BEHAVIORAL AND MOLECULAR CHARACTERIZATION OF OXYTOCIN'S EFFECT ON ALCOHOL CONSUMPTION

By

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List of Abbreviations

ACTH: Adrenocorticotropin hormone

AGE(s): Advanced glycation end product(s)

ANOVA- Analysis of variance

ASD: Autism Spectrum Disorder

AUD: Alcohol use disorder

AUDIT: Alcohol use disorder identification test

AVP: Arginine vasopressin

AVPR1a: Arginine vasopressin receptor 1a

BBB: Blood-brain barrier

CA-2BC: Continuous access two bottle choice

CeA: Central nucleus of the amygdala

CI: Confidence interval

CO_2: Carbon dioxide

CRF: Corticotropin releasing factor

CSF: Cerebrospinal fluid

CV: Consumptive visits

D: Day(s)

DA: Dopamine

DAB: Diaminobenzidine

DMSO: Dimethyl sulfoxide

DS: Drink size

D1R(s): Dopamine 1 receptor(s)

D2R(s): Dopamine 2 receptor(s)

EtOH: Ethanol/alcohol

ER: Estrogen receptor

FDA: Food and Drug Administration

G: Gram

GABA: Gamma-aminobutyric acid

GHS-R1a: Growth hormone secretagogue 1a receptor

GPCR: G protein coupled receptor

HM: Herdsman cage system

HR(S): Hours

H_2O: Water

ICV: Intracerebroventricular

IHC: Immunohistochemistry

IN: Inch

IN: Intranasal

IP: Intraperitoneal

IR: Immunoreactivity

IU: International units

LC-MS/MS: Liquid chromatography-tandem triple quadrupole mass spectrometry

LDCV(s): large-dense core vesicle(s)

LLOQ: Lower limit of quantification

MIN: Minute

µl: Microliter

ML: milliliter

µm: Micrometer

mM: Millimolar

MON(s): Magnocellular oxytocin neuron

mRNA: Messenger ribonucleic acid

NAcc: Nucleus Accumbens

ND: Not detected

NHP: Non-human primate

NIH: National Institutes of Health

NNV: Non-nutritive visits

OHSU: Oregon Health & Science University

OPRM1: Mu-opioid receptor

OXT: Oxytocin

D5 OXT: Oxytocin-(*leucine*-5,5,5-d_3, *glycine*-2,2-d_2) trifluoroacetate salt

OXTR: Oxytocin receptor

PBS: Phosphate buffered saline

PFA: Paraformaldehyde

PG: Picogram

PON(s): Parvocellular oxytocin neuron

PPT: Partner preference test

PVN: Paraventricular nucleus of the hypothalamus

RAGE: Receptor for advanced glycation end-products

RFID: Radiofrequency identification

S: Second(s)

SEM: Standard error of mean

SON: Supraoptic nucleus

SUD: Substance use disorder

TX: Treatment

ULOQ: Upper limit of quantification

VTA: Ventral tegmental area

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Abstract

Despite the prevalence and devastating impact of alcohol use disorder (AUD), treatment options remain inadequate with only three United States (U.S.) Food and Drug Administration (FDA) approved pharmacotherapies and limited efficacy across patient populations. Thus, development of new pharmacotherapies is necessary. Of crucial importance in designing such therapies are optimized preclinical, animal models for assessment of potential pharmacotherapies, including translationally relevant routes of administration and incorporation of the complex interactions of alcohol-related behaviors with the social environment.

Oxytocin (OXT), a hormone with crucial roles in a variety of social behaviors, has drawn attention as a promising pharmacotherapy for AUD with its implicated role in mediating the processes associated with alcohol use, as well as its social effects, which may serve to bolster abstinence and reduce harm. OXT has been shown to be effective in decreasing alcohol consumption in rodents and alcohol cue-reactivity in humans; however, results remain mixed and inconclusive. Previous investigation in the Ryabinin laboratory demonstrated robust effects of repeated OXT treatment in decreasing alcohol consumption in the prairie vole (*Microtus ochrogaster*) - a socially monogamous rodent species with demonstrated translational validity to humans through common mechanisms regulating social behaviors. Given the pharmacotherapeutic potential of OXT, it is essential to characterize the behavioral and molecular mechanisms of OXT's demonstrated ability to decrease alcohol consumption.

