HHS Public Access

Author manuscript

Psychopharmacology (Berl). Author manuscript; available in PMC 2019 December 01.

Published in final edited form as:

Psychopharmacology (Berl). 2018 December ; 235(12): 3363–3379. doi:10.1007/s00213-018-5099-x.

Vasopressin and alcohol: A multifaceted relationship

Kathryn M Harper[1,*], **Darin J. Knapp**[1,2], **Hugh E. Criswell**[1,2], and **George R. Breese**[1,2,3]

[1]Bowles Center for Alcohol Studies, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7178, USA

[2]Department of Psychiatry, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7178, USA

[3]Department of Pharmacology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7178, USA

Abstract

Background: Arginine vasopressin (VP) has been implicated in a number of neuropsychiatric disorders with an emphasis on situations were stress increased the severity of the disorder. Based on this hypothesized role for VP in neuropsychiatric disorders, much research is currently being undertaken in humans and animals to test VP as a target for treatment of a number of these disorders including alcohol abuse.

Objectives: To provide a summary of the literature regarding the role of VP in alcohol and stress related behaviors including the use of drugs that target VP in clinical trials.

Results: Changes in various components of the VP system occur with alcohol and stress. Manipulating VP or its receptors can alter alcohol and stress related behaviors including tolerance to alcohol, alcohol drinking, and anxiety-like behavior. Finally, the HPA axis response to alcohol is also altered by manipulating the VP system. However, clinical trials of VP antagonists have had mixed results.

Conclusions: A review of VP's involvement in alcohol's actions demonstrates that there is much to be learned about brain regions involved in VP-mediated effects on behavior. Thus, future work should focus on elucidating relevant brain regions. By using previous knowledge of the actions of VP and determining the brain regions and/or systems involved in its different behavioral effects, it may be possible to identify a specific receptor subtype target, drug treatment combination, or specific clinical contexts that may point toward a more successful treatment.

Keywords

ethanol; vasopressin; hypothalamus; amygdala; anxiety; alcohol intake; tolerance; clinical trials; antagonists; stress

[*]Correspondence: Dr. Kathryn M Harper, Bowles Center for Alcohol Studies, Thurston Bowles Building, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7178, US.

The authors have no conflicts of interest.

1. Introduction

Arginine vasopressin (VP; also known as antidiuretic hormone or ADH) is a nine amino acid peptide known to be involved in a number of physiological and behavioral effects. In both adult mice and rats detailed immunohistchemical studies mapped the location of VP immunopositive neurons and their fibers. VP containing neurons are found most abundantly in the paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN), and supraoptic nucleus (SON) of the hypothalamus. Smaller quantities have been found in the Bed nucleus of the stria terminalis (BST), and medial amygdala (DeVries et al. 1985; Otero-Garcia et al. 2016; Rood and De Vries 2011; Sofroniew 1983; Song and Albers 2017). Colchicine, which is thought to cause cell body accumulation of proteins normally localized to fibers, has revealed staining in other brain regions including the locus coeruleus and septum (DeVries et al. 1985; Sofroniew 1985). The colchicine experiments suggest that traditional immunohistochemistry techniques alone will not identify low expression VP neurons that might have functions outside the high expression regions, e.g. hypothalamus, amygdala or BST. Other studies using a VP reporter mouse have found VP neurons in the retina and olfactory bulbs (Moritoh et al. 2011; Tobin et al. 2010) and mRNA gene arrays have reported VP expression in the nucleus accumbens (Rodriguez-Borrero et al. 2010). These later studies suggest that VP may be synthesized in the cell body of neurons and rapidly transported to terminal regions. Thus, emerging studies using a combination of immunohistochemical procedures to localize protein and RNA can be expected to find a broad brain regional distribution of VP neurons beyond the traditionally known hypothalamic and extended amygdala regions.

A map showing known projections of the VP containing neurons can be seen in figure 1, however studies vary widely on which fibers come from which brain region. This variation seems to mostly stem from the technique used to identify the source of the fibers and perhaps species as well. The VP neurons in the medial amygdala have been found to project to the hippocampus, BST, and lateral septum (Caffe et al. 1987). The VP neurons in the BST project to the lateral septum, amygdala, ventral pallidum, lateral habenular nucleus, the periventricular gray, and the locus coeruleus (De Vries and Buijs 1983; Otero-Garcia et al. 2014). The VP neurons in the SCN project to the vascular organ of the lamina terminalis, the periventricular nucleus, the PVN, and the dorsomedial hypothalamus (Buijs 1978; 1980; Hoorneman and Buijs 1982; Rood et al. 2013; Sofroniew 1980; Sofroniew and Weindl 1978). The VP neurons in the PVN project to the pituitary, the spinal cord, and amygdala (Buijs 1978; 1980; Hernandez et al. 2016; Sofroniew 1980). The projection systems described above are based primarily on lesioning and retrograde-tracing (De Vries and Buijs 1983; Hoorneman and Buijs 1982; Otero-Garcia et al. 2014; Rood et al. 2013). Early studies where individual neurons were stained and followed to other brain sites showed many more possible projection pathways (Buijs 1978; 1980; Sofroniew 1980; Sofroniew and Weindl 1978). However, lesioning the nucleus of origin did not alter the VP fibers in the proposed projection region, a finding that suggested that the contribution was minor compared to inputs from other sites. These sites were not included in the above description. Therefore more detailed lesioning studies in a region by region fashion or site by site retrograde tracing

using techniques like fluorescent beads combined with immunohistochemistry might assist in confirming the existing data.

VP binds to one of three G protein-coupled receptors, vasopressin receptor 1a (V1a), vasopressin receptor 1b (V1b) and vasopressin receptor 2 (V2) (Barberis and Tribollet 1996; Hasunuma et al. 2013; Song and Albers 2017). The V1a receptor regulates a phosphatidylinositol 4,5-bisphosphate (PIP2) pathway and is expressed throughout the brain in the frontal cortex, piriform cortex, anterior olfactory nucleus, SCN, hippocampus, PVN, ventromedial hypothalamic nucleus, arcuate nucleus, lateral habenular nucleus, cerebellum, BST, nucleus accumbens, and amygdala (Song and Albers 2017; Szot et al. 1994; Veinante and Freund-Mercier 1997). The V1b receptor regulates PIP2 pathway and is expressed in the hippocampus, PVN, amygdala, lateral septum, BST, frontal cortex, cingulate cortex, entorhinal cortex, parietal cortex, nucleus accumbens, and pituitary (Stevenson and Caldwell 2012; Young et al. 2006). The V2 receptor regulates an adenylate cyclase/cyclic adenosine 3',5'-monophosphate (cAMP) pathway and by adulthood V2 receptors seem restricted to the cerebellum and outside the brain (Hirasawa et al. 1994; Kato et al. 1995). Most of the work involving vasopressin and alcohol has not focused on the V2 receptor, but rather on the V1a and V1b receptors, and given their distribution there are many potential brain regions through which VP can have effects on alcohol related behaviors.

There are sex differences in the VP system, which are partly thought to stem from the fact that many VP neurons also contain gonadal steroid hormone receptors and gonadal steroid hormones have been shown to control the synthesis of VP (Carter 2007; Pak et al. 2009; Thomas et al. 1996; van Leeuwen et al. 1985; Zhou et al. 1994). In many species, including humans, males have more VP immunoreactive fibers, more VP immunoreactive neurons, VP neurons of larger size, and altered V1a receptor binding densities (De Vries and Panzica 2006; DiBenedictis et al. 2017; Dumais and Veenema 2016; Ishunina and Swaab 1999; Rood et al. 2013; Smith et al. 2017; Winslow et al. 1993). This upregulation of the VP system in males is thought to underlie the findings that VP exposure in human and rodent males causes a behavioral response at a dose where females show either no response or a different behavioral outcome (Chen et al. 2016; Terranova et al. 2017; Thompson et al. 2006).

VP has neuromodulatory activities in brain regions suggesting it could affect behavior. For example VP can excite gamma-Aminobutyric acid (GABA) neurons in the central nucleus of the amygdala (CeA) (Huber et al. 2005), excite serotonin neurons in the dorsal raphe (Rood and Beck 2014), excite spinal motorneurons (Raggenbass 2008), excite GABA septal neurons (Raggenbass 2008), and excite GABA neurons in the hippocampus (Ramanathan et al. 2012), all of which are thought to be mediated by V1a. Additionally, VP has effects on plasticity modulating long term potentiation and long term depression in GABA neurons in the BST (Naughton 2016). In the hippocampus VP facilitates production of long term potentiation of CA1 and dentate gyrus neurons (Chen et al. 1993; Chepkova et al. 2001). Thus, VP is an excitatory transmitter at the level of the synapse and has been known to impact plasticity in multiple neurocircuits.

Effects of VP cannot be fully understood without looking at its interaction with oxytocin (OT) and these interactions occur at multiple levels. OT shares 7 of the 9 amino acids comprising VP and their receptors show a great deal of cross reactivity (Song and Albers 2017). Additionally, there are anatomical links between the VP and OT systems. For example, OT and VP receptors exist in many of the same brain regions but in different subdivisions within those brain regions (Huber et al. 2005; Veinante and Freund-Mercier 1997). In the case of the amygdala these OT and VP subdivisions even interact such that the OT receptor containing neurons in the centrolateral nucleus of the amygdala project to the VP receptor containing neurons in the centromedial nucleus of the amygdala and alter their firing pattern (Huber et al. 2005). Such serial interactions between OT and VP can explain why they have different or even opposite effects on the same behavior through the same brain regions (Huber et al. 2005; Neumann and Landgraf 2012; Stoop 2012). Therefore, in cases were VP antagonists have been suggested as a treatment OT agonists are also sometimes suggested as a treatment. This is also true of the alcohol field (Lee and Weerts 2016).

When discussing VP, another commonly discussed peptide hormone is corticotropin-releasing factor (CRF). Like VP, CRF is also made by neurons in the PVN. CRF has two receptors CRF-R1 and CRF-R2. CRF-R1 is expressed mainly in the brain and CRF binds CRF-R1 preferentially over CRF-R2. CRF from the PVN leads to release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. The released ACTH leads to secretion of cortisol/corticosterone (CORT) from the adrenal glands. Therefore, CRF is an important player in the hypothalamic pituitary adrenal (HPA) stress response, an outcome which is also true of VP. The same PVN neurons contain both CRF and VP which likely can lead to coordinated release of the two peptides (Whitnall et al. 1985). Simultaneous release of both CRF and VP enhances the effects of CRF on ACTH release (Gillies et al. 1982). Therefore, for stress-related neuropsychiatric disorders where VP antagonism is a suggested treatment, CRF antagonism is a suggested treatment as well.

2. Alcohol's effect on the VP system

An association between VP and alcohol was initially discovered in the context of alcohol-induced changes of plasma VP. Since neurons in the hypothalamus have terminals in the posterior pituitary which release VP into circulation, a change in plasma VP can be an indicator of changes in VP in the brain or at least in those specific hypothalamic neurons. In humans, plasma VP levels often decrease during alcohol consumption and increase upon cessation of consumption (Eisenhofer and Johnson 1982; Gill et al. 1982; Helderman et al. 1978; Linkola et al. 1978; Mander et al. 1989; Taivainen et al. 1995). Interestingly, individuals who abuse alcohol seem to have differences in their VP system compared to more alcohol naïve individuals. When comparing alcoholics to more alcohol naïve individuals, alcoholics were found to have a more pronounced decrease in plasma VP levels when drinking (Collins et al. 1992; Hirschl et al. 1994), suppressed VP levels even during alcohol withdrawal (Doring et al. 2003; Hirschl et al. 1994; Jahn et al. 2004; Trabert et al. 1992), and a lack of a VP increase in response to novelty (Ehrenreich et al. 1997). Similarly, differences in urine VP (urine VP being an approximate measure of plasma VP) are found between alcohol preferring (AA rats) and alcohol non-preferring rats (ANA) (Linkola and

Fyhrquist 1978; Linkola et al. 1977). However the two studies do not agree on the directionality of the difference as one study found a decrease in urine VP in AA rats compared to ANA rats and the other an increase in urine VP in AA rats compared to ANA rats. A possibly related variation found in alcohol abusers is increased promoter-related DNA methylation of VP DNA extracted from blood (Hillemacher et al. 2009). Additional studies are needed to clearly define how brain, blood and urine VP are modified during alcohol use and abstinence.

