effects, some of which are sex- and context specific. As in male prairie voles, there is some data suggesting a role in pair bonding; allelic variation within the RS3 domain of the promoter for the V1a receptor is associated with pair bond strength in men (27). Studies in which AVP is intranasally delivered, which elevates peptide levels in the brain (28), have more directly implicated AVP in social regulation. AVP selectively draws attention to sexual content in language (29) and increases empathic concern in both male and female subjects who had previously received high levels of warmth from their fathers (30). Intranasal AVP also facilitates cooperation in complex social decision tasks, effects that are dependent on sex, contexts of the task, and the personality of the individual (31–33).

The above-mentioned effects likely promote social engagement, consistent with the ability of nonapeptides to promote affiliative/courtship-related responses in other animals and/or in some contexts. However, AVP/AVT can also enhance aggression and social withdrawal. Consistent with the possibility that AVP might likewise have antisocial effects in humans, levels of AVP correlate positively with life histories of aggression in men (34). Also, intranasal AVP delivery not only affects the processing of positive emotions in faces but also negative ones (35, 36), and it decreases how friendly men rate the faces of unfamiliar men while enhancing facial expressions consistent with negative, and possibly even threat-related, responses (37, 38). However, we do not yet know if the negative effects in men depend on context, in this case of the stimulus sex. AVP might, as in male prairie voles, promote antisocial responses toward other males but facilitate affiliative responses toward potential mates. We also do not yet know if AVP might produce dose-dependent influences on the ratings of faces, or if any of its effects on subjective face ratings could have long-lasting consequences.

To address those questions, we compared the effects of two doses of AVP commonly used in intranasal studies, 20 and 40 IU, on subjective ratings of same- and other-sex faces in human males 50 min after drug or placebo delivery and again multiple days later (between 2 and 20 days after drug delivery). Because differences in tendencies to form emotional attachments are related to variation in the RS3 domain of the V1a receptor prompter, we also ran models that included variation in RS3 alleles. We had five primary predictions. First, that 20 IU would, as in our previous study, decrease ratings of same-sex faces, but possibly increase ratings of female faces if, as discussed above, mechanisms similar to those in prairie voles are operative in humans. Second, if AVP does enhance positive responses toward females, then the effects might persist on follow-up tests when no drug is delivered. Third, that influences of AVP on responses to male and female faces might differ in single men and those in relationships in light of findings that social experience, including

pairing, can induce changes in AVP circuitry in other animals. Fourth, that any negative effects of AVP would be greater in men who carry V1a RS3 risk alleles, and any positive responses smaller in those individuals. Fifth, that the higher dose would produce similar, but more pronounced effects than the lower dose if the dose–response function is linear, but divergent effects if it is not.

MATERIALS AND METHODS

Subjects

Male subjects between the ages of 18 and 30 were recruited through advertisements in Craig's list in Portland, Maine, the local gym, newspaper and community college, Maine Medical Center's electronic newsletter, as well as through referrals. Of those who responded, 94 passed our initial screenings and consented to participate. Seven subjects withdrew following Treatment Day 1 and one after Treatment Day 2 for various, non-study-related reasons. Of the 86 subjects who completed all 3 days, 2 were African-American, 3 were Asian, and 2 were Hispanic. The remaining 79 subjects were Caucasian. Data for those who only completed day 1 were used in between-subjects comparisons on that day, and data for subjects who only completed the first 2 days were used in within-subjects comparisons across those days.

All subjects were initially interviewed by phone for a pre-screen to exclude subjects that were prescribed serotonin reuptake inhibitors or had cardiovascular or neurological conditions, cancer, asthma, facial Botox, or substance abuse issues. Those who passed this pre-screen then came for an in-person screen at Maine Medical Center. At that time, verbal and written informed consent were obtained from each subject. Subjects were provided a copy of the informed consent document. Following consent, subjects were assigned an ID number and asked to provide a urine sample for drug testing. Demographic information, sexual orientation, and relationship status were recorded. Subjects were then given a physical exam, including EKG. Exclusion criteria were hypertension [systolic blood pressure (BP) >140 and/or diastolic BP >90], hypotension (systolic BP <90 and/or diastolic BP <50), temperature >100, and/or a positive drug screen. All subjects were examined by a board-certified psychiatrist and screened in a semi-structured interview for ongoing Axis I psychiatric or substance abuse. Any active Axis I disorder requiring ongoing treatment led to exclusion from participating in the study, as did any acute psychiatric symptoms (e.g., delusions, hallucinations, paranoia, mania, depression, obsessions, compulsions, or severe anxiety) evident at the time of the interview. Initially, subjects were paid $300 if they completed all visits, prorated to $50 at screening, $100 at treatment day 1 and 2 and $50 at non-treatment day if they did not complete all three test days in addition to the initial screening. However, due to difficulties recruiting subjects, we increased the amount paid to $500, prorated to $100 at screening, $150 on treatment days 1 and 2 and $100 on non-treatment day if they did not complete all three test days.

The study was approved by the Bowdoin College and Maine Medical Institutional Review Boards and by the U.S. Food and Drug Administration. None of the subjects developed any major side effects in response to AVP, including anaphylaxis.

Drugs

Sterile, lyophilized AVP was purchased from PolyPeptide Laboratories (Sweden). Drug was dissolved in sterile saline by the pharmacy at Maine Medical Center in two doses; 20 IU/0.5 ml and 40 IU/0.5 ml, drawn into 1 cc syringes, then immediately frozen and stored at −80°C until use. Placebo vials contained the same volume of sterile saline and were likewise stored at −80°C. Drugs were sent out for tests of efficacy every 6 months to Eagle Analytical Services. All tests showed that both doses retained their full efficacy throughout the test period (remained within 10% of appropriate international units). No drug was used after more than 12 months storage. The pharmacy also created randomization tables that assigned each subject to one of the two doses, to either getting drug or placebo on day 1, and to the stimulus sets that would be seen on each test day (see further explanation below). All study personnel were blind to whether the subject received placebo or drug on a given day and to what dose the subject would get on the drug day.

Stimuli

Photographs were taken of female and male models by a professional photographer. We chose Caucasian models because, given racial demographics in Maine, we anticipated the overwhelming majority of subjects would be Caucasian. For reasons related to our hypothesis that AVP would affect responses to specific individuals, we only wanted to show a single male and a single female after AVP and after placebo, and we did not include multiple models that differed by race in hopes of minimizing variation related to in-group/out-group influences. We used only neutral emotional expressions to determine if AVP can bias individuals to respond to ambiguous social stimuli more negatively or positively. Images from multiple models were initially piloted with Bowdoin undergraduates to select the two female and the two male models who appeared most similar in terms of basic features like hair color and whose pictures were rated most similarly on responses measured during the study. Measurements included Approachability (from −3, which indicated the face was threatening and not approachable, to 3 for faces subjects felt were friendly and very approachable), Willingness to Initiate Conversation with the person (Initiate; from −3, not likely at all, to 3, very likely), and Attractiveness (−3, very unattractive, to 3, very attractive). These responses were chosen to try to dissociate responses related to social perception (Approachability), social motivation (Initiate), and sexual/romantic potential (Attractiveness).

Five stimulus sets were created, four for the first two test days, and one for the third, final day (FD) of testing. Each of the four sets for days 1 and 2 contained 18 images, 9 of one female and 9 of one male, each taken in different lighting and with different postures to create some variability, in pseudorandomized order so the same individual was never presented more than twice in a row. The sets, therefore, consisted of the four possible combinations of individual male and female faces that could be seen together. The final day stimulus set consisted of 36 images and included the same 9 images of each of the four models seen previously on the first two test days, in a pseudorandomized order that ensured the same face was not presented more than twice in a row.