In this dissertation, I sought to further the translational relevance of the results by administering OXT intranasally, in line with human clinical trials. I aimed to characterize the behavioral mechanisms by recapitulating scenarios human patients face during medication-assisted maintenance of abstinence. Specifically, I employed a mixed-cage design- in which

treated and untreated (control) animals are housed in the same cage. I also utilized the Herdsman cage system to examine OXT's effect on alcohol consumption in socially-housed animals with unrestricted access to both alcohol (in a continuous access two-bottle choice, CA-2BC, design) and same-sex cagemates. Similarly, I assessed whether targeting the OXT system to decrease alcohol consumption can be improved by using an OXT receptor (OXTR) agonist, LIT-001, to optimize the pharmacokinetic profile. Moreover, with consideration of previous reports of limited central penetrance of systemically administered OXT, I also sought to test whether brain penetrance of exogenous OXT could be aided by the receptor for advanced glycation end products (RAGE). This was done by first confirming its presence in the prairie vole brain using immunohistochemistry (IHC). Finally, RAGE-facilitated transport of OXT into the brain was investigated using a selective antagonist, labeled OXT and liquid chromatography-tandem triple quadrupole mass spectrometry (LC-MS/MS). Taken together, I sought to test the hypotheses that: 1) intranasal (IN) OXT was efficacious in decreasing alcohol consumption in a translationally relevant rodent model of alcohol consumption, 2) a small molecule OXTR agonist could substitute for OXT for this effect, and 3) RAGE was involved in the transport of IN OXT into the brain. Given noted sex/gender differences in the OXT system, as well as conflicting preclinical and clinical reports on effects of sex/gender, I aimed to examine possible differences throughout the investigation.

Intranasally administered OXT was observed to selectively decrease measures of alcohol consumption- without effecting water consumption- 3hrs post-treatment and the small molecule OXTR agonist, LIT-001, was shown to selectively decrease measures of alcohol consumption up to 4hrs post-treatment. Importantly, both effects were sex-specific with significant decreases observed only in males. RAGE was both detected and widespread throughout the prairie vole

brain. Moreover, a RAGE antagonist was shown to significantly decrease exogenous OXT levels, suggesting a role for this receptor in the transport of IN OXT centrally. Ultimately, these experiments served to explicate OXT's role in mediating the reward processes associated with drug use and social behavior, particularly within the context of alcohol, and to contribute to the literature on OXT's potential as a promising target for pharmacotherapy for AUD.

Chapter 1: Introduction

1.1 The reciprocal nature of alcohol use and social influences

According to the 2020 National Survey on Drug Use and Health (NSDUH), 40.3 million people aged 12 and older (14.5% of this population) reported having a substance use disorder (SUD) in the past year, with alcohol use disorder (AUD) composing the largest share- affecting 28.3 million people (10.2% of this population) or nearly 3 out of 4 people with SUD (SAMHSA 2021). Alcohol use disorder (AUD) is a progressive disorder with detrimental consequences for both the individual and society. AUD is associated with a number of mental and physical comorbidities, motor vehicle crashes, fetal alcohol syndrome, financial difficulties, and negative social relationships (Lemoine, Harousseau et al. 2003, Mokdad, Marks et al. 2004, Hasin, Stinson et al. 2007, Rehm, Mathers et al. 2009). However, alcohol use can also increase social bonding, desire for socialization and verbal behaviors in those who drink socially as compared to those who drink non-alcoholic beverages (Sayette, Creswell et al. 2012). Moreover, social networks can influence alcohol drinking— fostering the initiation, escalation and de-escalation of alcohol drinking in adolescence (Prinstein, Boergers et al. 2001, Musher-Eizenman, Holub et al. 2003) and continuing to influence consumption in adulthood (Andrews, Tildesley et al. 2002, Delucchi, Matzger et al. 2008). Broadly, alcohol can affect social behavior and affiliations in humans (McLeod 1993, Leonard and Rothbard 1999, Sayette, Creswell et al. 2012).