It is now known that these alcohol induced changes in VP in the periphery extend to the brain. For example, it has been shown that there is a decrease in the number of VP immunoreactive neurons in the hypothalamus with alcohol exposure in rats and humans (Harding et al. 1996; Madeira et al. 1997; Paula-Barbosa et al. 2001; Silva et al. 2002; Sousa et al. 1995). However, these reports are conflicted as to whether withdrawal reverses the decrease in the number of VP neurons. Acute alcohol increases VP mRNA in the hypothalamus in rats (Ogilvie et al. 1997; Rivier and Lee 1996) whereas chronic alcohol decreases VP mRNA in hypothalamus and BST in both rats and mice (Gulya et al. 1991; Gulya et al. 1993; Hoffman and Dave 1991; Ishizawa et al. 1990; Madeira et al. 1997; Sanna et al. 1993). In contrast, with exposure to chronic alcohol there is an accumulation of VP protein in the hypothalamic neuronal nuclei of mice (Carmona-Calero et al. 1995). Thus, the amount of alcohol exposure as well as withdrawal impact brain VP.

3. VP and tolerance to alcohol

One of the first alcohol related behaviors to be associated with VP was tolerance (see table 1 for summary). This effect was shown by giving systemic injections of VP to C57BL/6 mice during and after alcohol treatment and seeing that tolerance to the hypothermic and sedative-hypnotic effects of alcohol was prolonged (Hoffman et al. 1978). Further support comes from studies that use DGAVP, the VP fragment (des- Gly^9-[Arg^8]-vasopressin dicitrate) which is thought to have reduced peripheral endocrinological activity compared to full VP (Hoffman 1982; Rigter et al. 1980a; Rigter et al. 1980b; Walter et al. 1975). Acute injection of DGAVP in mice (Rigter et al. 1980a), continuous systemic infusion of DGAVP in mice (Rigter et al. 1980b), daily systemic injections of DGAVP in mice (Hoffman 1982), and systemic daily injections of DGAVP in rats (Le et al. 1982) had similar effect on alcohol tolerance to that found with full length VP. Systemic injections of VP or DGAVP in mice also prolonged tolerance to the motor incoordinating effects of ethanol, but this effect was not prolonged as long as the hypothermic and sedative-hypnotic actions (Hoffman and Tabakoff 1984). Intraventricular injection of VP in mice also prolongs tolerance to the hypnotic effects of alcohol (Hung et al. 1984). Finally, it was found that V1 receptors are more likely to mediate this effect since intraventricular injections of the V1 agonist prolongs tolerance and V1 antagonists enhanced the rate of loss of alcohol tolerance. V2 receptor equivalents do not share these effects (Szabo et al. 1988). Intraventricular injections have also blocked the acquisition of environment dependent tolerance to the hypnotic and hypothermic effects of alcohol in a mouse learning paradigm (Mannix et al. 1986). The authors of this study highlight the use of alcohol injections in a standardized environment every day to create a specific type of tolerance, which they have demonstrated to be environment-dependent. This strategy differs from most of the other experiments which use

liquid diet, a method the authors claim does not produce environment dependent tolerance (Melchior and Tabakoff 1985). Therefore, while the mechanism of VP actions in alcohol tolerance may not be clear, these effects might depend on the type of tolerance with VP prolonging environment-independent tolerance and blocking environment-dependent tolerance.

Altogether the studies discussed above that used DGAVP as well as those studies that used intraventricular injections of VP, suggest VP has its effects on tolerance through actions in brain. However, what brain regions and receptor subtype (V1a vs V1b) mediate VP's effects of tolerance have thus far not been investigated. The brain regions mediating these effects most likely vary depending on which tolerance effects are studied. Future experiments on alcohol tolerance should focus on these earlier factors.

4. VP and alcohol drinking

Another alcohol related behavior to be associated with VP is alcohol drinking (see table 2 for summary). Some evidence for the role of VP in drinking behavior comes from the fact that rat strains that were selectively bred for their alcohol preference have higher levels of VP (Hwang et al. 1998; Zhou et al. 2011). For example, both alcohol-preferring (P) and high alcohol-drinking (HAD) rats have been shown to have elevated levels of VP mRNA in the hypothalamus (Hwang et al. 1998). Sardinian alcohol-preferring rats have more VP mRNA in the medial amygdala (MeA)/CeA and hypothalamus. Drinking alcohol decreased the levels of VP mRNA (Zhou et al. 2011). In agreement with the rat data, C57BL6/J mice with the highest levels of VP mRNA in the amygdala drank the greatest amount of alcohol (Nelson et al. 2018).

An association between the V1 receptor subtypes and alcohol drinking has also been established. In humans a modest association was found between a single nucleotide polymorphism in the V1a receptor gene and maximum drinks per in a 24 hour period in European-American males (Maher et al. 2011). A mouse knock-out of the V1a receptor on a mixed 129Sv and C57BL/6Cr Slc background increased alcohol consumption and preference for alcohol in the two bottle choice paradigm (Sanbe et al. 2008), but in a second study using a mixed C57BL/6J and 129/Sv-CP background only the heterozygote for the V1a knock-out showed increased alcohol consumption in the two bottle choice paradigm (Caldwell et al. 2006). Difference in genetic background of the knock-outs could account for the differences in results in the homozygous mice. A relationship has also been demonstrated between the V1b receptor and alcohol dependence when it was discovered that acute alcohol consumption in nondependent Wistar rats decreased V1b receptor protein in the basolateral amygdala (BLA), but once rats become dependent, alcohol consumption restores the protein levels of the receptor (Edwards et al. 2012). The authors of this study suggest that this change in the response of the V1b receptor to alcohol might be involved in the transition to dependence. On the other hand, when tested on the two bottle choice paradigm V1b knock-out mice showed similar alcohol consumption and preference as their wild-type littermates (Caldwell et al. 2006). In the case of knock-outs of either receptor subtype there is the chance of compensatory changes in the levels of other receptors that can conserve the behavior, for example increased expression of the oxytocin receptor can

compensate for the loss of the V1b receptor to allow for normal ACTH release in the V1b receptor knock-out (Nakamura et al. 2008). Thus, VP impacts drinking in complex ways needing further elucidation.

The studies discussed in the two paragraphs above use designs that mainly demonstrate a correlation, but leave cause and effect unclear. Evidence for a causal role of VP in drinking came from studies that used VP antagonists to alter drinking. When the V1b receptor antagonist is administered systemically, Sardinian alcohol-preferring rats decreased their alcohol intake (Zhou et al. 2011). This effect extends to other rats strains, as the V1b receptor antagonist given systemically also reduced drinking in alcohol dependent Wistar rats (Edwards et al. 2012). Additionally, the V1b antagonist blocked drinking in C57BL/6 mice alone at high doses and at lower doses in combination with naltrexone (Zhou et al. 2018). But perhaps, most importantly, a recent clinical trial demonstrated a significantly greater percentage of days abstinent following V1b receptor antagonism for individuals who met the criteria for DSM-IV alcohol dependence (Ryan et al. 2017). Conversely, 14 weeks of alcohol exposure with concurrent systemic DGAVP administration, the VP fragment that has reduced peripheral endocrinological activity compared to full VP, caused long lasting decreases in alcohol drinking in rhesus monkeys (Kornet et al. 1991; Kornet et al. 1992). One important difference between the Kornet et al (1991; 1992) studies and the other studies was that while the other studies initiated alcohol exposure followed by testing of the antagonist, this study did concurrent DGAVP administration with initial alcohol exposure. These results suggest that timing of the V1b antagonist might be important and therefore a V1b antagonist might have different effects before and after animals have transitioned to alcohol dependence. This interpretation is consistent with the findings of Edwards et al (2012) that there is a change in V1b receptor response after transition to alcohol dependence. Altogether these observations provide strong evidence for the role of VP in alcohol drinking behavior at least partially through the actions of the V1b receptor.

All of the pharmacological studies discussed above were done with systemic injections, and therefore do not give an indication as to which brain areas might be involved in these effects. As noted above, the amygdala is a source of VP (Otero-Garcia et al. 2016; Sofroniew 1983; Song and Albers 2017) and alcohol preferring rats have more VP in the amygdala than alcohol non-preferring rats (Zhou et al. 2011). The amygdala is a known contributor to drinking behavior (de Guglielmo et al. 2016; McBride 2002) and changes in the V1b receptor occur with drinking in the basolateral amygdala (Edwards et al. 2012). As V1b receptor antagonists have shown strong effects on drinking, these observations make the amygdala and the V1b receptor an attractive target for future work exploring the role of VP in alcohol drinking. The V1a receptor is a less attractive target as both heterozygous and homozygous V1a knock-outs increased alcohol consumption (Caldwell et al. 2006; Sanbe et al. 2008), but this work was only done in traditional knock-outs and more work using conditional knock-out approaches or pharmacological agents are warranted.

The diagnostic and statistical manual of mental disorders, 5th edition (DSM-5) criteria for alcohol use disorders (AUD) includes drinking related criteria like “had times when you ended up drinking more, or longer, than you intended?” and “spent a lot of time drinking? Or being sick or getting over other after effects” (APA 2013), so unsurprisingly alcohol

drinking paradigms like dependence induced excessive drinking have been suggested as models for testing treatments for alcoholism (Litten et al. 2012). Therefore, the effects of VP antagonists on dependence induced drinking could be a good indication for effectiveness as a treatment for AUD.

5. VP and stress-potential relationship to anxiety associated with alcohol

Interestingly, stress also alters the VP system. The forced swim test has been shown to cause release of VP in the amygdala (Ebner et al. 2002) and the septum (Ebner et al. 1999). Acute immobilization stress, repeated immobilization stress, chronic foot shock or long term social isolation all cause an increase in VP mRNA and VP immunoreactive neurons in the hypothalamus (Bartanusz et al. 1993; de Goeij et al. 1992; Ma and Lightman 1998; Makino et al. 1995; Pan et al. 2009; Pinnock and Herbert 2001; Sawchenko et al. 1993). Stress also increased the levels of V1b receptor mRNA (Aguilera and Rabadan-Diehl 2000; Litvin et al. 2011; Rabadan-Diehl et al. 1995) as well as V1a receptor binding and mRNA (Duque-Wilckens et al. 2016; Gray et al. 2012; Milik et al. 2014). Stress is also associated with alcohol withdrawal induced anxiety and with relapse in alcoholics (Breese et al. 2005a; Breese et al. 2011; Sinha 2007). The stress related changes in VP in alcoholics could lead to changes in the HPA and potentially impact alcohol related behaviors like drinking and alcohol withdrawal induced anxiety. Drugs that treat stress related disorders like anxiety could then potentially have an impact on treating alcoholics during stressful situations. In fact, the VP response to stress is related to the ability of VP to share a role with CRF in regulating the HPA axis response to stressors including alcohol. Blocking VP using either a passive immunoneutralization of VP or a V1 antagonist diminishes ACTH secretion caused by alcohol (Ogilvie et al. 1997; Rivier and Lee 1996), but other studies using the same immunoneutralization did not block alcohol-induced CORT or ACTH (Thiagarajan et al. 1989). Since using a CRF or VP antagonist alone could only blunt the alcohol induced ACTH secretion, one group gave both a CRF and VP antagonist and was able to abolish the alcohol induced ACTH section (Rivier and Lee 1996). The ability of VP to regulate release of ACTH and CORT in response to alcohol might also be partially mediated through the V1b receptor as the V1b receptor knock-out has reduced ACTH and CORT plasma levels compared to wild-types in response to acute alcohol (Lolait et al. 2007b). The V1b receptor knock-out mouse also has a reduced ACTH and CORT response to physical stressors such as restraint stress, the forced swim test, and insulin administration (Lolait et al. 2007a; Roper et al. 2010). Thus, stress and alcohol share adaptations in V1b regulation of the HPA axis.

Stressors like social isolation and social defeat that cause changes in the VP system also cause anxiety-like behaviors. Table 3 summarizes studies discussing the relation between VP and anxiety. The V1b receptor antagonist given systemically during stress blocks the effects of stressors on anxiety tasks like social defeat stress-induced anxiety in the elevated plus maze, chronic mild stress-induced anxiety in the elevated plus maze, and social defeat stress-induced anxiety on the social interaction task (Griebel et al. 2002; Litvin et al. 2011). Systemic administration of V1b receptor antagonists also alters nonstress related performance on elevated plus maze, light/dark box, punished drinking conflict test, conditioned lick suppression, social interaction test, and four-plate test (Griebel et al. 2002; Hodgson et al. 2007; Hodgson et al. 2014; Iijima et al. 2014; Overstreet and Griebel 2005;

Serradeil-Le Gal et al. 2002; Shimazaki et al. 2006). Systemic administration of a V1a receptor antagonist reduced anxiety on the elevated plus maze, elevated zero maze, marble burying test, and conditioned lick suppression (Bleickardt et al. 2009). Systemic administration of a VP agonist causes anxiety on the elevated plus maze, which is thought to be mediated through the V1a receptor (Mak et al. 2012). Mice bred for high anxiety were found to have a polymorphism in their VP gene leading to higher expression of VP while the reverse is true of the mice bred for low anxiety (Bunck et al. 2009; Murgatroyd et al. 2004). However, as with alcohol drinking, V1b knock-out mice show similar anxiety-like behavior on the open field, elevated plus maze and light/dark box as compared to their wild-type littermates (Egashira et al. 2005; Wersinger et al. 2002). On the other hand, male, but not female, V1a knock-out mice demonstrated reduced anxiety in the elevated open maze, light/dark box, open field, and marble burying test (Bielsky et al. 2004; Bielsky et al. 2005; Egashira et al. 2007). As mentioned above, interpretations of results of studies with knock-out animals are confounded by developmental shifts, but in general support VP involvement in anxiety.