Experimental Design

We employed a within-subjects design in which each subject received placebo on one test day and one of the two doses of AVP on the other, in counterbalanced order. The stimulus sets seen by each subject on placebo and drug days were assigned randomly. Thus, subjects saw one of the female models and one of the male models after placebo, and the other female and the other male after drug. On the final day, when no drug was administered, subjects saw the stimulus set that included all of the faces previously seen.

Procedure

Subjects were met at the study site by the research nurse who conducted the procedures. Adverse life events since screening, concomitant medications, fluid, and caffeine intake were reviewed. If all treatment criteria were met, the site investigator provided a written prescription for study drug to the pharmacy. The pharmacy then randomized the subject and delivered study drug syringe, with a MAD300 Nasal Atomization Device attached. Once the subject was settled and comfortable, the research nurse proceeded to prepare the subject for facial electromyographic, skin conductance, and heart rate recordings, but that data will not be presented in this paper. Subjects were attached to an automatic BP, pulse, and temperature monitor. Baseline (pre-study drug) readings were collected. Subjects were then asked to self-administer the study drug in a single dose to one nostril (20 IU/0.5 ml, 40 IU/0.5 ml, placebo-sterile saline). Subjects then viewed a neutral 30 min DVD, Blue Planet: Seas of Life. Serial BP, pulse, and temperature measurements were taken at baseline (pre-study drug administration), and again at 5, 20, 30, and 60 min post study drug administration, though only BP from baseline, 20 and 60 min were analyzed statistically.

Image presentation began 50–60 min after drug administration (Stroop Software; Coulbourn Instruments). Each face was presented on a computer screen 36 inches in front of the subject for 8 s, 20–30 s apart. The interval between images varied randomly between 20 and 30 s to keep subjects from anticipating exactly when each image would be presented within that window. Subjects observed a blank screen between images. Immediately after each image disappeared, the technician running the session asked the subject to say how approachable the face was, on the scale discussed above, how likely the subject would be to initiate conversation with the person whose face was shown, and how attractive the subject thought the face *was.* All verbal responses were recorded by the research nurse. Immediately following image viewing, 2 EDTA (ethylenediaminetetraacetic acid—a standard additive chelating agent that binds calcium and other metals, thus preventing coagulation of specimens) tubes of blood were collected *via* peripheral phlebotomy.

These procedures were repeated for treatment day 2, which occurred 2–7 days after day 1. Subjects reported back for the final test day no sooner than 2 days since the day 2 trial and no more than 21 days from the initial screening. Thus, the final test day occurred within 2–20 days of AVP administration. All of the procedures were repeated on the final test day, but subjects did not self-administer placebo or drug. They observed and responded to the stimulus set containing 36 images, 9 each of all the faces previously observed. Two female nurse/technicians collected data, but the same person collected all data across days from each individual.

Microsatellite Genotyping

Genotypes for the RS3 microsatellite at *AVPR1A* were determined according to the method described in Kim et al. (39). Briefly, a PCR with fluorophore-labeled primers was performed using the following conditions: 1XBuffer (Applied Biosystems), 2.5 mM $MgCl_2$, 0.5 mM forward-RS3 primer (6-FAM-TCCTGTAGAGATGTAAGTGC); 0.5 mM reverse-RS3 primer (gtttcttTCTGGAAGAGACTTAGATGG), 0.08 mM dNTP, 0.06 U Amplitaq Gold (Applied Biosystems). 5 µl of this assay mix was added to a 384 plate containing 10 ng of dried DNA. Amplification cycles were executed in a 9700 Gene Amp PCR System (Applied Biosystems) at the following conditions: 95°C for 5 min; 94°C for 30 s, 55°C for 30 s, 72°C for 1 min (35 cycles), and 72°C for 10 min. PCR products were then subjected to electrophoresis and laser detection of product on an ABI 3100 System, and data analyzed using *Gene Mapper Software (Applied Biosystems)*. Each electropherogram was checked visually to confirm calls assigned by the Software, and ambiguous calls were either resolved by consensus of two experienced readers, or discarded and repeated. Quality control included the analysis of positive and negative controls, duplicate samples and Hardy Weinberg Equilibrium tests.

Statistical Analysis

Statistical analyses were performed by the Biostatistics Center at Johns Hopkins University. Data were reviewed, and sequences of facial assessments were exluded on the drug day for two subjects for whom the nursing log indicated that substantial amounts of drug had been lost during delivery due to problems the subjects had with the self-administration. For BP, the percent change from baseline 20 and 60 min after placebo and drug administration were used as the outcome measure, with initial baseline BP as a covariate. For behavioral scores, medians of scores within each stimulus sex under each condition were calculated where there were at least five scores available (equipment/software problems caused the Stroop program to stop running before the trial was complete in 1 case). Medians were considered the outcome measure for all analyses.

General linear models were performed on all analyses using IBM SPSS Statistics v 24 (IBM, Inc., Armonk, NY, USA). Relationship Status was a factor in our initial models of behavioral responses because we predicted AVP might differentially affect responses in single and coupled men. That prediction was supported in our initial models, so subsequent models were stratified by Relationship Status.

For day 1 analyses, Dose (0, 20, 40 IU) was a between-subjects factor; repeated measure was Stimulus Sex. For analyses across test days and on the final day, factors in the model were Drug Order and Dose; repeated measures were Stimulus Sex and Drug (AVP, Placebo). For those models, significant ($p < 0.05$) main effects and significant or marginal ($0.1 < p < 0.05$) interactions were evaluated, as were pairwise comparisons that tested specific predictions. Marginal interactions were interrogated to evaluate potentially important relationships between the factors/repeated measures and outcomes, recognizing that the power to

detect a significant interaction was likely not adequate given the sample size (40, 41). For the highest order interactions, pairwise comparisons of Dose and Drug were made at each level of Stimulus Sex and other factors or repeated measures within the interaction using the Sidak adjustment for multiple comparisons (42). *p*-Values noted are adjusted. We do not report significant interactions in cases where pairwise comparisons failed to detect significant differences between treatment condition (drug vs placebo) or Dose (20 vs 40 IU).

To control for individual differences in responses potentially associated with V1a allelic variation, we included whether subjects had 0, 1, or 2 copies of the 335 allele, which corresponds to the 334 risk allele identified by others (39, 43) with the primers we used, in our models. We also included whether subjects had 0, 1, or 2 long alleles (≥335). To determine if variation in RS3 influences responsiveness to AVP, we ran models for men who received 20 or 40 IU on day 1, which our initial analyses indicated was the only time when drug administration produced effects, that included whether or not subjects had at least one copy of the 335 allele as a factor.

RESULTS

Blood Pressure

There were no significant differences in mean percent change in systolic or diastolic BP 20 or 60 min after administration across subjects who received placebo, 20 or 40 IU on day 1 of testing. Across test days, there were not main effects for Dose or interactions with Drug Order for either dose for mean percent changes in systolic or diastolic pressure from baseline 20 min after administration, nor for percent changes in diastolic pressure 60 min after administration. The mean percent change in systolic pressure, however, was significantly lower 60 min after the administration of 40 IU than 60 min after the administration of placebo (Drug main effect, $p = 0.01$; mean ± SEM: placebo, −0.3% ± 1.1, 40 IU; −2.0% ± 1.4).

Behavior

We ran nine models that included Relationship Status as a factor, three for responses on the first day across subjects who got placebo, 20 or 40 IU (1 model for each variable), three for responses across the first two test days when subjects received placebo on one day and 20 or 40 IU on the other, and three for responses on the final day. There were significant interactions between Dose, Stimulus Sex, and Relationship Status for seven of the nine models ($p < 0.01$ for all but Approachability on day 1 and Willingness to Initiate Conversation across days 1 and 2), supporting the stratification by Relationship Status. We, therefore, report the results from the same nine models stratified by Relationship Status.