1.2 The shared neural substrates of social affiliation and alcohol reward

Social bonding and alcohol reward share a number of neural substrates, neuropeptides and neurotransmitters. Principle among them are the hormone oxytocin (OXT) and the neural transmitter dopamine (DA). OXT is a nonapeptide hormone with a myriad of known roles—such as in lactation, parturition and maternal behaviors, social behaviors and attachments, fear and

anxiety, food consumption, body temperature, neuroinflammation, and learning and memory (Lee, Macbeth et al. 2009, Stoop 2012, Carter, Kenkel et al. 2020, Grinevich and Neumann 2021). In recent years, OXT has drawn attention as a promising pharmacotherapy for neuropsychiatric disorders with associated social behavioral deficits (e.g. Autism Spectrum Disorder, schizophrenia, SUD; (Baskerville and Douglas 2010, Lee and Weerts 2016).

OXT is synthesized in the paraventricular nucleus (PVN) of the hypothalamus and supraoptic nucleus (SON). Classical categorization of OXT synthesizing neurons described two main types- magnocellular and parvocellular (Swanson and Sawchenko 1980, Swanson and Sawchenko 1983, Althammer and Grinevich 2017)- which differ in size, shape, anatomical location, projections, function, manner of release, and electrophysiological characteristics (Hatton 1990, Stern and Armstrong 1995). Magnocellular OXT neurons (MONs) project from the PVN to the pituitary, where they act to release OXT into systemic circulation. MONs also project collaterals from axons of this tract to more than 50 forebrain regions (Knobloch, Charlet et al. 2012, Mitre, Marlin et al. 2016, Zhang, Qiu et al. 2021). These areas are involved in the modulation of social and contextual fear responses, transfer of emotional information between conspecifics and aggression between females (Menon, Grund et al. 2018, Ferretti, Maltese et al. 2019, Oliveira, Lukas et al. 2021).

Parvocellular OXT neurons (PONs) have been suggested to be the "master regulators" of MONs (Tang, Benusiglio et al. 2020). PONs synapse onto MONs within the PVN and SON, regulating activity-dependent systemic release of OXT (Eliava, Melchior et al. 2016, Hasan, Althammer et al. 2019). PONs emanating in the PVN also project to the midbrain, brainstem and spinal cord, and consequently have roles in the regulation of food intake, autonomic, sexual, cardiovascular and gastric functions, and the perception of pain (Melis, Argiolas et al. 1986,

Mack, Kc et al. 2002, Petersson 2002, Blevins, Schwartz et al. 2004, Sabatier, Leng et al. 2013, Rash, Aguirre-Camacho et al. 2014, Eliava, Melchior et al. 2016). The convergence of somatosensory information onto PONs, which in turn activate MONs, allows for coordinated, context-dependent release from the MONs, resulting in robust and dynamic activation of the OXT system (Eliava, Melchior et al. 2016).

The classical understanding of oxytocinergic activation of neuronal circuits suggested OXT acted primarily through events at receptors that are preferentially localized to synaptic areas on either the pre- or post-synaptic side to regulate neurotransmission via conventional synapses (e.g. glutamate, gamma-aminobutyric acid (GABA), serotonin synapses), and ultimately modify the "gain" of these synapses (Brussaard, Kits et al. 1996, Dölen, Darvishzadeh et al. 2013, Mairesse, Gatta et al. 2015, Mitre, Marlin et al. 2016, Hung, Neuner et al. 2017). It has been suggested that OXT operates synergistically with traditional neurotransmitters - such as glutamate- whereby action potentials can evoke synaptic release of glutamate, resulting in excitation of post-synaptic neurons, while sustained excitation of MONs results in release of OXT and prolonged, downstream effects on both neuronal networks and associated behaviors (MacGregor and Leng 2013, Maícas-Royo, Leng et al. 2018).