A V1a antagonist administered via microdialysis probe (Liebsch et al. 1996) or antisense to the V1a receptor mRNA administered via osmotic minipump (Landgraf et al. 1995) in the Septum decreased anxiety related behaviors on the elevated plus maze, but microinjection of the V1b antagonist into the Septum had no effect on the elevated plus maze or the punished drinking conflict test (Stemmelin et al. 2005). Surprisingly injections of VP into the septum and intraperitoneally also decreased anxiety-like behavior in the elevated plus maze (Appenrodt et al. 1998) while general VP antagonism in the lateral septum increased anxiety in the elevated plus maze (Everts and Koolhaas 1999). As the studies overlap in anxiety test and rat strains used, one author suggested that the opposite findings between studies might be methodical variations that can affect behavior such as when microdialysis was done in relation to when behavior was tested. It should also be noted that in the case of Liebsch et al (1996) and Landgraf et al (1995) the rats were purchased from Charles River in Germany while Everts & Koolhaas (1999) breed their rats in house. Interestingly, microinjections of the V1b antagonist into the basolateral amygdala, but not the CeA, decreased anxiety on the elevated plus maze (Salome et al. 2006), but only microinfusion of the V1b antagonist and not the V1a antagonist into the CeA blocked the effects of VP in Wistar rats in the shock-probe burying test and the light-dark box (Hernandez-Perez et al. 2018). V1a receptor antagonists in the BST cause anxiety in the open field and social interaction test (Duque-Wilckens et al. 2016). Altogether there appears to be a complex relationship between brain region, receptor subtype and type of anxiety tested.

The role of VP in alcohol associated anxiety is being investigated as well. Intermittent alcohol exposure causes long term changes after exposure to alcohol has ended including increased drinking and anxiety on the social interaction test (Crews et al. 2016; Dannenhoffer et al. 2018). Twenty-five to twenty-seven days after the end of alcohol exposure anxiety is still apparent in males only on the social interaction test and this anxiety can be blocked by systemic V1b antagonists, but not V1a antagonist (Dannenhoffer et al. 2018). In contrast to this study, V1a and V1b knock-out mice of both sexes show no difference in time in the open arm of the elevated plus maze in response to acute alcohol (Caldwell et al. 2006). As the intermittent alcohol exposure paradigm takes place in

adolescence, e.g. ages P25 to P45, the developmental period as well as the length of alcohol exposure could cause changes in the VP system, such as increased V1b receptors (Dannenhoffer et al. 2018), that lead to a different VP related anxiety pathway than anxiety related to acute alcohol exposure. Adolescent alcohol has been found to have long lasting effects not found with similar adult alcohol exposure (Crews et al. 2016).

A second chronic intermittent alcohol paradigm has a relationship to both alcohol withdrawal and stress, both of which have profound effects on the VP system. This paradigm requires three withdrawals to obtain robust anxiety-like behavior, a mild stressor can substitute for the first two withdrawals to cause anxiety-like behavior (Breese et al. 2005a; Overstreet et al. 2002). Additionally, in an extended withdrawal which alone no longer causes anxiety, a mild stressor can reinstate the anxiety (Breese et al. 2005b). Different pharmacological agents, like CRF, that activate systems associated with stress and alcohol withdrawal have also been shown to substitute for alcohol/stress in these paradigms (Breese and Knapp 2016). We predict that VP would have a similar effect, and that blocking V1b receptors during withdrawal could block alcohol withdrawal induced anxiety-like behavior (see Dannenhoffer et al. 2018). Along with examining alcohol withdrawal induced anxiety, more work needs to be done to examine brain regions involved in the role in VP's effects on alcohol associated anxiety. The adolescent study that used an alcohol paradigm to cause anxiety found changes in the V1b receptor in the hypothalamus suggesting a role for this brain region while also showing that the anxiety caused by this paradigm could be reversed by a systemically injected V1b antagonist. Thus, this finding suggests, but does not confirm, that the V1b antagonist might have its effects through the hypothalamus (Dannenhoffer et al. 2018). Studies showing anxiety not associated with alcohol as discussed above would suggest the amygdala and septum as strong targets as well (Everts and Koolhaas 1999; Hernandez-Perez et al. 2018; Landgraf et al. 1995; Liebsch et al. 1996; Salome et al. 2006; Stemmelin et al. 2005).

6. VP and clinical trials

The evidence discussed above and evidence from animal models of other neuropsychiatric disorders has led to four human drug trials for VP antagonists in stress related neuropsychiatric disorders. Unfortunately only one of these antagonists has been tested for treatment of alcohol abuse. It is important to note two things: first that the four drugs are orally active and second that they passed safety trials with mild side effects at most (Fabio et al. 2013; Griebel et al. 2012; Katz et al. 2016). The clinical trials are summarized in table 4.

ABT-436 is a V1b receptor antagonist that has reached phase II clinical trials for major depressive disorder and has thus far been the only VP antagonist tested against alcohol abuse. In trials for patients with major depressive disorder, patients were excluded if they had current or recent drug or alcohol abuse, or a psychotic disorder. After one week ABT-436 significantly improved performance on the Mood and Anxiety Symptom Questionnaire, but not the 17-item Hamilton Depression Rating Scale (Katz et al. 2017). In trials to test the effects of ABT-436 on alcohol abuse, participants were chosen that were heavy drinkers that had been diagnosed with alcohol dependence in the past year. Participants were excluded if they were diagnosed with a DSM axis I disorder or were

currently abusing or dependent on a psychoactive drug other than alcohol. When tested using the Spielberger trait anxiety index (STAI) and profile of mood states (POMS) total mood disturbance, participants selected for the study had near normal anxiety with slightly elevated mood disturbance. ABT-436 reduced the frequency of drinking without reducing the amount of alcohol consumed (Ryan et al. 2017). It should also be noted that the drug seemed to have its greatest effect on patients with high stress levels as measured by the POMS tension-anxiety, the STAI, and peak cortisol levels during an ACTH stimulation test. This finding lends support for the authors' idea that ABT-436 might have its effects through the VP stress response. Despite these somewhat promising outcomes, AbbVie has discontinued the development of ABT-436.

SSR149415 is a V1b receptor antagonist that has undergone phase II clinical trials for generalized anxiety disorder and major depressive disorder. In the clinical trial for generalized anxiety disorder the drug caused no significant changes on the Hamilton Anxiety Rating Scale or the Clinical Global Impressions-Severity of Illness score (Griebel et al. 2012). Two of the clinical trials in depressed patients showed no improvement on the Hamilton Depression Rating Scale or the Clinical Global Impressions-Severity of Illness score, although a third trial in depressed patients showed improvement on the Hamilton Depression Rating Scale without effects on Montgomery-Asberg Depression Rating Scale (Griebel et al. 2012). Sanofi has halted further drug development, but the drug continues to be used for animal experiments including many of the experiments discussed above, which show strong effects on behavior. These clinical trials have not directly tested alcohol induced anxiety, but would appear to provide safe antagonists for such a clinical trial

Two other VP antagonists that have been pursued are TS-121 and SRX46. TS-121 is a V1b antagonist developed by Taisho Pharmaceutical. As TS-121 has only begin phase II clinical trials for major depressive disorder- it is too early to draw any conclusions about this drug. SRX246 was developed by Azevan Pharmaceuticals. SRX246 is unique in that it is the only V1a antagonist that has gone to phase II clinical trials for these stress related neuropsychiatric disorders. These phase II clinical trials for anxiety disorder and post-traumatic stress disorder are currently ongoing and therefore we cannot draw conclusions. However, these results will be interesting in that V1a receptors are expressed throughout the brain while the V1b receptor has a more limited expression.

7. Conclusions and future directions

It is clear from the evidence provided that VP plays a role in many alcohol related behaviors and might therefore make an excellent target for treating alcohol abuse disorders. Evidence from human studies examining the effects of the V1b antagonists on drinking have shown promising results (Ryan et al. 2017) and more VP antagonists are currently being tested for other neuropsychiatric disorders. Nonetheless, future testing of VP antagonists in a variety of human disorders is needed. Additionally, there is still much to be accomplished to identify which brain regions are involved in the effects of VP on alcohol related behaviors. It is clear that alcohol changes the levels of VP in the hypothalamus and perhaps other brain regions (Carmona-Calero et al. 1995; Gulya et al. 1991; Ogilvie et al. 1997; Sanna et al. 1993; Zhou et al. 2011), a finding which implicates these brain regions in alcohol related

behaviors associated with release of VP (summarized in figure 1). What is less clear is in which brain regions the V1b receptor antagonist is having its effects as most studies give the antagonist systemically and whether V1a antagonism would be effective on any of these alcohol related behaviors as it has been effective in nonalcohol related anxiety.

Despite the existence of sex differences in the VP system, few alcohol studies have included both sexes. Most of these studies have not found any differences between the sexes though these studies were mostly targeting the V1b system (Caldwell et al. 2006; Ryan et al. 2017; Zhou et al. 2018). The data from Dannenhoffer et al. (2018) are harder to interpret as their alcohol paradigm caused a behavioral and molecular changes in males that were not found in females, and those changes could relate to the differential effect of the V1b antagonist on males and females. However, the data from the other studies examining both sexes would suggest that VP, or at least the V1b receptor, would be a valid treatment option for both sexes, but more work needs to be done including both sexes.

There are likely multiple factors that contribute to drinking in alcoholics as suggested by the study showing that a V1b antagonist decreased the number of drinking days, but not the total alcohol intake in humans (Ryan et al. 2017). It is reasonable that variables controlling an abstinent alcoholic taking their first drink since becoming abstinent (number of drinking days) are different than those responsible for continued drinking after that first drink (total alcohol intake) and that separate treatments are needed for each. Thus a multidrug program may be needed to effectively treat alcoholism as suggested by the work of Zhou et al (2018) showing a decrease in alcohol intake in mice with a combination of naltrexone and a V1b antagonist. Given the results in the human trials of the V1b antagonist, it might be best to combine it with a drug that affects the total alcohol intake. However, in order to successfully employ the correct combination of drugs, a better understanding is needed of how VP affects alcohol related behaviors. As mentioned in Zhou et al (2018), naltrexone and the V1b antagonist seem to effect different systems, which likely contributes to their combined effectiveness.

An additional future direction of this research would be to focus on the stress related aspects of drinking based on the results in the human trials showing the greatest effects of the V1b antagonists in humans with high stress (Ryan et al. 2017). Further support for this research direction comes from studies showing a correlation between response to stress, the measurement of VP levels in the amygdala and alcohol consumption (Nelson et al. 2018). Others have suggested that VP effects alcohol drinking through its effects on the HPA axis, and therefore plays a role in the relationship between stress-reactivity and alcoholism (Zhou and Kreek 2018), which is supported by evidence that blocking the VP system decreases the HPA response to alcohol (Lolait et al. 2007b; Ogilvie et al. 1997; Rivier and Lee 1996). Altogether these ideas suggest that perhaps VP antagonists might be most beneficial in situations with a stress aspect and that feature might be the best target for future human trails.

Finally, looking to OT and CRF human drug trial failures and successes can help strengthen the agreement for further avenues for VP trials. For example, OT has been successful in treating the symptoms of withdrawal perhaps due to its rapid reversal of tolerance (Pedersen

et al. 2013), therefore given the ability of a V1 antagonist to enhance the rate of loss of tolerance maybe V1 antagonists should be tested for use during alcohol withdrawal as well. It has been suggested that there are many reasons for the failure of the CRF antagonist human trials among them are timing (the drug should have been giving during withdrawal) and selective treatment populations (for example high stress individuals) both of which are most likely relevant to the VP antagonists as well (Schreiber and Gilpin 2018; Spierling and Zorrilla 2017). Therefore, using VP antagonists during withdrawal as well as in high stress patients should be considered as future direction in human drug trials.