Day 1 Between-Subjects Comparisons Stratified by Relationship Status

Approachability

In single men, there was a significant main effect of Dose ($p = 0.047$) associated with significantly lower responses in men given 20 IU than in men given 40 IU (−0.91, 95%CI: −1.79 to −0.03, $p = 0.042$; see **Figure 1A**). We cannot resolve whether this indicates that 20 IU decreased responses, 40 IU increased them, or both. Because we previously observed that 20 IU decreases ratings of male faces relative to placebo, we did examine the pairwise comparison of placebo vs 20 IU for male faces, which was not significant (see **Figure 1A**). There were no differences between doses for coupled men.

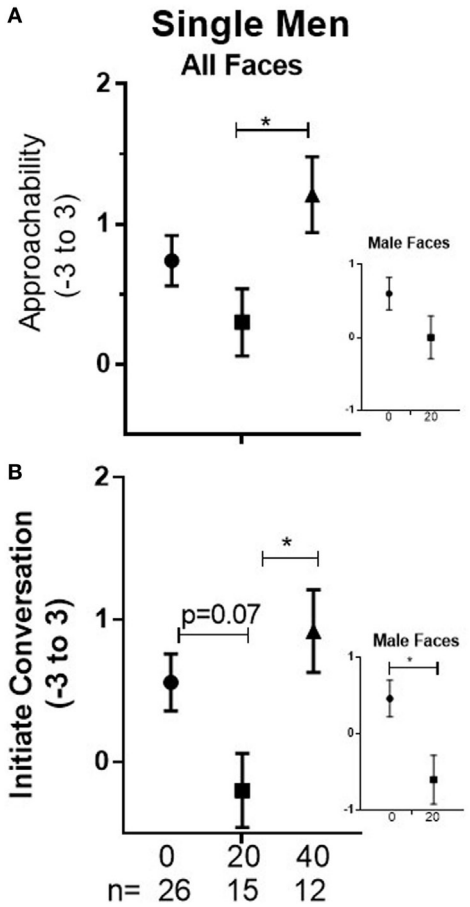

FIGURE 1 | Men ± SEM of Approachability **(A)** and Initiate ratings **(B)** of faces (averaged across male and female) on day 1 in single men who received placebo (0), 20, or 40 on that day. Inserts show planned, focused comparison between placebo and 20 IU for responses to male faces.

Initiate

In single men, there was a significant main effect of Dose ($p = 0.02$) that was associated with a marginally lower mean in subjects given 20 IU than placebo (0.76, 95%CI: −1.6 to 0.05, $p = 0.07$) and a significantly lower mean in subjects given 20 than 40 IU (−1.12, 95%CI: −2.09 to −0.15, $p = 0.02$; see **Figure 1B**). Because we previously observed that 20 IU decreases ratings of male faces relative to placebo, we did examine the pairwise comparison of placebo vs 20 IU for male faces; mean responses were significantly lower in men given 20 IU than in men given placebo (−1.06, 95%CI: −2.05 to −0.08, $p = 0.03$; see **Figure 1B**). There were no differences between doses for coupled men.

Attractiveness

There were no significant main effects or interactions for single men, nor was the planned comparison of responses to male faces

between men given 20 IU and placebo significant. In coupled men, the Dose × Stimulus Sex interaction was significant ($p = 0.003$). For male faces, the mean response after 20 IU was marginally higher than after 40 IU (1.17, 95%CI: −0.05 to 2.39, $p = 0.06$). The significant interaction was largely due to differences in how coupled men processed female faces relative to male faces; those given placebo and 40 IU rated female faces significantly higher than male faces (placebo: 1.09; 95%Cl: 0.31 to 1.87, $p = 0.008$; 40 IU: 2, 95%Cl: 1.31 to 2.69, $p < 0.001$; see **Figure 2**), whereas those given 20 IU did not. This pattern could reflect a tendency for men given 20 IU to rate female faces lower, male faces higher, or both.

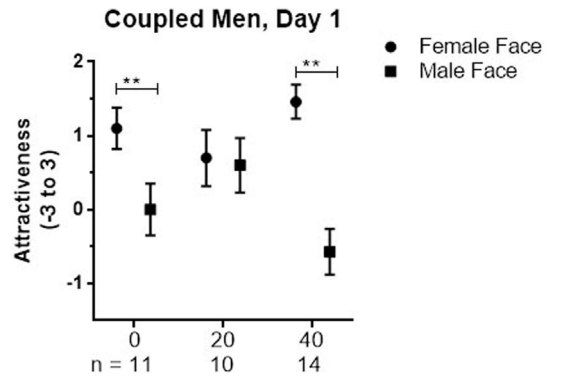

FIGURE 2 | Mean ± SEM of Attractiveness ratings of female and male faces on day 1 in coupled men who received placebo (0), 20, or 40 IU on that day.

Days 1 and 2 Within- and Between-Subjects Comparisons Stratified by Relationship Status

Approachability

Single Men

There was no drug effect for either the 20 or 40 IU dose compared with placebo in the within-subject comparison. However, there was evidence for dose differences that persisted over time, particularly if AVP was administered on day 1. There was a significant Dose × Drug Order interaction ($p = 0.01$). The mean for responses to the faces across days and Stimulus Sex was significantly lower in men given 20 IU than in men given 40 IU if drug was given on day 1 (−0.90, 95%CI −1.53 to −0.28; $p = 0.006$; not shown, but see further analysis below, as summarized in **Figure 3**). Additionally, the mean response to faces across days

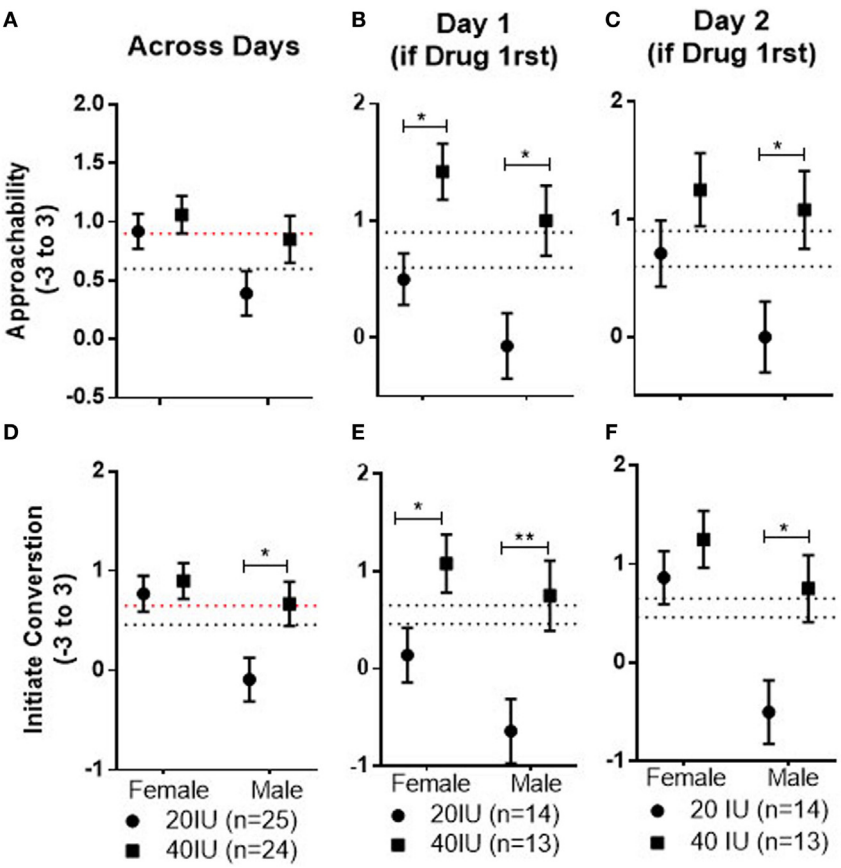

FIGURE 3 | Mean ± SEM of Approachability (top) and Initiate (bottom) ratings to female and male faces, averaged across both test days, in men who received 20 or 40 IU on either day **(A,D)**, to female and male faces observed after drug on day 1 in men who received 20 or 40 IU **(B,E)**, and to female and male faces observed after placebo on day 2 in men who received 20 or 40 IU on day 1 **(C,F)**. The top dotted line shows the mean response to female faces on day 1 in men who received placebo on that day, the bottom dotted line the mean response to male faces on day 1 in men who received placebo on that day.