Evidence indicates OXT can also be released non-axonally from the somata and dendrites of MONs (Landgraf and Neumann 2004, Ludwig and Leng 2006). Interestingly, evidence of MONs forming "true", functional synapses with other neurons is scarce outside of the central nucleus of the amygdala (CeA; Knobloch, Charlet et al. 2012). These two modes of release (axonal and dendritic) may be evoked by the same stimulus, but may be regulated separately and operate on different time scales (Landgraf and Neumann 2004, Ludwig and Leng 2006, Hrabovszky and Liposits 2008). OXT can both prime dendritic stores of OXT, to ready them for

activity-dependent release, and mobilize intracellular Ca^{2+} to evoke dendritic release of OXT independent of electrical activity (Grinevich and Ludwig 2021). This dendritically released OXT may have actions similar to those of hormones (Son, Filosa et al. 2013) and involve general diffusion of OXT through the extracellular space to an assorted population of neurons, with signaling specificity dependent upon the presence of transmitter-specific receptors on these neurons. In addition, it has been suggested MONs are capable of *en passant* release (i.e. at a connection between neurons not at the axonal terminal) of OXT -- resulting in the diffusion of a small number of OXT large-dense core vesicles (LDCVs) within a discrete, target region (Chini, Verhage et al. 2017). This may partly explain previously reported delays in OXT activity following evoked release in optogenetic studies (Knobloch, Charlet et al. 2012, Hasan, Althammer et al. 2019).

Release of OXT is thought to involve typical Ca^{2+}-mediated exocytosis of LDCVs (Burbach, Luckman et al. 2001). Briefly, LDCVs are transported to axon terminals and stored in pools which fuse with the pre-synaptic membrane in SNARE-mediated exocytosis. Each LDCV can contain up to 85,000 molecules of OXT (Morris 1976, Nordmann and Morris 1984). Released OXT can then bind post-synaptically. OXT exerts its effect primarily through the OXT receptor (OXTR), a Ca^{2+} dependent G protein coupled receptor (GPCR) coupled to various subtypes/pathways (e.g. Gaq, Ga11, Gi/o, β-arrestin; (Chini, Verhage et al. 2017). The OXT signaling pathway is immensely complex -- with 66 molecules, including 9 protein-protein interactions, 39 post-translational modifications, 14 protein translocation events and 22 activation/inhibition events (Chatterjee, Patil et al. 2016). The various paths of oxytocinergic activation of neuronal circuits appear to allow for the coordinated and robust responses of the OXT system.

OXT receptors (OXTR) are widely distributed throughout the brain. However, species- and sex-specific differences have been noted -- serving evolutionarily to tailor physiological and behavioral responses (Pedersen and Prange 1979, Ferris, Albers et al. 1984, Kendrick, Keverne et al. 1987, Argiolas and Melis 2005). On a species level, OXTR distributions vary both within and across species (Anacker and Beery 2013, Albers 2015) and patterns align with their role in the integration and processing of salient stimuli. For example, in rodents expression is prominent in the olfactory pathway (as rodents primarily navigate their world using olfaction) -- in areas such as olfactory bulb, tubercle and accessory olfactory nucleus- as well as downstream regions receiving projections such as bed nucleus of the stria terminalis (BNST), nucleus accumbens (NAcc), central and lateral amygdala, hippocampal formation (particularly subiculum), neocortex, endopiriform cortex, and ventromedial hypothalamus (VMH). In contrast, human (in which the primary sense is vision) OXTR expression is prominent in the primary visual pathway- including the superior colliculus, pulvinar and primary visual cortex- as well as associated areas involved in signal integration and processing such as the pre-optic/anterior and posterior hypothalamic areas, ventral portion of the lateral septal nucleus, substantia nigra pars compacta, basal nucleus of Meynert, nucleus of the vertical limb of the diagonal band of Broca, substantia gelatinosa of the caudal spinal trigeminal nucleus, medio-dorsal region of the nucleus of the solitary tract, and dorsal horn of the spinal cord (Loup, Tribollet et al. 1989, Loup, Tribollet et al. 1991). Importantly, in rodents and primates, many of these areas have been implicated in the processing of social stimuli information, such as the lateral septum, hippocampus, PFC and NAcc (Freeman and Young 2016).