In conclusion, VP is an attractive target for treatment of alcohol disorders, but more basic research needs to be done to understand how VP effects alcohol related behaviors, so that the correct application for VP treatment might be found.

Acknowledgements

This work was supported in part by the Bowles Center for Alcohol Studies, and the National Institute on Alcoholism and Alcohol Abuse (P60AA01165; R01AA022234; R01AA021275). The authors would like to thank Dr. Fulton Crews for his comments on this manuscript.

List of Abbreviations

AA	alcohol preferring
ACTH	Adrenocorticotropic hormone
AMY	Amygdala
ANA	alcohol non-preferring rats
AUD	alcohol use disorders
BST	Bed nucleus of the stria terminalis
cAMP	cyclic adenosine 3’,5’-monophosphate
CeA	central nucleus of the amygdala
CORT	corticosterone
CRF	corticotropin- releasing factor
CRF-R1	corticotropin-releasing factor receptor 1
CRF-R2	corticotropin-releasing factor receptor 2
DGAVP	des- Gly^{9}-[Arg^{8}]-vasopressin dicitrate
DMH	Dorsomedial hypothalamic nucleus
DR	Dorsal raphe
DSM-5	diagnostic and statistical manual of mental disorders, 5^{th} edition
GABA	gamma-Aminobutyric acid

HAD	high alcohol-drinking rats
HPA	hypothalamic pituitary adrenal
HPC	Hippocampus
LBH	Lateral habenular nucleus
LC	Locus coeruleus
LS	Lateral septum
MeA	medial amygdala
MPO AH	Medial preoptic area – anterior hypothalamus
OT	oxytocin
***OT**	Olfactory tubercle
VOLT	vascular organ of the lamina terminalis
P	alcohol-preferring rats
PAG	Periaqueductal grey
PIP2	phosphatidylinositol 4,5-bisphosphate
POMS	profile of mood states (POMS) total mood disturbance
PV	Periventricular nucleus hypothalamus
PVN	Paraventricular nucleus
SCN	Suprachiasmatic nucleus
SON	Supraoptic nucleus
STAI	Spielberger trait anxiety index
V1a	vasopressin receptor 1a
V1b	vasopressin receptor 1b
V2	vasopressin receptor 2
VOLT	vascular organ of the lamina terminalis
VP	Arginine vasopressin
***VP**	Ventral pallidum

References

Aguilera G, Rabadan-Diehl C (2000) Vasopressinergic regulation of the hypothalamic-pituitary-adrenal axis: implications for stress adaptation. Regulatory peptides 96: 23–9. [PubMed: 11102648]

APA (2013) Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub

Appenrodt E, Schnabel R, Schwarzberg H (1998) Vasopressin administration modulates anxiety-related behavior in rats. Physiol Behav 64: 543–7. [PubMed: 9761230]

Barberis C, Tribollet E (1996) Vasopressin and oxytocin receptors in the central nervous system. Critical reviews in neurobiology 10: 119–54. [PubMed: 8853957]

Bartanusz V, Aubry JM, Jezova D, Baffi J, Kiss JZ (1993) Up-regulation of vasopressin mRNA in paraventricular hypophysiotrophic neurons after acute immobilization stress. Neuroendocrinology 58: 625–9. [PubMed: 8127391]

Bielsky IF, Hu SB, Szegda KL, Westphal H, Young LJ (2004) Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. Neuropsychopharmacology 29: 483–93. [PubMed: 14647484]

Bielsky IF, Hu SB, Young LJ (2005) Sexual dimorphism in the vasopressin system: lack of an altered behavioral phenotype in female V1a receptor knockout mice. Behav Brain Res 164: 132–6. [PubMed: 16046007]

Bleickardt CJ, Mullins DE, Macsweeney CP, Werner BJ, Pond AJ, Guzzi MF, Martin FD, Varty GB, Hodgson RA (2009) Characterization of the V1a antagonist, JNJ-17308616, in rodent models of anxiety-like behavior. Psychopharmacology (Berl) 202: 711–8. [PubMed: 18923820]

Breese GR, Chu K, Dayas CV, Funk D, Knapp DJ, Koob GF, Le DA, O'Dell LE, Overstreet DH, Roberts AJ, Sinha R, Valdez GR, Weiss F (2005a) Stress enhancement of craving during sobriety: a risk for relapse. Alcohol Clin Exp Res 29: 185–95. [PubMed: 15714042]

Breese GR, Knapp DJ (2016) Persistent adaptation by chronic alcohol is facilitated by neuroimmune activation linked to stress and CRF. Alcohol 52: 9–23. [PubMed: 27139233]

Breese GR, Overstreet DH, Knapp DJ, Navarro M (2005b) Prior multiple ethanol withdrawals enhance stress-induced anxiety-like behavior: inhibition by CRF1- and benzodiazepine-receptor antagonists and a 5-HT1a-receptor agonist. Neuropsychopharmacology 30: 1662–9. [PubMed: 15726114]

Breese GR, Sinha R, Heilig M (2011) Chronic alcohol neuroadaptation and stress contribute to susceptibility for alcohol craving and relapse. Pharmacol Ther 129: 149–71. [PubMed: 20951730]

Buijs RM (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Pathways to the limbic system, medulla oblongata and spinal cord. Cell and tissue research 192: 423–35. [PubMed: 699026]

Buijs RM (1980) Immunocytochemical demonstration of vasopressin and oxytocin in the rat brain by light and electron microscopy. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 28: 357–60. [PubMed: 6989899]

Bunck M, Czibere L, Horvath C, Graf C, Frank E, Kessler MS, Murgatroyd C, Muller-Myhsok B, Gonik M, Weber P, Putz B, Muigg P, Panhuysen M, Singewald N, Bettecken T, Deussing JM, Holsboer F, Spengler D, Landgraf R (2009) A hypomorphic vasopressin allele prevents anxiety-related behavior. PLoS One 4: e5129. [PubMed: 19357765]

Caffe AR, van Leeuwen FW, Luiten PG (1987) Vasopressin cells in the medial amygdala of the rat project to the lateral septum and ventral hippocampus. J Comp Neurol 261: 237–52. [PubMed: 3305600]

Caldwell HK, Stewart J, Wiedholz LM, Millstein RA, Iacangelo A, Holmes A, Young WS, 3rd, Wersinger SR (2006) The acute intoxicating effects of ethanol are not dependent on the vasopressin 1a or 1b receptors. Neuropeptides 40: 325–37. [PubMed: 17049983]

Carmona-Calero E, del Mar Perez-Delgado M, Banuelos-Pineda J, Marrero-Gordillo N, Ferres-Torres R, Castaneyra-Perdomo A (1995) Effects of chronic alcohol intake on the vasopressin content in the hypothalamic paraventricular and supraoptic nuclei of the mouse. An immunohistochemical and morphometric study. Drug and alcohol dependence 38: 19–24. [PubMed: 7648993]

Carter CS (2007) Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders? Behav Brain Res 176: 170–86. [PubMed: 17000015]

Chen C, Diaz Brinton RD, Shors TJ, Thompson RF (1993) Vasopressin induction of long-lasting potentiation of synaptic transmission in the dentate gyrus. Hippocampus 3: 193–203. [PubMed: 8394770]

Chen X, Hackett PD, DeMarco AC, Feng C, Stair S, Haroon E, Ditzen B, Pagnoni G, Rilling JK (2016) Effects of oxytocin and vasopressin on the neural response to unreciprocated cooperation within brain regions involved in stress and anxiety in men and women. Brain imaging and behavior 10: 581–93. [PubMed: 26040978]

Chepkova AN, Kapai NA, Skrebitskii VG (2001) Arginine vasopressin fragment AVP(4–9)facilitates induction of long-term potentiation in the hippocampus. Bulletin of experimental biology and medicine 131: 136–8. [PubMed: 11391395]

Collins GB, Brosnihan KB, Zuti RA, Messina M, Gupta MK (1992) Neuroendocrine, fluid balance, and thirst responses to alcohol in alcoholics. Alcohol Clin Exp Res 16: 228–33. [PubMed: 1590544]

Crews FT, Vetreno RP, Broadwater MA, Robinson DL (2016) Adolescent Alcohol Exposure Persistently Impacts Adult Neurobiology and Behavior. Pharmacological reviews 68: 1074–1109. [PubMed: 27677720]

Dannenhoffer CA, Kim EU, Saalfield J, Werner DF, Varlinskaya EI, Spear LP (2018) Oxytocin and vasopressin modulation of social anxiety following adolescent intermittent ethanol exposure. Psychopharmacology (Berl).

de Goeij DC, Jezova D, Tilders FJ (1992) Repeated stress enhances vasopressin synthesis in corticotropin releasing factor neurons in the paraventricular nucleus. Brain Res 577: 165–8. [PubMed: 1521142]

de Guglielmo G, Crawford E, Kim S, Vendruscolo LF, Hope BT, Brennan M, Cole M, Koob GF, George O (2016) Recruitment of a Neuronal Ensemble in the Central Nucleus of the Amygdala Is Required for Alcohol Dependence. J Neurosci 36: 9446–53. [PubMed: 27605618]

De Vries GJ, Buijs RM (1983) The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain Res 273: 307–17. [PubMed: 6311351]

De Vries GJ, Panzica GC (2006) Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: different mechanisms, similar endpoints. Neuroscience 138: 947–55. [PubMed: 16310321]

DeVries GJ, Buijs RM, Van Leeuwen FW, Caffe AR, Swaab DF (1985) The vasopressinergic innervation of the brain in normal and castrated rats. J Comp Neurol 233: 236–54. [PubMed: 3882778]

DiBenedictis BT, Nussbaum ER, Cheung HK, Veenema AH (2017) Quantitative mapping reveals age and sex differences in vasopressin, but not oxytocin, immunoreactivity in the rat social behavior neural network. J Comp Neurol 525: 2549–2570. [PubMed: 28340511]

Doring WK, Herzenstiel MN, Krampe H, Jahn H, Pralle L, Sieg S, Wegerle E, Poser W, Ehrenreich H (2003) Persistent alterations of vasopressin and N-terminal proatrial natriuretic peptide plasma levels in long-term abstinent alcoholics. Alcohol Clin Exp Res 27: 849–61. [PubMed: 12766631]

Dumais KM, Veenema AH (2016) Vasopressin and oxytocin receptor systems in the brain: Sex differences and sex-specific regulation of social behavior. Frontiers in neuroendocrinology 40: 1–23. [PubMed: 25951955]

Duque-Wilckens N, Steinman MQ, Laredo SA, Hao R, Perkeybile AM, Bales KL, Trainor BC (2016) Inhibition of vasopressin V1a receptors in the medioventral bed nucleus of the stria terminalis has sex- and context-specific anxiogenic effects. Neuropharmacology 110: 59–68. [PubMed: 27452721]

Ebner K, Wotjak CT, Holsboer F, Landgraf R, Engelmann M (1999) Vasopressin released within the septal brain area during swim stress modulates the behavioural stress response in rats. Eur J Neurosci 11: 997–1002. [PubMed: 10103093]

Ebner K, Wotjak CT, Landgraf R, Engelmann M (2002) Forced swimming triggers vasopressin release within the amygdala to modulate stress-coping strategies in rats. Eur J Neurosci 15: 384–8. [PubMed: 11849304]

Edwards S, Guerrero M, Ghoneim OM, Roberts E, Koob GF (2012) Evidence that vasopressin V1b receptors mediate the transition to excessive drinking in ethanol-dependent rats. Addict Biol 17: 76–85. [PubMed: 21309953]

Egashira N, Tanoue A, Higashihara F, Fuchigami H, Sano K, Mishima K, Fukue Y, Nagai H, Takano Y, Tsujimoto G, Stemmelin J, Griebel G, Iwasaki K, Ikeda T, Nishimura R, Fujiwara M (2005)

Disruption of the prepulse inhibition of the startle reflex in vasopressin V1b receptor knockout mice: reversal by antipsychotic drugs. Neuropsychopharmacology 30: 1996–2005. [PubMed: 15956991]

Egashira N, Tanoue A, Matsuda T, Koushi E, Harada S, Takano Y, Tsujimoto G, Mishima K, Iwasaki K, Fujiwara M (2007) Impaired social interaction and reduced anxiety-related behavior in vasopressin V1a receptor knockout mice. Behav Brain Res 178: 123–7. [PubMed: 17227684]

Ehrenreich H, tom Dieck K, Gefeller O, Kaw S, Schilling L, Poser W, Ruther E (1997) Sustained elevation of vasopressin plasma levels in healthy young men, but not in abstinent alcoholics, upon expectation of novelty. Psychoneuroendocrinology 22: 13–24. [PubMed: 9141148]