and Stimulus Sex was significantly lower in men given 20 IU on day 1 than in men given 20 IU on day 2 (−0.74; 95%CI: −1.38 to −0.1; $p = 0.03$; not shown). This pattern is consistent with the possibility that the dose difference is, at least in part, associated with decreased responses induced by 20 IU if given on day 1. Because responses across days differed as a function of the dose given on day 1, we wanted to dissociate and test for acute and carry-over effects through which AVP, if given on day 1, may have affected responses across the days. Therefore, we examined pairwise comparisons between men given 20 and 40 IU on day 1 for faces seen after drug on day 1 and after placebo on day 2, even though the Drug × Dose × Stimulus Sex × Drug Order interaction was not significant. Consistent with acute differences on day 1, the mean for responses to female and male faces were both significantly lower after 20 IU than after 40 IU on day 1 (female faces: −0.92, 95%CI: −1.57 to −0.26, $p = 0.007$; male faces: −1.07, 95%CI: −1.9 to −0.18, $p = 0.01$; see **Figure 3B**). Consistent with carryover effects that generalized to new male faces, the mean response to male faces seen after placebo on day 2 was significantly lower for men that had been given 20 than 40 IU on day 1 (−1.08, 95%CI: −1.98 to −0.18, $p = 0.02$; **Figure 3C**). In contrast, the mean for female faces seen after placebo on day 2 did not differ between subjects that had been given 20 and 40 IU on day 1 (−0.54, 95%CI: −1.37 to 0.32, $p = 0.2$).

Coupled Men

There was no drug effect for either the 20 or 40 IU dose compared with placebo in the within-subject comparison. However, here was a significant Dose × Stimulus Sex interaction ($p = 0.01$). Regarding female faces, the mean across test days was significantly lower in coupled men given 20 IU than in coupled men given 40 IU (−0.72, 95%CI: −1.23 to −0.16, $p = 0.01$; data not shown, but see **Figure 4** for a similar pattern for Attractiveness). We cannot resolve whether that reflects lowered responses in men given 20 IU or higher responses in men given 40 IU. The Dose × Stimulus Sex interaction was qualified by a marginal Dose × Stimulus Sex × Drug Order interaction ($p = 0.06$). If placebo was given first, the mean response to female faces across days was significantly lower in coupled men given 20 IU on day 2 than in those given 40 IU (−1.09, 95%CI: −2.0 to −0.17, $p = 0.02$). This pattern is difficult to interpret, but it suggests that of the coupled men given placebo first, there may have been initial differences in how those who subsequently received 20 and 40 IU on day 2-rated female faces. The possibility for such sampling error was high for that comparison because only six coupled men received placebo on day 1 and 20 IU on day 2, and only four received placebo on day 1 and 40 IU on day 2. Thus, it is important to exercise caution when interpreting order effects associated with placebo administered on day 1 and AVP on day 2 in coupled men.

Willingness to Initiate Conversation Stratified by Relationship Status

Single Men

There was no drug effect for either the 20 or 40 IU dose compared with placebo in the within-subject comparison. However, there was evidence for dose differences that persisted over time, particularly if AVP was administered first. There was a significant Dose × Stimulus Sex interaction ($p = 0.008$); the mean response to male faces seen across both days was significantly lower in men given 20 IU than in those given 40 IU (−0.76, 95%CI: −1.39 to −0.13, $p = 0.02$; see **Figure 3D**). There was also a significant Dose × Drug Order interaction ($p = 0.04$); the mean response to all faces across both test days was significantly lower for subjects given 20 IU on day 1 than for subjects given 40 IU on day 1 (−0.99, 95%CI: −1.72 to −0.27, $p = 0.008$; not shown, but see further analysis below, as summarized in **Figure 3**). Additionally, the mean response, across days and sexes, was significantly lower in men given 20 IU on day 1 than in men given 20 IU on day 2 (−0.75, 95%CI: −1.49 to −0.01, $p = 0.046$; not shown), again suggesting the dose differences are, at least in part, associated with decreased responses induced by 20 IU on day 1. Because responses across days differed as a function of the dose given on day 1, we wanted

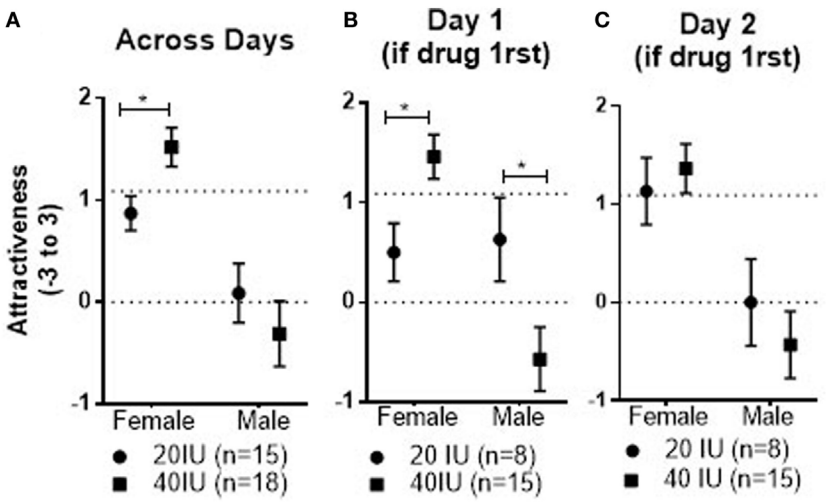

FIGURE 4 | Mean ± SEM of Attractiveness ratings of male and female faces across days in coupled men who received 20 or 40 IU on either day **(A)**, as well as responses on day 1 **(B)** and day 2 **(C)** in coupled men who received 20 or 40 IU on day 1. The top dotted line shows the mean response to female faces on day 1 in coupled men who received placebo on that day, the bottom dotted line the mean response to male faces in men who received placebo on day 1.

to dissociate and test for acute and carryover effects. We, therefore, examined pairwise comparisons between subjects given 20 and 40 IU on day 1 for faces seen after drug on day 1 and after placebo on day 2, even though the Drug × Dose × Stimulus Sex × Drug Order interaction was not significant. The mean responses to female and male faces on day 1 were significantly lower for subjects given 20 IU than for subjects given 40 IU (female faces: −0.97, 95%CI; −1.77 to −0.11, $p = 0.03$; male faces: −1.39, 95%CI: −2.37 to −0.42, $p = 0.006$; see **Figure 3E**), consistent with predicted acute differences. Additionally, the mean response to male faces seen after placebo on day 2 was significantly lower for men that had been given 20 than 40 IU on day 1(−1.25, 95%CI: −2.19 to −0.31, $p = 0.01$; see **Figure 3F**), consistent with carry-over effects that generalized to the new male faces. In contrast, the mean for female faces seen after placebo on day 2 did not differ between subjects that had been given 20 and 40 IU on day 1 (−0.39, 95%CI: −1.18 to 0.4, $p = 0.32$).

Coupled Men

No significant main effects or interactions were detected for which follow-up, pairwise comparisons revealed significant differences between treatment conditions (AVP vs placebo) or doses.

Attractiveness

Single Men

No significant main effects or interactions were detected for which follow-up, pairwise comparisons revealed significant differences between treatment conditions (AVP vs placebo) or doses.