Sex differences in OXT and OXTR distribution have been widely reported in animal models (including mice, rats, prairie voles and NHPs; (Insel, Gelhard et al. 1991, Tribollet, Dubois-Dauphin et al. 1992, Tribollet, Barberis et al. 1997, Carter 2007). OXT and OXTR expression is typically higher in females (Zingg and Laporte 2003, Carter 2007), but only a few areas are noted to have sexually-dimorphic expression of OXT binding (such as the VMH) while others show no sexual-dimorphism (such as the CeA; (Uhl-Bronner, Waltisperger et al. 2005). However, OXT's central functions on physiology and behavior are critically dependent on steroid hormones, in particular the hormone estrogen.

The estrogen receptor (ER) β is expressed on magnocellular neurons in the PVN and SON (Forsling, Kalló et al. 2003, Hrabovszky, Kalló et al. 2004). OXT gene promoters in both humans and rats contain estrogen-response elements and are activated by estrogen, as well as thyroid hormones (Richard and Zingg 1990, Mohr and Schmitz 1991). However, a recent in vivo study provided evidence for a DNA-independent mechanism (rather than direct regulation) of estrogen action on the OXT gene (Stedronsky, Telgmann et al. 2002). Treatment with either estrogen or testosterone increased OXTR binding and mRNA levels in rat brains, while castration decreased levels (Tribollet, Audigier et al. 1990, Stevenson, Riley et al. 1994, Larcher, Neculcea et al. 1995, Breton and Zingg 1997); however, this may be species-dependent (Insel, Young et al. 1993). OXTR is particularly sensitive to these gonadal steroids within the VMH- an area noted for its role in the regulation of sexual behaviors- in both females and males (De Kloet, Voorhuis et al. 1985, de Kloet, Voorhuis et al. 1986, Coirini, Johnson et al. 1989, Johnson, Coirini et al. 1991, Bale and Dorsa 1995, Bale, Dorsa et al. 1995, Quiñones-Jenab, Jenab et al. 1997).

The mesolimbic dopaminergic pathway is the predominant pathway in natural (e.g. social) and drug-mediated reward and motivational processes; as such, it is also the primary pathway for the development and maintenance of substance use disorders. Mesolimbic DA activity serves to code saliency information of stimuli (De Luca 2014). Thus, it is intricately involved in a variety of physiological and behavioral processes, such as feeding, reproduction and parturition, stress response, aversion, learning, and social interactions (Carlezon and Thomas 2009, Borland, Grantham et al. 2018, Melis, Sanna et al. 2019). Consequently, it is also associated with pathologies such as compulsions and SUD (Koob and Volkow 2016). Neurons in this pathway project from the ventral tegmental area (VTA) to structures of the limbic forebrain such as the amygdala, NAcc and prefrontal cortex (PFC; Vengeliene, 2013). Importantly, the oxytocinergic system and mesocorticolimbic dopaminergic reward pathway share a number of key brain regions such as the PVN, VTA, NAcc, amygdala and PFC.

OXT and OXTR expression in areas such as the VTA, amygdala, NAcc, PFC, hippocampus and BNST have been shown to be crucial in the regulation of motivated behaviors (Goto and Grace 2005). Moreover, many of the OXT PVN projections overlap with DA systems critical in processing of motivationally relevant stimuli (Gordon, Jack et al. 2016). Notably, OXT PVN projections onto VTA DA neurons and NAcc DA terminals affect social reward (Hung, Neuner et al. 2017, Peris, MacFadyen et al. 2017). Projections have also been demonstrated to areas that directly interact with the VTA and NAcc DA neurons, namely the amygdala and medial PFC (Brill-Maoz and Maroun 2016, Maroun and Wagner 2016). The Social Salience Hypothesis of OXT functioning suggests an overarching role of OXT in the regulation of social cue saliency via its actions in the mesocorticolimbic dopaminergic systems (Shamay-Tsoory and Abu-Akel 2016). Significantly, emerging literature suggests that OXT can alter DA

neurotransmission within mesocorticolimbic systems; thereby altering motivational aspects of not only social and maternal behavior, but also alcohol drinking and alcohol-related behaviors (Baskerville and Douglas 2010, Burkett and Young 2012, Groppe, Gossen et al. 2013, Love 2014).