Eisenhofer G, Johnson RH (1982) Effect of ethanol ingestion on plasma vasopressin and water balance in humans. The American journal of physiology 242: R522–7. [PubMed: 7081477]

Everts HG, Koolhaas JM (1999) Differential modulation of lateral septal vasopressin receptor blockade in spatial learning, social recognition, and anxiety-related behaviors in rats. Behav Brain Res 99: 7–16. [PubMed: 10512567]

Fabio KM, Guillon CD, Lu SF, Heindel ND, Brownstein MJ, Lacey CJ, Garippa C, Simon NG (2013) Pharmacokinetics and metabolism of SRX246: a potent and selective vasopressin 1a antagonist. Journal of pharmaceutical sciences 102: 2033–43. [PubMed: 23471831]

Gill GV, Baylis PH, Flear CT, Skillen AW, Diggle PH (1982) Acute biochemical responses to moderate beer drinking. British medical journal (Clinical research ed) 285: 1770–3. [PubMed: 6816364]

Gillies GE, Linton EA, Lowry PJ (1982) Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. Nature 299: 355–7. [PubMed: 6287293]

Gray M, Innala L, Viau V (2012) Central vasopressin V1A receptor blockade impedes hypothalamic-pituitary-adrenal habituation to repeated restraint stress exposure in adult male rats. Neuropsychopharmacology 37: 2712–9. [PubMed: 22828750]

Griebel G, Beeske S, Stahl SM (2012) The vasopressin V(1b) receptor antagonist SSR149415 in the treatment of major depressive and generalized anxiety disorders: results from 4 randomized, double-blind, placebo-controlled studies. J Clin Psychiatry 73: 1403–11. [PubMed: 23146246]

Griebel G, Simiand J, Serradeil-Le Gal C, Wagnon J, Pascal M, Scatton B, Maffrand JP, Soubrie P (2002) Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. Proc Natl Acad Sci U S A 99: 6370–5. [PubMed: 11959912]

Gulya K, Dave JR, Hoffman PL (1991) Chronic ethanol ingestion decreases vasopressin mRNA in hypothalamic and extrahypothalamic nuclei of mouse brain. Brain Res 557: 129–35. [PubMed: 1747746]

Gulya K, Orpana AK, Sikela JM, Hoffman PL (1993) Prodynorphin and vasopressin mRNA levels are differentially affected by chronic ethanol ingestion in the mouse. Brain Res Mol Brain Res 20: 1–8. [PubMed: 8255170]

Harding AJ, Halliday GM, Ng JL, Harper CG, Kril JJ (1996) Loss of vasopressin-immunoreactive neurons in alcoholics is dose-related and time-dependent. Neuroscience 72: 699–708. [PubMed: 9157316]

Hasunuma I, Toyoda F, Okada R, Yamamoto K, Kadono Y, Kikuyama S (2013) Roles of arginine vasotocin receptors in the brain and pituitary of submammalian vertebrates. International review of cell and molecular biology 304: 191–225. [PubMed: 23809437]

Helderman JH, Vestal RE, Rowe JW, Tobin JD, Andres R, Robertson GL (1978) The response of arginine vasopressin to intravenous ethanol and hypertonic saline in man: the impact of aging. Journal of gerontology 33: 39–47. [PubMed: 618965]

Hernandez-Perez OR, Crespo-Ramirez M, Cuza-Ferrer Y, Anias-Calderon J, Zhang L, Roldan-Roldan G, Aguilar-Roblero R, Borroto-Escuela DO, Fuxe K, Perez de la Mora M (2018) Differential activation of arginine-vasopressin receptor subtypes in the amygdaloid modulation of anxiety in the rat by arginine-vasopressin. Psychopharmacology (Berl) 235: 1015–1027. [PubMed: 29306965]

Hernandez VS, Hernandez OR, Perez de la Mora M, Gomora MJ, Fuxe K, Eiden LE, Zhang L (2016) Hypothalamic Vasopressinergic Projections Innervate Central Amygdala GABAergic Neurons:

Implications for Anxiety and Stress Coping. Frontiers in neural circuits 10: 92. [PubMed: 27932956]

Hillemacher T, Frieling H, Luber K, Yazici A, Muschler MA, Lenz B, Wilhelm J, Kornhuber J, Bleich S (2009) Epigenetic regulation and gene expression of vasopressin and atrial natriuretic peptide in alcohol withdrawal. Psychoneuroendocrinology 34: 555–60. [PubMed: 19046820]

Hirasawa A, Hashimoto K, Tsujimoto G (1994) Distribution and developmental change of vasopressin V1A and V2 receptor mRNA in rats. Eur J Pharmacol 267: 71–5. [PubMed: 8206132]

Hirschl MM, Derfler K, Bieglmayer C, Roggla H, Zeiner A, Seidler D, Laggner AN (1994) Hormonal derangements in patients with severe alcohol intoxication. Alcohol Clin Exp Res 18: 761–6. [PubMed: 7943688]

Hodgson RA, Higgins GA, Guthrie DH, Lu SX, Pond AJ, Mullins DE, Guzzi MF, Parker EM, Varty GB (2007) Comparison of the V1b antagonist, SSR149415, and the CRF1 antagonist, CP-154,526, in rodent models of anxiety and depression. Pharmacol Biochem Behav 86: 431–40. [PubMed: 17291571]

Hodgson RA, Mullins D, Lu SX, Guzzi M, Zhang X, Bleickardt CJ, Scott JD, Miller MW, Stamford AW, Parker EM, Varty GB (2014) Characterization of a novel vasopressin V1b receptor antagonist, V1B-30N, in animal models of anxiety-like and depression-like behavior. Eur J Pharmacol 730: 157–63. [PubMed: 24602808]

Hoffman PL (1982) Structural requirements for neurohypophyseal peptide maintenance of ethanol tolerance. Pharmacol Biochem Behav 17: 685–90. [PubMed: 7178182]

Hoffman PL, Dave JR (1991) Chronic ethanol exposure uncouples vasopressin synthesis and secretion in rats. Neuropharmacology 30: 1245–9. [PubMed: 1775224]

Hoffman PL, Ritzmann RF, Walter R, Tabakoff B (1978) Arginine vasopressin maintains ethanol tolerance. Nature 276: 614–6. [PubMed: 723943]

Hoffman PL, Tabakoff B (1984) Neurohypophyseal peptides maintain tolerance to the incoordinating effects of ethanol. Pharmacol Biochem Behav 21: 535–43. [PubMed: 6504951]

Hoorneman EM, Buijs RM (1982) Vasopressin fiber pathways in the rat brain following suprachiasmatic nucleus lesioning. Brain Res 243: 235–41. [PubMed: 7049323]

Huber D, Veinante P, Stoop R (2005) Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. Science 308: 245–8. [PubMed: 15821089]

Hung CR, Tabakoff B, Melchior CL, Hoffman PL (1984) Intraventricular arginine vasopressin maintains ethanol tolerance. Eur J Pharmacol 106: 645–8. [PubMed: 6519181]

Hwang BH, Froehlich JC, Hwang WS, Lumeng L, Li TK (1998) More vasopressin mRNA in the paraventricular hypothalamic nucleus of alcohol-preferring rats and high alcohol-drinking rats selectively bred for high alcohol preference. Alcohol Clin Exp Res 22: 664–9. [PubMed: 9622448]

Iijima M, Yoshimizu T, Shimazaki T, Tokugawa K, Fukumoto K, Kurosu S, Kuwada T, Sekiguchi Y, Chaki S (2014) Antidepressant and anxiolytic profiles of newly synthesized arginine vasopressin V1B receptor antagonists: TASP0233278 and TASP0390325. Br J Pharmacol 171: 3511–25. [PubMed: 24654684]

Ishizawa H, Dave JR, Liu LI, Tabakoff B, Hoffman PL (1990) Hypothalamic vasopressin mRNA levels in mice are decreased after chronic ethanol ingestion. Eur J Pharmacol 189: 119–27. [PubMed: 2253699]

Ishunina TA, Swaab DF (1999) Vasopressin and oxytocin neurons of the human supraoptic and paraventricular nucleus: size changes in relation to age and sex. The Journal of clinical endocrinology and metabolism 84: 4637–44. [PubMed: 10599731]

Jahn H, Doring WK, Krampe H, Sieg S, Werner C, Poser W, Brunner E, Ehrenreich H (2004) Preserved vasopressin response to osmostimulation despite decreased basal vasopressin levels in long-term abstinent alcoholics. Alcohol Clin Exp Res 28: 1925–30. [PubMed: 15608610]

Kato Y, Igarashi N, Hirasawa A, Tsujimoto G, Kobayashi M (1995) Distribution and developmental changes in vasopressin V2 receptor mRNA in rat brain. Differentiation; research in biological diversity 59: 163–9. [PubMed: 7589900]

Katz DA, Liu W, Locke C, Dutta S, Tracy KA (2016) Clinical safety and hypothalamic-pituitary-adrenal axis effects of the arginine vasopressin type 1B receptor antagonist ABT-436. Psychopharmacology (Berl) 233: 71–81. [PubMed: 26407603]

Katz DA, Locke C, Greco N, Liu W, Tracy KA (2017) Hypothalamic-pituitary-adrenal axis and depression symptom effects of an arginine vasopressin type 1B receptor antagonist in a one-week randomized Phase 1b trial. Brain and behavior 7: e00628. [PubMed: 28293470]

Kornet M, Goosen C, Ribbens LG, Van Ree JM (1991) The effect of desglycinamide-(Arg8)-vasopressin (DGAVP) on the acquisition of free-choice alcohol drinking in rhesus monkeys. Alcohol Clin Exp Res 15: 72–9. [PubMed: 2024735]

Kornet M, Goosen C, Thyssen JH, Van Ree JM (1992) Endocrine profile during acquisition of free-choice alcohol drinking in rhesus monkeys; treatment with desglycinamide-(Arg8)-vasopressin. Alcohol Alcohol 27: 403–10. [PubMed: 1329787]

Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F, Engelmann M (1995) V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities, and anxiety-related behavior in rats. J Neurosci 15: 4250–8. [PubMed: 7790909]

Le AD, Kalant H, Khanna JM (1982) Interaction between des-glycinamide9-[Arg8]vasopressin and serotonin on ethanol tolerance. Eur J Pharmacol 80: 337–45. [PubMed: 7106190]

Lee MR, Weerts EM (2016) Oxytocin for the treatment of drug and alcohol use disorders. Behavioural pharmacology 27: 640–648. [PubMed: 27603752]

Liebsch G, Wotjak CT, Landgraf R, Engelmann M (1996) Septal vasopressin modulates anxiety-related behaviour in rats. Neurosci Lett 217: 101–4. [PubMed: 8916082]

Linkola J, Fyhrquist F (1978) Urinary cyclic AMP and vasopressin excretion in rat strains selected for their alcohol intake. Acta physiologica Scandinavica 102: 364–7. [PubMed: 206097]

Linkola J, Fyhrquist F, Forsander O (1977) Effects of ethanol on urinary arginine vasopressin excretion in two rat strains selected for their different ethanol preferences. Acta physiologica Scandinavica 101: 126–8. [PubMed: 906857]

Linkola J, Ylikahri R, Fyhquist F, Wallenius M (1978) Plasma vasopressin in ethanol intoxication and hangover. Acta physiologica Scandinavica 104: 180–7. [PubMed: 362827]

Litten RZ, Egli M, Heilig M, Cui C, Fertig JB, Ryan ML, Falk DE, Moss H, Huebner R, Noronha A (2012) Medications development to treat alcohol dependence: a vision for the next decade. Addict Biol 17: 513–27. [PubMed: 22458728]

Litvin Y, Murakami G, Pfaff DW (2011) Effects of chronic social defeat on behavioral and neural correlates of sociality: Vasopressin, oxytocin and the vasopressinergic V1b receptor. Physiol Behav 103: 393–403. [PubMed: 21397619]

Lolait SJ, Stewart LQ, Jessop DS, Young WS, 3rd, O'Carroll AM (2007a) The hypothalamic-pituitary-adrenal axis response to stress in mice lacking functional vasopressin V1b receptors. Endocrinology 148: 849–56. [PubMed: 17122081]

Lolait SJ, Stewart LQ, Roper JA, Harrison G, Jessop DS, Young WS, 3rd, O'Carroll AM (2007b) Attenuated stress response to acute lipopolysaccharide challenge and ethanol administration in vasopressin V1b receptor knockout mice. Journal of neuroendocrinology 19: 543–51. [PubMed: 17561882]

Ma XM, Lightman SL (1998) The arginine vasopressin and corticotrophin-releasing hormone gene transcription responses to varied frequencies of repeated stress in rats. The Journal of physiology 510 (Pt 2): 605–14. [PubMed: 9706007]

Madeira MD, Andrade JP, Lieberman AR, Sousa N, Almeida OF, Paula-Barbosa MM (1997) Chronic alcohol consumption and withdrawal do not induce cell death in the suprachiasmatic nucleus, but lead to irreversible depression of peptide immunoreactivity and mRNA levels. J Neurosci 17: 1302–19. [PubMed: 9006974]

Maher BS, Vladimirov VI, Latendresse SJ, Thiselton DL, McNamee R, Kang M, Bigdeli TB, Chen X, Riley BP, Hettema JM, Chilcoat H, Heidbreder C, Muglia P, Murrelle EL, Dick DM, Aliev F, Agrawal A, Edenberg HJ, Kramer J, Nurnberger J, Tischfield JA, Devlin B, Ferrell RE, Kirillova GP, Tarter RE, Kendler KS, Vanyukov MM (2011) The AVPR1A gene and substance use disorders: association, replication, and functional evidence. Biol Psychiatry 70: 519–27. [PubMed: 21514569]

Mak P, Broussard C, Vacy K, Broadbear JH (2012) Modulation of anxiety behavior in the elevated plus maze using peptidic oxytocin and vasopressin receptor ligands in the rat. Journal of psychopharmacology (Oxford, England) 26: 532–42.