Coupled Men

There was no drug effect for either the 20 or 40 IU dose compared with placebo in the within-subject comparison. However, there was a significant Dose × Stimulus Sex interaction ($p = 0.03$); the mean response to female faces observed across test days was significantly lower in men given 20 IU than in those given 40 IU (−0.65, 95%CI: −1.17 to −0.13, $p = 0.02$; see **Figure 4A**). There was also a significant Drug × Dose × Stimulus Sex × Drug Order interaction ($p = 0.04$). For female faces, the mean for responses to faces observed after drug on day 1 was significantly lower in men given 20 IU than in men given 40 IU (−0.96, 95%CI: −1.7 to 0.23, $p = 0.01$; see **Figure 4B**). We cannot resolve whether that reflects lower responses in men givne 20 IU or higher responses in men given 40 IU. In contrast, the mean response to male faces seen after 20 IU on day 1 was significantly higher than the mean response after 40 IU on day 1 (1.2, 95%CI: 0.11 to 2.28, $p = 0.03$; see **Figure 4B**). Unlike what we observed in single men, there were not any carry-over effects of the doses in coupled men, as neither the mean responses to the female nor the male faces seen after placebo on day 2 were significantly different in men who had received 20 and 40 IU on day 1 (see **Figure 4C**).

Final Day Comparisons Stratified by Relationship Status

Approachability

Single Men

There was no drug effect for either the 20 or 40 IU dose compared with placebo in the within-subject comparison. However, there was evidence for dose differences that persisted over time, particularly if AVP had been administered first, as indicated by a marginal Dose × Drug Order interaction ($p = 0.05$). The mean for responses to all faces previously seen across test days was marginally lower in men given 20 IU on day 1 than in men given 40 IU on day 1 (−0.67, 95%CI: −1.37 to 0.04, $p = 0.07$; see **Figure 5A**). Additionally, the mean response in men given 20 IU was significantly lower if they had received drug on day 1 than on day 2 (−0.82, 95%CI −1.56 to −0.08, $p = 0.03$; not shown). Together, these results suggest lasting influences of AVP that appear, at least in part, associated with decreased responses induced by 20 IU on the first test day. Consistent with that possibility, responses in men given 20 IU on day 1 remained below the "baseline" responses on day 1 for men given placebo on that day (see dotted lines in **Figure 5**). In our parallel fMRI study, Approachability ratings of male faces increased across days in single men given placebo or 40 IU on day 1 as a function of experience seeing the faces or simply going through the task (44), which did not happen in single men given 20 IU in the current study.

Coupled Men

There was no drug effect for either the 20 or 40 IU dose compared with placebo in the within-subject comparison. However, there was a significant Dose × Stimulus Sex interaction ($p = 0.001$), suggestive of dose differences that extended beyond the time of drug delivery, though not exclusively associated with delivery on day 1. Independent of drug order, the mean response was marginally lower for female faces previously observed across both trials in men given 20 IU than in men given 40 IU (−0.68, 95%CI: −1.36 to 0.1, $p = 0.05$; not shown). Again, we cannot determine if that dose difference is associated with lower responses in men given 20 IU, higher responses in men given 40 IU, or both. There was also a significant Drug × Dose × Stimulus Sex × Drug Order interaction ($p = 0.01$). For female faces, the mean for responses to the faces previously seen after placebo on day 1 was significantly lower in men given 20 IU on day 2 than in men given 40 IU on day 2 (−1.58, 95%CI: −2.94 to −0.23, $p = 0.02$). However, the small number of coupled men who received placebo on day 1 and drug on day 2, as already discussed, make it necessary to exercise caution in interpreting that difference, which may be associated with starting differences between coupled men who received placebo on day 1 and 20 or 40 IU on day 2.

Willingness to Initiate Conversation

Single Men

There was no drug effect for either the 20 or 40 IU dose compared with placebo in the within-subject comparison. However, there was evidence for dose differences that persisted over time, particularly if AVP was administered on day 1. There was a significant Dose × Drug Order interaction ($p = 0.03$); independent of Stimulus Sex, the mean for responses to all the faces previously observed was significantly lower in men given 20 IU on day 1 than in men given 40 IU on day 1 (−0.92, 95%CI: −1.63 to −0.22, $p = 0.01$; see **Figure 5B**). Additionally, for subjects given 20 IU, the mean response was significantly lower if the drug had been given on day 1 than if it had been given on day 2 (−0.8, 95%CI: −1.54 to −0.05, $p = 0.03$; not shown).

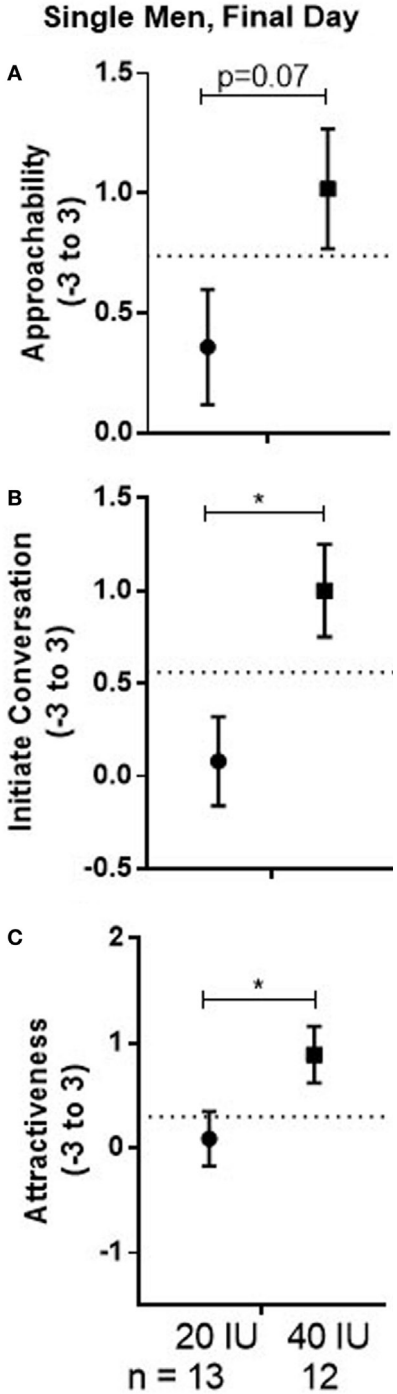

FIGURE 5 | Mean ± SEM of Approachability **(A)**, Initiate **(B)**, and Attractiveness **(C)** ratings, averaged across sex, on the final day of testing when no drug was given in men who received 20 or 40 IU on day 1. The dotted line shows mean response to all faces on day 1 in men who received placebo on that day.

Coupled Men

There was a significant Drug × Dose × Stimulus Sex × Drug Order interaction ($p = 0.04$). The mean for responses to faces previously seen after placebo on day 1 was marginally lower in men given 20 IU on day 2 than in men given 40 IU on day 2 (−1.08, 95%CI: −2.22 to 0.06, $p = 0.06$). However, for reasons discussed above related to small sample sizes and potential starting differences in responsiveness to faces in coupled men who received placebo first and drug second, these differences should be interpreted cautiously.

Attractiveness

Single Men

There was no drug effect for either the 20 or 40 IU dose compared with placebo in the within-subject comparison. However, there was evidence for dose differences that persisted over time, particularly if AVP was administered on day 1. There was a marginal Dose × Drug Order interaction ($p = 0.09$); the mean response to all faces previously observed on both test days was significantly lower in men who received 20 IU on day 1 than in men who received 40 IU on day 1 (−0.8, 95%CI: −1.55 to −0.05, $p = 0.04$; see **Figure 5C**). There was also a significant Dose × Stimulus Sex interaction ($p = 0.03$), though pairwise comparisons only detected a marginally lower mean for responses to male faces in men given 20 IU than in men given 40 IU (−0.71, 95%CI: −1.50 to 0.81, $p = 0.08$).