1.3 Prairie voles as a translational animal model of social neuroscience and alcohol use

Modeling social influences on alcohol use in animals has proven difficult as traditional laboratory rodents (i.e. mice and rats) do not alter alcohol consumption based on the drinking status of a conspecific (see (Anacker and Ryabinin 2010) for review). The prairie vole (*Microtus ochrogaster*)- a rodent species with demonstrated translational validity to humans through common mechanisms regulating social behaviors (Insel and Hulihan 1995, Wang, Yu et al. 1999, Pitkow, Sharer et al. 2001, Walum, Lichtenstein et al. 2012)- emerged as an animal model in which social influences on alcohol consumption can be demonstrated. While traditional laboratory rodents have provided foundational insight into the underlying biological mechanisms of various human social behaviors such as mating, maternal care and aggression (Leckman and Herman 2002, Burns-Cusato, Scordalakes et al. 2004, Ferrari, Palanza et al. 2005, Blaustein 2008), these animals do not form long-term social attachments as adults- a social behavior which is ubiquitous in humans- resulting in the understudy of this aspect of social behavior.

In fact, prairie voles and humans belong to an exclusive group of approximately 3-5% of all species of mammals which display social monogamy (Lukas and Clutton-Brock 2013, Johnson and Young 2015), another behavior mice and rats do not typically display (Kleiman 1977). Importantly, the behaviors of social monogamy appear to be facilitated by distinct and evolutionary conserved neural mechanisms mediating selective social attachments. As reviewed above, significantly, a number of homologous neuropeptides, neurotransmitters, and receptors

(e.g. OXT, OXTR, CRF, DA, D1/2Rs) are involved in both affiliative behaviors and alcohol consumption in prairie voles and humans (Walum et al., 2012; Walum et al., 2008). Yet, principal amongst them are the OXT and DA systems (for review see (Potretzke and Ryabinin 2019) and Appendix). These long-term attachments in prairie voles are called pair bonds and were first discovered through observation of wild prairie voles which were repeatedly captured in female-male pairs throughout the breeding and non-breeding seasons, suggesting long-term associations (Getz, Carter et al. 1981). Pair bonds have also been extensively studied in the laboratory. Importantly, the oxytocinergic system and mesocorticolimbic dopaminergic reward pathways interact in their functions related to pair bonds (Liu and Wang 2003) and OXTR expression within the mesolimbic pathways has been shown to be essential for pair-bonding (Young, Gobrogge et al. 2011).

Male prairie voles also display aggression towards unfamiliar males and females (a behavior called mate-guarding), and both sexes participate in the care of offspring (aka biparental; (Kleiman 1977, Thomas and Birney 1979, Buss 1988, Fraley, Brumbaugh et al. 2005). These behaviors can be studied and quantified on macro- and micro-levels within the laboratory, providing insight into the mechanisms regulating these behaviors. For example, the Partner Preference Test (PPT) is used to assess the strength of a pair bond between female-male pairs by quantifying the amount of time spent with the partner animal versus a stranger animal (i.e. partner preference; (Getz, Carter et al. 1981, Williams, Catania et al. 1992, Carter and Getz 1993) following a cohabitation period during which the bond is formed (Insel, Preston et al. 1995). This test can similarly be used in same-sex prairie voles (DeVries, Cho et al. 1997). Using this test, researchers have shown that intracerebroventricular (ICV) injections of OXT facilitate

partner preference formation in female prairie voles, while an OXTR antagonist prevents this formation (Williams, Insel et al. 1994).