Makino S, Smith MA, Gold PW (1995) Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. Endocrinology 136: 3299–309. [PubMed: 7628364]

Mander AJ, Young A, Merrick MV, Morton JJ (1989) Fluid balance, vasopressin and withdrawal symptoms during detoxification from alcohol. Drug and alcohol dependence 24: 233–7. [PubMed: 2605999]

Mannix SA, Hoffman PL, Melchior CL (1986) Intraventricular arginine vasopressin blocks the acquisition of ethanol tolerance in mice. Eur J Pharmacol 128: 137–41. [PubMed: 3758185]

McBride WJ (2002) Central nucleus of the amygdala and the effects of alcohol and alcohol-drinking behavior in rodents. Pharmacol Biochem Behav 71: 509–15. [PubMed: 11830185]

Melchior CL, Tabakoff B (1985) Features of environment-dependent tolerance to ethanol. Psychopharmacology (Berl) 87: 94–100. [PubMed: 3933044]

Milik E, Szczepanska-Sadowska E, Dobruch J, Cudnoch-Jedrzejewska A, Maslinski W (2014) Altered expression of V1a receptors mRNA in the brain and kidney after myocardial infarction and chronic stress. Neuropeptides 48: 257–66. [PubMed: 25169016]

Moritoh S, Sato K, Okada Y, Koizumi A (2011) Endogenous arginine vasopressin-positive retinal cells in arginine vasopressin-eGFP transgenic rats identified by immunohistochemistry and reverse transcriptase-polymerase chain reaction. Molecular vision 17: 3254–61. [PubMed: 22194651]

Murgatroyd C, Wigger A, Frank E, Singewald N, Bunck M, Holsboer F, Landgraf R, Spengler D (2004) Impaired repression at a vasopressin promoter polymorphism underlies overexpression of vasopressin in a rat model of trait anxiety. J Neurosci 24: 7762–70. [PubMed: 15342744]

Nakamura K, Fujiwara Y, Mizutani R, Sanbe A, Miyauchi N, Hiroyama M, Yamauchi J, Yamashita T, Nakamura S, Mori T, Tsujimoto G, Tanoue A (2008) Effects of vasopressin V1b receptor deficiency on adrenocorticotropin release from anterior pituitary cells in response to oxytocin stimulation. Endocrinology 149: 4883–91. [PubMed: 18583426]

Naughton M (2016) Electrophysiological study of the effects of arginine vasopressin in the rat juxtacapsular nucleus of the bed nucleus of the stria terminalis. Queen's University (Canada)

Nelson BS, Sequeira MK, Schank JR (2018) Bidirectional relationship between alcohol intake and sensitivity to social defeat: association with Tacr1 and Avp expression. Addict Biol 23: 142–153. [PubMed: 28150369]

Neumann ID, Landgraf R (2012) Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci 35: 649–59. [PubMed: 22974560]

Ogilvie KM, Lee S, Rivier C (1997) Role of arginine vasopressin and corticotropin-releasing factor in mediating alcohol-induced adrenocorticotropin and vasopressin secretion in male rats bearing lesions of the paraventricular nuclei. Brain Res 744: 83–95. [PubMed: 9030416]

Otero-Garcia M, Agustin-Pavon C, Lanuza E, Martinez-Garcia F (2016) Distribution of oxytocin and co-localization with arginine vasopressin in the brain of mice. Brain structure & function 221: 3445–73. [PubMed: 26388166]

Otero-Garcia M, Martin-Sanchez A, Fortes-Marco L, Martinez-Ricos J, Agustin-Pavon C, Lanuza E, Martinez-Garcia F (2014) Extending the socio-sexual brain: arginine-vasopressin immunoreactive circuits in the telencephalon of mice. Brain structure & function 219: 1055–81. [PubMed: 23625152]

Overstreet DH, Griebel G (2005) Antidepressant-like effects of the vasopressin V1b receptor antagonist SSR149415 in the Flinders Sensitive Line rat. Pharmacol Biochem Behav 82: 223–7. [PubMed: 16181667]

Overstreet DH, Knapp DJ, Breese GR (2002) Accentuated decrease in social interaction in rats subjected to repeated ethanol withdrawals. Alcohol Clin Exp Res 26: 1259–68. [PubMed: 12198403]

Pak TR, Chung WC, Hinds LR, Handa RJ (2009) Arginine vasopressin regulation in pre- and postpubertal male rats by the androgen metabolite 3beta-diol. American journal of physiology Endocrinology and metabolism 296: E1409–13. [PubMed: 19383875]

Pan Y, Liu Y, Young KA, Zhang Z, Wang Z (2009) Post-weaning social isolation alters anxiety-related behavior and neurochemical gene expression in the brain of male prairie voles. Neurosci Lett 454: 67–71. [PubMed: 19429056]

Paula-Barbosa MM, Silva SM, Andrade JP, Cadete-Leite A, Madeira MD (2001) Nerve growth factor restores mRNA levels and the expression of neuropeptides in the suprachiasmatic nucleus of rats submitted to chronic ethanol treatment and withdrawal. Journal of neurocytology 30: 195–207. [PubMed: 11709626]

Pedersen CA, Smedley KL, Leserman J, Jarskog LF, Rau SW, Kampov-Polevoi A, Casey RL, Fender T, Garbutt JC (2013) Intranasal oxytocin blocks alcohol withdrawal in human subjects. Alcohol Clin Exp Res 37: 484–9. [PubMed: 23025690]

Pinnock SB, Herbert J (2001) Corticosterone differentially modulates expression of corticotropin releasing factor and arginine vasopressin mRNA in the hypothalamic paraventricular nucleus following either acute or repeated restraint stress. Eur J Neurosci 13: 576–84. [PubMed: 11168566]

Rabadan-Diehl C, Lolait SJ, Aguilera G (1995) Regulation of pituitary vasopressin V1b receptor mRNA during stress in the rat. Journal of neuroendocrinology 7: 903–10. [PubMed: 8745267]

Raggenbass M (2008) Overview of cellular electrophysiological actions of vasopressin. Eur J Pharmacol 583: 243–54. [PubMed: 18280467]

Ramanathan G, Cilz NI, Kurada L, Hu B, Wang X, Lei S (2012) Vasopressin facilitates GABAergic transmission in rat hippocampus via activation of V(1A) receptors. Neuropharmacology 63: 1218–26. [PubMed: 22884625]

Rigter H, Dortmans C, Crabbe JC, Jr. (1980a) Effects of peptides related to neurohypophyseal hormones on ethanol tolerance. Pharmacol Biochem Behav 13 Suppl 1: 285–90. [PubMed: 7243830]

Rigter H, Rijk H, Crabbe JC (1980b) Tolerance to ethanol and severity of withdrawal in mice are enhanced by a vasopressin fragment. Eur J Pharmacol 64: 53–68. [PubMed: 7192631]

Rivier C, Lee S (1996) Acute alcohol administration stimulates the activity of hypothalamic neurons that express corticotropin-releasing factor and vasopressin. Brain Res 726: 1–10. [PubMed: 8836539]

Rodriguez-Borrero E, Rivera-Escalera F, Candelas F, Montalvo J, Munoz-Miranda WJ, Walker JR, Maldonado-Vlaar CS (2010) Arginine vasopressin gene expression changes within the nucleus accumbens during environment elicited cocaine-conditioned response in rats. Neuropharmacology 58: 88–101. [PubMed: 19596360]

Rood BD, Beck SG (2014) Vasopressin indirectly excites dorsal raphe serotonin neurons through activation of the vasopressin1A receptor. Neuroscience 260: 205–16. [PubMed: 24345477]

Rood BD, De Vries GJ (2011) Vasopressin innervation of the mouse (Mus musculus) brain and spinal cord. J Comp Neurol 519: 2434–74. [PubMed: 21456024]

Rood BD, Stott RT, You S, Smith CJ, Woodbury ME, De Vries GJ (2013) Site of origin of and sex differences in the vasopressin innervation of the mouse (Mus musculus) brain. J Comp Neurol 521: 2321–58. [PubMed: 23239101]

Roper JA, Craighead M, O'Carroll AM, Lolait SJ (2010) Attenuated stress response to acute restraint and forced swimming stress in arginine vasopressin 1b receptor subtype (Avpr1b) receptor knockout mice and wild-type mice treated with a novel Avpr1b receptor antagonist. Journal of neuroendocrinology 22: 1173–80. [PubMed: 20846299]

Ryan ML, Falk DE, Fertig JB, Rendenbach-Mueller B, Katz DA, Tracy KA, Strain EC, Dunn KE, Kampman K, Mahoney E, Ciraulo DA, Sickles-Colaneri L, Ait-Daoud N, Johnson BA, Ransom J, Scott C, Koob GF, Litten RZ (2017) A Phase 2, Double-Blind, Placebo-Controlled Randomized Trial Assessing the Efficacy of ABT-436, a Novel V1b Receptor Antagonist, for Alcohol Dependence. Neuropsychopharmacology 42: 1012–1023. [PubMed: 27658483]

Salome N, Stemmelin J, Cohen C, Griebel G (2006) Differential roles of amygdaloid nuclei in the anxiolytic- and antidepressant-like effects of the V1b receptor antagonist, SSR149415, in rats. Psychopharmacology (Berl) 187: 237–44. [PubMed: 16779555]

Sanbe A, Takagi N, Fujiwara Y, Yamauchi J, Endo T, Mizutani R, Takeo S, Tsujimoto G, Tanoue A (2008) Alcohol preference in mice lacking the Avpr1a vasopressin receptor. Am J Physiol Regul Integr Comp Physiol 294: R1482–90. [PubMed: 18305023]

Sanna PP, Folsom DP, Barizo MJ, Hirsch MD, Melia KR, Maciejewski-Lenoir D, Bloom FE (1993) Chronic ethanol intake decreases vasopressin mRNA content in the rat hypothalamus: a PCR study. Brain Res Mol Brain Res 19: 241–5. [PubMed: 8412569]

Sawchenko PE, Arias CA, Mortrud MT (1993) Local tetrodotoxin blocks chronic stress effects on corticotropin-releasing factor and vasopressin messenger ribonucleic acids in hypophysiotropic neurons. Journal of neuroendocrinology 5: 341–8. [PubMed: 8401558]

Schreiber AL, Gilpin NW (2018) Corticotropin-Releasing Factor (CRF) Neurocircuitry and Neuropharmacology in Alcohol Drinking Handbook of experimental pharmacology.