Coupled Men

There was a significant Dose × Stimulus Sex interaction ($p = 0.04$); the mean response to female faces was marginally lower in subjects given 20 than 40 IU (−0.53, 95%CI: −1.17 to 0.1, $p = 0.097$). There was also a significant Drug × Dose × Stimulus Sex × Drug Order interaction ($p = 0.02$). For female faces, the mean response to faces previously seen after placebo on day 1 was significantly lower in men given 20 IU on day 2 than in men given 40 IU on day 2 (−1.42, 95%CI: −2.65 to −0.15, $p = 0.02$). Again, we suspect this difference may reflect starting differences between the small numbers of men given 20 and 40 IU on day 2.

Model Variations

None of the patterns in the models were affected by dropping four subjects with extreme emotional trauma (more than 2.5 SD from the mean for the average scores of emotional neglect and emotional abuse). Nor were they altered by dropping individuals who were not exclusively heterosexual, or adding RS3 allelic variation as a covariate (whether individuals had 0, 1, or 2 long alleles, and whether they had 0, 1, or 2 versions of the 335 allele). However, when we specifically compared responses to faces in men who had received 20 or 40 IU on day 1, the day when AVP effects were evident, between those who carried at least one 335 risk allele and those who did not, we found preliminary evidence that the allele may influence responsiveness to AVP. For single men, the mean Approachability rating of faces observed on day 1 was marginally lower in single men who received 20 IU and carried at least one copy of 335 ($n = 6$) than in men who received 20 IU but did not have a copy ($n = 9$; −1.02. 95%CI; −2.06 to −0.2, $p = 0.05$). For coupled men, the mean Initiate rating of female faces on day 1 was marginally higher in those who received 40 IU and did not carry a 335 copy ($n = 6$) than in men who received 40 IU but did carry at least one copy ($n = 8$; 0.92, 95%CI; −0.02 to

1.85, $p = 0.05$). Similarly, the mean Attractiveness rating of female faces observed on day 1 was marginally higher in coupled men who received 40 IU and did not carry a 335 copy ($n = 6$) than in those who received 40 IU and did carry at least one copy ($n = 8$; 1.13, 95%CI: 0.02 to 2.24, $p = 0.05$). Together, these preliminary findings are generally consistent with the hypothesis that carrying 335 increases acute, negative responses induced by low doses of AVP and decreases positive influences potentially induced by higher doses. However, we note that while there were dose differences associated with AVP delivery on day 1 in coupled men, we were unable to determine to what extent, if at all, those differences were associated with increased responses induced by 40 IU.

DISCUSSION

In this study two doses of intranasal AVP administration differentially influenced subjective responses to faces in men, and those influences differed between men who reported being single and those who reported being in a relationship. In single men, the lower dose, relative to the higher dose and, for Initiate, relative to placebo, generally decreased ratings of faces, although the most persistent differences were selective for male faces. On the other hand, lowered responses associated with 20 IU relative to 40 IU were more selectively associated with female faces in coupled men, and the dose differences toward men even reversed, with 20 IU associated with higher ratings than 40 IU for Attractiveness. Although we could not show that the higher dose increased positive ratings of the faces relative to placebo in this study, it did, several days after drug delivery, in a parallel fMRI study that measured some of the same behavioral responses [(44), see further discussion below]. Together, these studies suggest that different doses of AVP produce opposing effects on some social responses, perhaps as a function of different patterns of peptide receptor activation in the brain. Although the higher dose did appear to influence one peripheral response, it decreased, rather than increased, systolic BP, an effect inconsistent with peripheral vasoconstrictive influences and therefore suggestive of a central mechanism of action. Most importantly, and consistent with our parallel fMRI studies (44), some of the effects of intranasal AVP administration appeared potentially long lasting.

Similar dose differences were observed in single men for responses related to how approachable other faces appeared and how likely they would be to initiate conversation with those individuals. On the other hand, differences for Attractiveness, a potential index of sexual/romantic interest, were not different in single men given 20 and 40 IU, nor different from placebo for either dose. In contrast, the higher doses of AVP did increase Attractiveness ratings several days after drug adminstration in our parallel study in single, heterosexual men, though only to same-sex faces, suggesting that effect was also unrelated to sexual/romantic interest (44). All three behavioral responses were highly correlated and each likely reflects a global social assessment of the faces that the different populations of subjects in the two studies, primarily urban Caucasian men in this study and a diverse group of college students in our parallel study, may have emphasized/used differently. We are, therefore, hesitant to try to speculate on AVP influences on specific psychological parameters, but rather suggest that AVP manipulations generally affected tendencies to see others more or less positively, with lower doses promoting less positive assessments relative to high doses, and for Initiate, at least on day 1 in single men, relative to placebo. This pattern is consistent with the negative effects of 20 IU in our previous study (38). Unfortunately, we could not conclusively determine if 20 IU decreased, and/or 40 IU increased, ratings in the contrasts across test days, likely because of variation related to ratings of different individuals across test days and/or carry-over drug order effects that will be discussed below. However, it is worth noting that Approachability and Initiate ratings across the 2 days, and on the final day, remained lower in men given 20 IU than the "baseline" ratings of faces after placebo on day 1. We have observed that Approachability ratings of male faces generally increase across days in men because of experience with the faces and/or procedure [(44); Initiate was not measured in that study]. However, that did not happen in men given 20 IU in the current study, consistent with negative, lasting effects of the lower dose.

We were unable to conclusively demonstrate acute or lasting positive effects of 40 IU on ratings of male or female faces in the present study, but that dose did enhance Attractiveness ratings in our parallel fMRI study on a follow-up test days after drug delivery. The inability to detect similar effects of 40 IU in the present study may be the result of differences in experimental design and/or drug delivery methods in the two studies. The within-subjects nature of the current design may have obscured any such effects (see further discussion below), and the two studies used different drug delivery devices. We used a device that delivered the complete dose in a single, small volume of spray in an effort to avoid the leakage that we have observed sometimes accompanies repeated sniffs, which would decrease accuracy of the doses delivered. In our parallel fMRI study (44), in contrast, the dose was delivered *via* multiple sniffs. If effective entry into the brain depends on repeated sniffs and saturation of the nasal mucosa, as was suggested in a critical review of intranasal delivery methods (45), then higher central elevations may have occurred in subjects given 40 IU in the fMRI study than in subjects given 40 IU in this study. However, even with those delivery differences and associated difficulties comparing elevations of AVP likely induced within the central nervous system between the studies, together they suggest that different doses of intranasal AVP may produce opposing effects on some behaviors. We suggest lower doses decrease social assessments of others and higher doses increase them.

Arginine vasopressin and its non-mammalian homolog AVT dose-dependently affect social behaviors in other species. For at least some behaviors, there is an optimal dose associated with an inverted U dose relationship (8, 46, 47). This suggests that particular patterns of receptor activation have unique behavioral effects and that increasing doses produce patterns that counteract those induced by lower doses or produce different behavioral outcomes altogether. In this and our parallel study, the higher dose could have more broadly activated different types of receptors to which AVP has lower binding affinities, including OT receptors. OT can stimulate affiliative interactions in numerous species, including humans [reviewed in Ref. (48)], so increasing cross talk with such receptors at higher doses could negate

antisocial effects of lower doses, which we propose happened in the present study, or produce positive responses, as observed in our parallel study. Dose-dependent receptor cross talk may not be purely pharmacological. Studies in rodents have not only shown that exogenous administration of AVP or OT can affect social behavior through promiscuous receptor activation (49, 50), but also that endogenous AVP and OT exert influences on some behaviors, including affiliative interactions, through receptor cross-talk mechanisms (18, 51).