Serradeil-Le Gal C, Wagnon J, Simiand J, Griebel G, Lacour C, Guillon G, Barberis C, Brossard G, Soubrie P, Nisato D, Pascal M, Pruss R, Scatton B, Maffrand JP, Le Fur G (2002) Characterization of (2S,4R)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl)-2-oxo- 2,3-dihydro-1H-indol-3-yl]-4-hydroxy-N,N-dimethyl-2-pyrrolidine carboxamide (SSR149415), a selective and orally active vasopressin V1b receptor antagonist. J Pharmacol Exp Ther 300: 1122–30. [PubMed: 11861823]

Shimazaki T, Iijima M, Chaki S (2006) The pituitary mediates the anxiolytic-like effects of the vasopressin V1B receptor antagonist, SSR149415, in a social interaction test in rats. Eur J Pharmacol 543: 63–7. [PubMed: 16843459]

Silva SM, Paula-Barbosa MM, Madeira MD (2002) Prolonged alcohol intake leads to reversible depression of corticotropin-releasing hormone and vasopressin immunoreactivity and mRNA levels in the parvocellular neurons of the paraventricular nucleus. Brain Res 954: 82–93. [PubMed: 12393236]

Sinha R (2007) The role of stress in addiction relapse. Curr Psychiatry Rep 9: 388–95. [PubMed: 17915078]

Smith CJ, Poehlmann ML, Li S, Ratnaseelan AM, Bredewold R, Veenema AH (2017) Age and sex differences in oxytocin and vasopressin V1a receptor binding densities in the rat brain: focus on the social decision-making network. Brain structure & function 222: 981–1006. [PubMed: 27389643]

Sofroniew MV (1980) Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 28: 475–8. [PubMed: 7381192]

Sofroniew MV (1983) Morphology of vasopressin and oxytocin neurones and their central and vascular projections. Progress in brain research 60: 101–14. [PubMed: 6198686]

Sofroniew MV (1985) Vasopressin- and neurophysin-immunoreactive neurons in the septal region, medial amygdala and locus coeruleus in colchicine-treated rats. Neuroscience 15: 347–58. [PubMed: 4022328]

Sofroniew MV, Weindl A (1978) Projections from the parvocellular vasopressin- and neurophysin-containing neurons of the suprachiasmatic nucleus. The American journal of anatomy 153: 391–429. [PubMed: 360814]

Song Z, Albers HE (2017) Cross-talk among oxytocin and arginine-vasopressin receptors: Relevance for basic and clinical studies of the brain and periphery. Frontiers in neuroendocrinology.

Sousa N, Madeira MD, Ruela C, Paula-Barbosa MM (1995) Structural reorganization in the supraoptic nucleus of withdrawn rats following long-term alcohol consumption. Alcohol Clin Exp Res 19: 879–85. [PubMed: 7485834]

Spierling SR, Zorrilla EP (2017) Don't stress about CRF: assessing the translational failures of CRF1antagonists. Psychopharmacology (Berl) 234: 1467–1481. [PubMed: 28265716]

Stemmelin J, Lukovic L, Salome N, Griebel G (2005) Evidence that the lateral septum is involved in the antidepressant-like effects of the vasopressin V1b receptor antagonist, SSR149415. Neuropsychopharmacology 30: 35–42. [PubMed: 15367924]

Stevenson EL, Caldwell HK (2012) The vasopressin 1b receptor and the neural regulation of social behavior. Horm Behav 61: 277–82. [PubMed: 22178035]

Stoop R (2012) Neuromodulation by oxytocin and vasopressin. Neuron 76: 142–59. [PubMed: 23040812]

Szabo G, Tabakoff B, Hoffman PL (1988) Receptors with V1 characteristics mediate the maintenance of ethanol tolerance by vasopressin. J Pharmacol Exp Ther 247: 536–41. [PubMed: 2972830]

Szot P, Bale TL, Dorsa DM (1994) Distribution of messenger RNA for the vasopressin V1a receptor in the CNS of male and female rats. Brain Res Mol Brain Res 24: 1–10. [PubMed: 7968346]

Taivainen H, Laitinen K, Tahtela R, Kilanmaa K, Valimaki MJ (1995) Role of plasma vasopressin in changes of water balance accompanying acute alcohol intoxication. Alcohol Clin Exp Res 19: 759–62. [PubMed: 7573805]

Terranova JI, Ferris CF, Albers HE (2017) Sex Differences in the Regulation of Offensive Aggression and Dominance by Arginine-Vasopressin. Front Endocrinol (Lausanne) 8: 308. [PubMed: 29184535]

Thiagarajan AB, Mefford IN, Eskay RL (1989) Single-dose ethanol administration activates the hypothalamic-pituitary-adrenal axis: exploration of the mechanism of action. Neuroendocrinology 50: 427–32. [PubMed: 2554177]

Thomas A, Kim NB, Amico JA (1996) Sequential exposure to estrogen and testosterone (T) and subsequent withdrawal of T increases the level of arginine vasopressin messenger ribonucleic acid in the hypothalamic paraventricular nucleus of the female rat. Journal of neuroendocrinology 8: 793–800. [PubMed: 8910810]

Thompson RR, George K, Walton JC, Orr SP, Benson J (2006) Sex-specific influences of vasopressin on human social communication. Proc Natl Acad Sci U S A 103: 7889–94. [PubMed: 16682649]

Tobin VA, Hashimoto H, Wacker DW, Takayanagi Y, Langnaese K, Caquineau C, Noack J, Landgraf R, Onaka T, Leng G, Meddle SL, Engelmann M, Ludwig M (2010) An intrinsic vasopressin system in the olfactory bulb is involved in social recognition. Nature 464: 413–7. [PubMed: 20182426]

Trabert W, Caspari D, Bernhard P, Biro G (1992) Inappropriate vasopressin secretion in severe alcohol withdrawal. Acta psychiatrica Scandinavica 85: 376–9. [PubMed: 1605058]

van Leeuwen FW, Caffe AR, De Vries GJ (1985) Vasopressin cells in the bed nucleus of the stria terminalis of the rat: sex differences and the influence of androgens. Brain Res 325: 391–4. [PubMed: 3978433]

Veinante P, Freund-Mercier MJ (1997) Distribution of oxytocin- and vasopressin-binding sites in the rat extended amygdala: a histoautoradiographic study. J Comp Neurol 383: 305–25. [PubMed: 9205043]

Walter R, Hoffman PL, Flexner JB, Flexner LB (1975) Neurohypophyseal hormones, analogs, and fragments: their effect on puromycin-induced amnesia. Proc Natl Acad Sci U S A 72: 4180–4. [PubMed: 1060098]

Wersinger SR, Ginns EI, O'Carroll AM, Lolait SJ, Young WS, 3rd (2002) Vasopressin V1b receptor knockout reduces aggressive behavior in male mice. Mol Psychiatry 7: 975–84. [PubMed: 12399951]

Whitnall MH, Mezey E, Gainer H (1985) Co-localization of corticotropin-releasing factor and vasopressin in median eminence neurosecretory vesicles. Nature 317: 248–50. [PubMed: 3900740]

Winslow JT, Hastings N, Carter CS, Harbaugh CR, Insel TR (1993) A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 365: 545–8. [PubMed: 8413608]

Young WS, Li J, Wersinger SR, Palkovits M (2006) The vasopressin 1b receptor is prominent in the hippocampal area CA2 where it is unaffected by restraint stress or adrenalectomy. Neuroscience 143: 1031–9. [PubMed: 17027167]

Zhou L, Blaustein JD, De Vries GJ (1994) Distribution of androgen receptor immunoreactivity in vasopressin- and oxytocin-immunoreactive neurons in the male rat brain. Endocrinology 134: 2622–7. [PubMed: 8194487]

Zhou Y, Colombo G, Carai MA, Ho A, Gessa GL, Kreek MJ (2011) Involvement of arginine vasopressin and V1b receptor in alcohol drinking in Sardinian alcohol-preferring rats. Alcohol Clin Exp Res 35: 1876–83. [PubMed: 21575018]

Zhou Y, Kreek MJ (2018) Involvement of Activated Brain Stress Responsive Systems in Excessive and "Relapse" Alcohol Drinking in Rodent Models: Implications for Therapeutics. J Pharmacol Exp Ther 366: 9–20. [PubMed: 29669731]

Zhou Y, Rubinstein M, Low MJ, Kreek MJ (2018) V1b Receptor Antagonist SSR149415 and Naltrexone Synergistically Decrease Excessive Alcohol Drinking in Male and Female Mice. Alcohol Clin Exp Res 42: 195–205. [PubMed: 29105118]

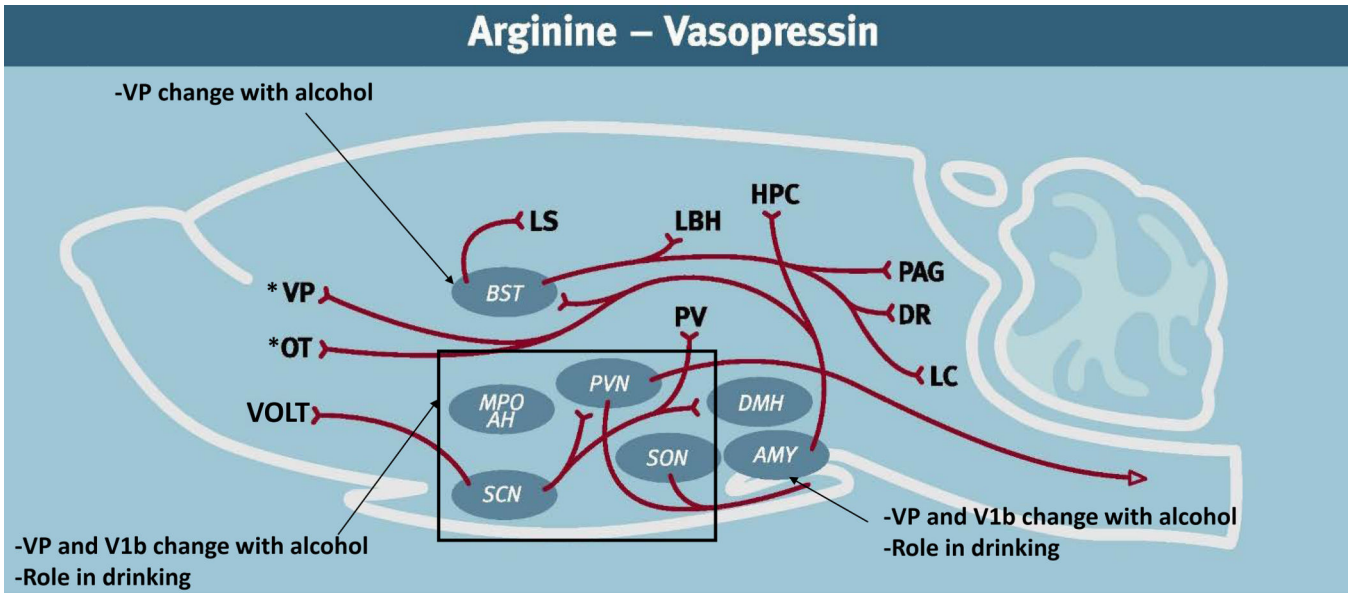

Figure 1: Summary of current evidence of VP related brain regions involved in alcohol related behaviors.

The bed nucleus of the stria terminalis has been shown to have changes in VP mRNA with alcohol consumption. The amygdala exhibits changes in the levels of V1b receptors with alcohol. The hypothalamus shows many VP related changes with alcohol consumption and likely plays a role in alcohol drinking as rats breed for alcohol preference have higher levels of VP in the PVN and SON.

Table 1:

Summary of studies exploring the effects of VP on alcohol tolerance

Paper	Species/sex	Ethanol Paradigm	Tolerance Paradigm	Drug	Results
Hoffman et al. 1978	Mice male	7 days 7% (v/v) ethanol liquid diet and 3g/kg ip challenge	hypothermic; sedative-hypnotic	SC VP	Prolongs tolerance
Rigter et al. 1980a Rigter et al. 1980b	Mice male	3 day ethanol vapor and 3g/kg ip challenge	hypothermic	SC DGAVP	Enhanced tolerance
Lê et al. 1982	Mice male	25 days of oral 5g/kg ethanol and 3g/kg ip challenge	hypothermic; motor coordination	SC DGAVP	Prolongs tolerance
Hoffman 1982	Mice male	7 days 7% (v/v) ethanol liquid diet and 3.1g/kg ip challenge	sedative-hypnotic	SC DGAVP	Prolongs tolerance
Hoffman & Tabakoff 1984	Mice male	7 days 7% (v/v) ethanol liquid diet and 2.3g/kg (14.9% v/v ethanol) ip challenge	motor coordination	SC VP & DGAVP	Prolongs tolerance
Hung et al. 1984	Mice male	5 days 7% (v/v) ethanol liquid diet and 3.1g/kg ip challenge	sedative-hypnotic	ICV VP	Prolongs tolerance
Mannix et al. 1986	Mice male	3.5g/kg ip twice daily for two 5 days blocks separated by 2 days; 3.5g/kg ip twice daily for 4 days and 10μl icv 20% ethanol (2.0mg) challenge	sedative-hypnotic; hypothermic	ICV VP	Blocked the acquisition of tolerance
Szabo et al. 1988	Mice male	5 or 7 days 7% (v/v) ethanol liquid diet and 3.2g/kg ip challenge	sedative-hypnotic	ICV V1 agonist & antagonist or V2 agonist & antagonist	Only the V1 agonist prolonged tolerance and only the V1 antagonist enhanced the rate of loss of tolerance

IP=intraperitoneal injection, SC=subcutaneous injection, ICV=intraventricular injection

VP=arginine vasopressin

DGAVP=des-Gly^{9}-[Arg^{8}]-vasopressin dicitrate, VP fragment with reduced peripheral endocrinological activity (Hoffman 1982; Rigter et al. 1980; Walter et al. 1975).