On the other hand, it is possible that the effects of one or both doses are purely pharmacological. We do not yet know what the local concentrations of AVP in different brain circuits are following intranasal delivery of either dose or even what physiologically relevant concentrations of AVP are within individual circuits during social interactions in humans, though levels in extracellular space may be quite high, as they are in rodents (52). Furthermore, it is possible, if not probable, that intranasal peptide delivery simultaneously affects multiple circuits and induces patterns of brain activity, directly or as a result of feedback from the periphery, which could have happened in response to the higher dose in this study, which decreased systolic BP, that are not typical of any that occur in natural contexts. Those "unique" patterns could then produce behavioral influences that are not reflective of endogenous AVP functions. Thus, while this and other studies that have utilized intranasal methods do shed some light on potential roles that endogenous AVP systems play in social regulation, they should be interpreted cautiously. However, such studies do highlight the complexity and diversity of effects that pharmacologically targeting AVP systems may have upon behavior in clinical settings, some of which could be quite unintended.

Our results suggest some stimulus specificity for intranasal AVP's influences on face processing, though they are complex, depending on duration (acute vs long term), relationship status, and possibly genotype. In single men, comparisons on day 1 indicated that while 20 IU, relative to 40 IU, generally decreased ratings of faces, the only significant difference between 20 IU and placebo was for Initiate responses to male faces. We also identified what appeared to be carry-over differences between single men given 20 and 40 IU on day 1 to new faces observed on day 2, though only toward new male faces. In our parallel study, long-term effects of AVP in single men were selective for male faces (44). Likewise, 20 IU AVP has been shown to selectively affect men's ability to process emotional cues in the faces of other men, but not women (36). Together, these studies indicate that AVP plays a predominant role in the processing of same-sex faces in men, but that its effects are not exclusive to males face processing. Indeed, in coupled men at least some of AVP's influences appeared more selective for female faces, the ratings of which, at least for Attractiveness, were lower in men given 20 IU than in men given 40 IU.

It is possible the differences in stimulus specificity for AVP influences in single and coupled men were related to different perceptions of the social context of the rating task. The juxtaposed presentation of male and female faces, rated on attributes associated with interpersonal interactions, including those potentially related to sexual interest, could have created a context of reproductive competition that was perceived differently in single and coupled men. In single men, the male faces may have represented a source of threat/competition for the female pictured, and thus the most persistent influences of AVP in single men, which we argue reflect decreased ratings associated with 20 IU, were antisocial responses toward other male faces. Of course, as mentioned, not all of the dose differences were exclusive for male faces, suggesting that at least some of AVP's influences may be part of a more generalized response that decreases assessments of faces, perhaps in relation to AVP's ability to increase stress responses in conditions of social threat (53). It is also possible that some of the dose differences reflect, in part, increased ratings of faces, particularly male faces, induced by the higher dose. If so, that would be consistent with positive effects induced by 40 IU in our parallel study, albeit on a slower time scale (44).

In men who reported being in a romantic relationship, on the other hand, in which the lower dose, relative to the higher dose, was selectively associated with decreased ratings of female faces, the perceived social context may have been different. It is possible that the novel female was the larger perceived threat to those men, who were rating the attractiveness of unfamiliar women in the absence of their partner. Thus, 20 IU may have lowered social assessments of this threat to their current relationship. Similarly, intranasal OT selectively promotes withdrawal from unfamiliar women in men who are in relationships (54). The faces of other men, on the other hand, would presumably not be a rival for the absent partner. In those contexts, the dose differences even appeared to change, with the lower dose increasing ratings of the male faces relative to the higher dose. It is also possible the dose difference in responses to female faces reflect, in part, increased responses induced by 40 IU. Although 40 IU did not increase ratings of female faces in single men in our parallel study, it did selectively increase neural responds in the ventral striatum and septum to female faces (44), both areas in which nonapeptides induce affiliative responses related to pair bonding in prairie voles (see further discussion below).

This context-dependent explanation for potential differences in stimulus specificity between single and coupled men would suggest that AVP has a common effect on the brains of single and coupled men, and that the divergent behavioral outcomes of that effect are a function of differences in perceived social contexts. Alternatively, it is possible that AVP differentially affects the brains of single and coupled men. Pairing can influence vasopressin receptor expression and change responsiveness to social stimuli in prairie voles (17, 55), so such a mechanism is possible. In our parallel study, we only measured the effects of 40 IU in single men on brain responses and not of the lower dose that we suggest decreased social assessments in this and our previous study (38). It will, therefore, be interesting to determine if AVP produces different patterns of brain responses to female and male faces in single and coupled men, particularly in nodes of the Social Brain Network, or if it induces similar patterns across those subject populations. If the latter occurs, it would suggest that behavioral differences between single and coupled men are a downstream consequence of that common activation, filtered by social context.

We did not detect specific effects of AVP relative to placebo for either dose in our within-subjects comparisons across days, and

many of the dose differences were qualified by drug order. Those interactions were largely associated with AVP delivery on the first test day. Responses to faces on the first day were lower in single men given 20 than 40 IU, as were responses to the faces seen after placebo on the second test day, and responses to female faces were lower in coupled men given 20 IU relative to 40 IU on the first day. Additionally, responses on the final day to the faces previously seen across the first 2 days were lower in men who had received 20 IU on day 1 than in men who had received 40 IU. These patterns suggest that acute AVP effects may be most pronounced in novel/ambiguous test contexts (the first day), and that some of its effects may be long lasting and potentially generalizable to faces seen subsequent to AVP's initial administration. We had originally predicted that AVP would acutely affect responses to faces independent of the day of drug delivery, and that any lasting influences would be selective for the faces paired with drug. We did find some evidence for more selective, long-term effects of AVP for faces seen immediately after AVP in our parallel study (44). In that study 40 IU AVP increased positive ratings of the male faces paired with AVP 2–21 days after AVP delivery, but not of a novel face seen for the first time on that final test day. It remains to be resolved whether differences in how selective or generalized the lasting influences of AVP were in the two studies reflect unique mechanisms induced by the different doses in relation to their promotion of positive [high dose (44)] and negative (low dose, current study) responses or simple differences in study design.

The mechanisms through which either dose of intranasal AVP may produce prolonged negative and/or positive effects on face evaluations are entirely unclear. Acute elevations of AVP enhance social learning and memory processes in rodents, presumably by altering connectivity within neural networks (24, 56–58). However, those influences are selective for specific individuals encountered immediately before AVP administration; in the current study, at least, AVP effects appeared to influence responses to faces seen after drug delivery and to generalize to new faces seen on subsequent days, which suggest an alternative mechanism that could involve lasting, general influences on social stimulus processing. It seems unlikely that a single dose of AVP could induce epigenetic changes within those circuits, yet it does remain a possibility, especially if intranasal AVP can trigger feed-forward mechanisms that facilitate further and possibly prolonged release (59). Recent studies have demonstrated epigenetic modifications induced by the cohabitation/mating experiences that trigger AVP release and, as a result, induce pair bonding in prairie voles (25, 26), but it is not yet known if the AVP released during those interactions contributes to the epigenetic modifications. It is also possible the prolonged behavioral influences in this and our parallel study do not reflect lasting effects of acute elevations on the brain, which is easy to presume because of the short half-life of AVP in tissue (60). Rather, they could be a function of lasting elevations of AVP induced by the intranasal administration. Despite its short half-life in tissue, levels of AVP in cerebrospinal fluid were still elevated 120 min after drug delivery in the original studies by Born et al. (28). As mentioned, AVP has been shown to facilitate feed-forward mechanisms that can promote further release within the brain (59), raising the possibility of a surprisingly long-window in which intranasally delivered AVP could influence social/emotional processes.

AVP did not promote positive ratings of female faces, acutely or over time, even when we restricted the model to heterosexual, single men. We had hypothesized that AVP might, in single men as in unpaired male prairie voles, stimulate affiliative processes that would promote interactions with potential reproductive/romantic partners, manifested as more positive ratings of female faces. Our parallel fMRI studies also failed to detect AVP effects on behavioral responses to female faces in single men, though 40 IU AVP did selectively increase activation in the ventral striatum and septum, areas in which AVP and OT modulation influences affiliative behaviors related to pair bonding in prairie voles, when males looked at female faces. Thus, it is possible that our behavioral measures simply did not capture responses related to tendencies to form emotional attachments in reproductive contexts. It should also be noted that continuous infusions of AVP, while males have extended contact with females, are required to enhance partner preferences in male prairie voles; single injections are not effective (18). We only delivered AVP once, and interactions with females were limited to brief exposures to their faces, which were devoid of positive emotional cues that are likely necessary to promote affiliative interactions. Thus, it is likely these tests did not promote the concomitant dopamine release that normally occurs during cohabitation in voles and that is necessary for AVP to induce partner preferences (61). Of course, life histories associated with pair bond formation in reproductive contexts evolved independently in most lineages in which such behaviors are evident, including humans, and there is not yet conclusive evidence for convergent AVP/AVT mechanisms that promote pair bonding in males across those species (62–64). Thus, it is also possible that endogenous AVP does not play a role in pair bond formation in reproductive contexts in humans, or that it plays a role in relationship maintenance, rather than pair bond formation. In another primate that forms long-term pair bonds, titi monkeys, intranasal AVP increases social contact with already established mates in males (65).

We also ran models that included RS3 allelic variation previously associated with differences in social responses (27, 66–71). Inclusion as a covariate of whether men had one or two long alleles (≥335), or one or two copies of a previously identified risk allele (335 with our primers), did not influence the pattern of results. However, our preliminary, focused analysis of whether having at least one copy of the 335 risk allele in men given AVP on the first test day, when the drug appeared most likely to influence behavior, suggest the allele may, as predicted, be associated with more negative, antisocial responses to AVP. In single men, Approachability responses were lower in men given 20 IU on the first day if they carried at least one copy of 335, and Attractiveness responses were not as high in coupled men given 40 IU who carried at least one copy as in those who did not. These patterns suggest the risk allele may increase negative social assessments associated with low doses and/or decrease positive assessments associated with higher doses. Consistent with the possibility that AVP might induce more negative/less positive social assessments in carriers of the risk allele, men with that allele show heightened amygdala responses to faces (43).

Unfortunately, we did not have sample sizes sufficient to evaluate more fully the relationship between V1a gene variation and responsiveness to AVP.

Study Limitations

There are several limitations with the current study and with intranasal delivery studies more generally, some of which have already been discussed. Intranasal delivery is likely highly variable as a simple function of individual competence with self-delivery. Furthermore, we know nothing about local elevations in individual brain regions that follow the delivery of different doses, or even about elevations within cerebral spinal fluid that result from the same dose delivered with different applicators. We agree with Churchland and Winkielman's (47) argument that a systematic study comparing elevations in CSF using different applicators would be quite helpful in that regard, as would determining how long the elevations persist. More specifically related to this study, the stimuli used were all Caucasian, and the study population was largely limited to Caucasians. Therefore, we suggest caution about generalizing results related to dose-dependencies or long-term influences to non-Caucasian populations or to influences on responses toward more diverse groups of subjects that could be moderated by in-group/out-group perceptions. Perhaps most importantly, the unexpected carry-over/lasting influences of AVP discovered in this and our parallel study suggest that within-subjects, repeated measures designs, even when drug order is counterbalanced and statistically accounted for, are difficult to interpret. Such designs are common in non-human studies of peptide effects, under the presumption that these peptides do not produce long-term influences on behavior in adults. We suggest future studies should consider potential long-term and drug-order effects more carefully.

CONCLUSION

The present results suggest that AVP produces dose-dependent influences on face processing in men, that those influences differ as a function of relationship status, and that some may be long lasting and potentially generalizable to faces seen after the initial drug delivery. The potential for intranasal AVP to induce long-lasting effects on behavior, in particular, warrants further discussion on the use of this method for basic research and the implications that might be associated with clinical interventions that pharmacologically target the AVP system.

ETHICS STATEMENT

All subjects received informed consent, and the protocol was approved by the Bowdoin and Maine Medical Institutional Review Boards, as well as by the US Food and Drug Administration.

AUTHOR CONTRIBUTIONS

DP participated in study design, evaluated all subjects, oversaw day-to-day running of the experiment, and wrote the paper. DB and AC ran all subjects, kept all logs, and advised on technical aspects of study implementation. CT ran all statistics and wrote the paper. JR participated in study design and wrote the paper. RT participated in study design, data interpretation, and wrote the paper.

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Corrigendum: Dose-Dependent and Lasting Influences of Intranasal Vasopressin on Face Processing in Men

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A corrigendum on

Dose-Dependent and Lasting Influences of Intranasal Vasopressin on Face Processing in Men
by Price D, Burris D, Cloutier A, Thompson CB, Rilling JK, Thompson RR. Front Endocrinol (2017) 8:220. doi: 10.3389/fendo.2017.00220

In the original article, there was a mistake in the legend for **Figure 5** as published. The legend should have only alluded to one dotted line in each panel indicating average responses to all faces on day 1 in single men who received placebo on that day. The correct legend appears below.

In the original article, there was a mistake in **Figure 5** as published. The subject numbers at the bottom of the graph were incorrect. The corrected **Figure 5** appears below.

The authors apologize for these errors and state that these do not change the scientific conclusions of the article in any way. The original article was updated.

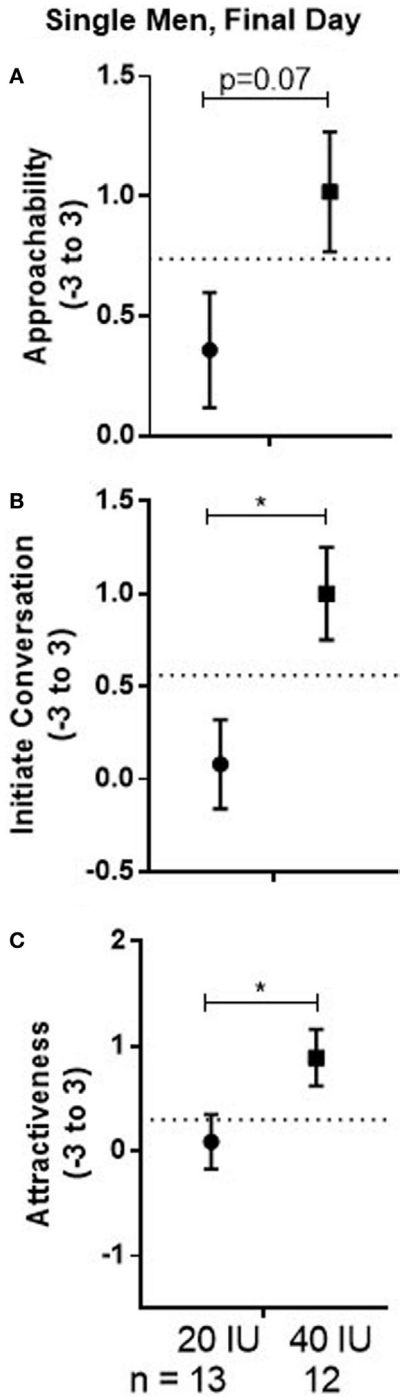

FIGURE 5 | Mean ± SEM of approachability **(A)**, initiate **(B)**, and attractiveness **(C)** ratings, averaged across sex, on the final day of testing when no drug was given in men who received 20 or 40 IU on day 1. The dotted line shows mean response to all faces on day 1 in men who received placebo on that day.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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