Table 2:

Summary of the studies examining the effects of VP on alcohol drinking

Paper	Species/sex	Paradigm	Drug	Results
Kornet et al. 1991	Rhesus monkey male	Free choice (1% and 2% v/v) or (4% and 8% v/v) alcohol solution for 14 days with drug treatment, followed by 14 days without treatment	IM DGVAP	Reduced alcohol intake
Kornet et al. 1991	Rhesus monkey male	Free choice 1% and 2% v/v alcohol solution for 14 days with drug treatment followed by 14 days without treatment	IM DGVAP	Reduced alcohol intake
Hwang et al. 1998	HAD & P rats male	N/A	N/A	Increased VP mRNA in hypothalamus of HAD and P rats
Caldwell et al. 2006	V1a & V1b KO mice male & female	2-bottle choice (3 to 11% v/v alcohol vs water) for 30–36 days	N/A	V1a heterozygotes consumed more alcohol; V1b KO no difference from WT; no sex related differences
Sanbe et al. 2008	V1a KO mice male	2-bottle choice (4 to 16% v/v alcohol vs water) for 21 days	N/A	KO consumed more alcohol and showed a higher preference for alcohol
Zhou et al. 2011	sP rats male	2-bottle choice (10% v/v alcohol vs water) for 4 weeks	IP SSR149415	Increased VP mRNA in hypothalamus and amygdala of sP rats; Drug reduced alcohol intake
Maher et al. 2011	Humans	N/A	N/A	V1a receptor gene SNP had a modest association with maximum drinks per day
Edwards et al. 2012	Wistar rats male	Operant self-administration of 10% w/v alcohol solution then intermittent exposure to ethanol vapor 4–6 weeks followed by operant self-administration	IP SSR149415	Drug reduced alcohol intake; Self-administration lowered V1b in the amygdala, but restored V1b levels if rat was alcohol dependent
Ryan et al. 2017	Humans male & female	Individuals who met DSM-IV criteria for alcohol dependence received 12 weeks of drug treatment	Oral ABT-436	Drug reduced frequency of drinking; no gender related differences
Zhou et al. 2018	C57BL/6J mice male & female	Paradigm 1– 3 weeks 2-bottle choice paradigm with chronic alcohol (7.5, 15, or 30%) exposure every other day; Paradigm 2– 3 weeks 1 bottle choice of 15% alcohol every day for 4 hours in the dark	IP SSR149415	Drug reduced alcohol intake alone or in combination with Naltrexone; no sex related differences
Nelson et al. 2018	C57BL/6J mice male	2-bottle choice paradigm with 20 % v/v alcohol solution	N/A	VP levels in the amygdala correlated to the amount of alcohol consumed

IP=intraperitoneal injection, SC=subcutaneous injection, IM=intramuscular injection

VP=arginine vasopressin

DGAVP=des-Gly^{9}-[Arg^{8}]-vasopressin dicitrate, VP fragment with reduced peripheral endocrinological activity (Hoffman 1982; Rigter et al. 1980; Walter et al. 1975)

V1b antagonist=SSR149415 & ABT-436

Table 3:

Summary of the studies examining the effects of VP on anxiety

Paper	Species/sex	Paradigm	Drug	Results
Landgraf et al. 1995	Wistar rats male	On the 4th day of infusion rats were tested in the elevated plus maze	Intra-LS V1a antisense	Loss of V1a receptor reduced anxiety on the elevated plus maze
Liebsch et al. 1996	Wistar rats male	Infusions via microdialysis were performed over 30 min and immediately following infusions rats were tested on the elevated plus maze	Intra-LS VP or manning compound	VP into the LS did not alter anxiety on the elevated plus maze; V1a antagonism decreased anxiety on the elevated plus maze
Appenrodt et al. 1998	Wistar rats male	Infusions via microdialysis were performed over 30 min, 25 min into infusion rats were tested for 5 min on the elevated plus maze; For IP injections elevated plus maze testing was done 30 min post-injection	Intra-LS VP or VP antagonist or IP VP	Both injections of VP into the LS and IP caused decreased anxiety in the elevated plus maze; General VP antagonism had no effect on anxiety on the elevated plus maze
Everts and Koolhaas 1999	Wistar rats male	On the 11th day of drug infusion rats were tested in the shock probe burying test and elevated plus maze	Intra-LS VP antagonist	General VP receptor antagonism increased anxiety on the elevated plus maze, but no increase noted in the shock probe burying test
Griebel et al. 2002	Sprague Dawley or Wistar rats male	Punished drinking test 30 mins post-drug injection; elevated plus-maze 1, 1.4, 3 or 6 hrs post-drug injection	IP or PO SSR149415	V1b antagonism decreased anxiety-like behavior on both tests
Griebel et al. 2002	CD1, OF1, and BALB/c mice male	Paradigm 1- light-dark test 30 min post-drug injection; Paradigm 2– 60 mins post-drug administration mice exposed for 60 min in residents cage prior to testing on the elevated plus maze; Paradigm 3– 7 weeks chronic mild stress treatment once a day and beginning at 4 weeks mice received drug treatment that continued through testing on the elevated plus maze	IP or PO SSR149415	V1b antagonism decreased anxiety-like behavior on light-dark test; V1b antagonism partially reversed the anxiety-like behaviors caused by social defeat and chronic mild stress
Serradeil-Le et a.l 2002	NMRI mice male	Four plate test was done. 5, 1, 2, 4, or 6 hr after drug was given	IP or PO SSR149415	V1b antagonism decreased anxiety in the four plate test
Wersinger et al. 2002	V1b KO mice male	Mice were tested on the elevated plus maze	N/A	Loss of V1b receptor did not alter anxiety on the elevated plus maze
Bielsky et al. 2004	V1a receptor KO mice male	KOs were tested on the elevated plus maze, open field, and light-dark box	N/A	Loss of V1a receptor reduced anxiety on the elevated plus maze, open field and the light-dark box
Murgatroyd et al. 2004	Wistar rats male	Wistar rats were bred for high (HAB) or low (LAB) anxiety-like behavior on the elevated plus maze	N/A	HAB rats have increased expression of VP gene in the PVN compared to LAB rats
Bielsky et al. 2004	V1a KO mice female	Mice were tested in the elevated plus maze, open field and the light-dark box	N/A	Loss of V1a receptor did not change anxiety on the elevated plus maze, open field or light-dark box
Egashira et al. 2005	V1b KO mice male	Mice were tested on the elevated plus maze and light-dark box	N/A	Loss of V1b receptor did not change anxiety on the elevated plus maze or light-dark box

Psychopharmacology (Berl). Author manuscript; available in PMC 2019 December 01.

Paper	Species/sex	Paradigm	Drug	Results
Stemmelin et al. 2005	Sprague Dawley rats male	10 min post-injections rats were tested on the punished drinking test and the elevated plus maze	Intra-LS SSR149415	V1b antagonism in the LS did not change anxiety on the punished drinking test or the elevated plus maze
Overstreet & Griebel 2005	Flinders Sensitive Line rats	Rats injected once daily for 14 days, rats were then tested on the social interaction test 20 hrs after the last injection	IP SSR149415	V1b antagonisms decreased anxiety behavior in the social interaction test
Shimazaki et al. 2006	Sprague Dawley rats male	Following 7 days of isolate housing rats were administered drug followed by social interaction test 1 hr post-drug administration	PO SSR149415	V1b antagonism decreased anxiety in the social interaction test
Salome et al. 2006	Sprague Dawley rats male	10 min post-injection rats were tested on the elevated plus maze	Intra-CeA & Intra-BLA SSR149415	V1b antagonism only in the BLA reduced anxiety on the elevated plus maze
Caldwell et al. 2006	**V1a & V1b KO mice male & female**	**Alcohol 20%v/v in.9% saline IP at 1.0, 1.25 or 1.5 g/kg immediately before being placed in the elevated plus maze**	**N/A**	**V1a and V1b KO show no difference in anxiety on the elevated plus maze compared to WT; no sex related differences**
Egashira et al. 2007	V1a KO mice male	Mice were tested on the elevated plus maze and marble burying test	N/A	Loss of V1a receptor reduced anxiety on the elevated plus maze and marble burying test
Hodgson et al. 2007	CD rats & CD1 mice male	Tests were performed 15 min post-injection of drug; Rats were tested in the elevated plus maze & conditioned lick suppression; Mice were tested in the marble burying task	IP SSR149415	V1b antagonism decreased anxiety in the elevated plus maze and lick suppression task in rats, but not the marble burying in mice
Bleickardt et al. 2009	CD or Sprague-Dawley rats	Drug was injected 30 min before all behavioral tests were given; Rats performed	IP JNJ-17308616	V1a antagonism decreased anxiety in all tests of rats and mice
	male & CD1 mice male	elevated plus-maze, elevated zero-maze and conditioned lick and mice performed the marble burying task		
Bunck et al. 2009	Swiss CD1 mice male & female	Mice were selectively bred for high (HAB) and low (LAB) anxiety-like behavior on the elevated plus maze	N/A	LAB mice have reduced expression of VP gene in the PVN and SON; no sex related differences
Litvin et al. 2011	Swiss-Webster mice male	For 10 days mice received 10 min exposure to a novel resident male followed by 10 min separation by partition for 10 days. On the 11th day mice received 24 hours single housing followed by a drug injection one hour prior to the social investigation paradigm	IP SSR149415	Socially defeated mice showed anxiety-like behaviors in the social investigation paradigm which was partly reversed by V1b antagonist
Mak et al. 2012	Sprague Dawley rats male	Rats were tested on the elevated plus maze 15 min post-drug injection	IV desmopressin	VP agonist causes anxiety on the elevated plus maze
Iijima et al. 2014	Sprague Dawley rats male	Paradigm 1- Social interaction test was performed 1 hr post-drug administration; Paradigm 2– 1 hr post-drug administration the elevated plus maze measured stress induced by swim stress which was performed 5 min post-swim	PO TASP0233278	V1b antagonism decreased anxiety in the social interaction test and reversed anxiety caused by swim stress

Paper	Species/sex	Paradigm	Drug	Results
Hodgson et al. 2014	CD rats male	Conditioned lick suppression 30 min postdrug injection	IP V1B-30N	V1b antagonism decreased anxiety in the conditioned lick suppression test
Duque-Wilckens et al. 2016	California mice male & female	30 min post-injection mice were tested in the social interaction test	Intra-BST manning compound	V1a antagonism in the BNST caused anxiety on the social interaction test; no sex related differences
Hernandez-Perez et al. 2018	Wistar rats male	Immediately following microinjections rats were tested on either the light-dark box or the shock probe burying test	Intra-CeA VP, VP/SSR149415 or VP/manning compound	VP alone caused anxiety only in the shock probe burying test; V1b antagonisms blocked the effects of VP on the shock probe burying test
Dannenhoffer et al. 2018	**SpragueDawley rats male & female**	**4g/kg every 48 h from postnatal day 25–45. At least 25 days after last exposure rats were tested on the social interaction test**	**IP SR-49059 SSR149415**	**Only males showed anxiety in the social interaction test and it was blocked by the V1b antagonist**

Studies in bold are related to alcohol associated anxiety.

IP=intraperitoneal injection, IV=intravenous injection, PO=oral

VP=arginine vasopressin

V1a antagonist=manning compound, SR-49059 & JNJ-17308616 V1b antagonist=SSR149415, TASP0233278 & V1B-30N desmopressin=nonselective VP agonist

BLA=basolateral amygdala, BST=bed nucleus of the stria terminalis, CeA=central nucleus of the amygdala, LS=lateral septum, PVN=Paraventricular nucleus

Table 4:

Summary of VP antagonist clinical trials

Drug	Receptor target	Trial	Disorder	Status
ABT-436	V1b	NCT01613014 NCT01741142 NCT01380704	-Alcohol Abuse -Major Depressive Disorder	Completed
SSR149415	V1b	NCT00374166 NCT00358631 NCT00361491 NCT01606384	-Anxiety Disorders -Major Depressive Disorder	Completed
TS-121	V1b	NCT03093025	-Major Depressive Disorder	Ongoing
SRX246	V1a	NCT02055638 NCT02733614 NCT03036397 NCT02922166	-Intermittent Explosive Disorder -PTSD -Anxiety Disorders/Fear	Ongoing