Critically, prairie voles voluntarily consume and metabolize alcohol at levels similar to those observed in C57BL/6J mice- an established inbred strain which consume high amounts of alcohol (Anacker, Loftis et al. 2011)- as well as exhibit signs of hyperalgesia following acute withdrawal (i.e. a 24 hour, hr, period; (Walcott, Smith et al. 2018), similar to the increased pain sensitivity observed in alcohol-dependent humans (Jochum, Boettger et al. 2010, Egli, Koob et al. 2012). Prairie voles also prefer alcohol solutions to water (Anacker, Loftis et al. 2011, Ryabinin and Hostetler 2016). In addition, because of the substantial degree of genetic diversity found in outbred prairie voles, high variability in alcohol intake between individuals has been observed (ranging from ~5 g/kg to over 30 g/kg per day)- which, although absent in other inbred rodent models, is prevalent in humans. Although prairie voles metabolize alcohol significantly faster than humans (Anacker, Loftis et al. 2011), BECs have been reported to range between 1.8 and 132.1mg/dL -- well within range of intoxication (80mg/dL; (Walcott and Ryabinin 2020).

Prairie voles also consume greater amounts of alcohol when housed in same-sex pairs compared to isolate-housed voles (Anacker, Loftis et al. 2011), and this social facilitation of alcohol drinking is similar to that observed in humans (de Castro 1990). Moreover, alcohol consumption in prairie voles has been demonstrated to be socially influenced by a non-familiar drinking same-sex conspecific (Anacker, Loftis et al. 2011, Anacker and Ryabinin 2013). Specifically, high drinking voles will decrease their consumption levels when paired with a low drinking vole and occasionally, low drinking voles will increase their consumption levels when paired with a higher drinking vole (Anacker, Loftis et al. 2011).

Importantly, alcohol can affect social behavior and affiliation in prairie voles, as it can in humans (McLeod 1993, Leonard and Rothbard 1999, Sayette, Creswell et al. 2012). In female-male pairs consuming alcohol during the 24hr pair bond formation period, males consumed an average of 11.20 ± 0.81 g/kg of alcohol, with an average alcohol : water preference of 74.00 ± 2.80% and displayed no preference for the partner female compared to males drinking only water, while females consumed an average of 12.48 ± 1.03 g/kg of alcohol, with an average preference of 58.00 ± 5.70%, and showed an increased preference for partner males compared to females drinking only water (Anacker, Smith et al. 2014). Moreover, the social effects of discordant and concordant drinking in female-male pairs appear to be sex-specific. Female prairie voles have been shown to consume more alcohol if their male partner is also consuming alcohol, but not if their male partner is consuming water, and no effect on partner preference was observed in alcohol consuming females (Walcott and Ryabinin 2019), suggesting a resiliency of the pair bond to alcohol consumption in females. However, in males, while a similar increase in alcohol consumption was observed if their female partner was also consuming alcohol (compared to female drinking only water), discordant drinking resulted in a decrease in partner preference, with no effect on partner preference observed in concordant drinking (Walcott and Ryabinin 2017). These studies highlight the influence of social environments on alcohol intake and the reciprocal influence of alcohol consumption on social behaviors. Moreover, these studies serve to emphasize the translational validity of the prairie vole model to behaviors also observed in humans.

With consideration of all of this- from the noted common, distinct, conserved mechanisms regulating social affiliations, to the observed reciprocal influence of alcohol and social environments- prairie voles provide a unique opportunity to study the underlying

mechanisms of social influences on alcohol consumption. Because of this, prairie voles also serve as an ideal model for assessing pharmacotherapies for AUD in a translationally-validated species and in translationally relevant paradigms incorporating social influences (Ryabinin and Hostetler 2016, Ryabinin and Walcott 2018). In fact, naltrexone- one of three United States (U.S.) Food and Drug Administration (FDA) approved pharmacotherapies for AUD- has been shown to significantly decrease preference for alcohol in a limited access procedure in semi-socially housed prairie voles (Anacker and Ryabinin 2010). Moreover, a growth hormone secretagogue 1 receptor (GHS-R1a) antagonist has also been shown to decrease alcohol consumption in a concentration-dependent manner in semi-socially housed prairie voles (Stevenson, Buirkle et al. 2015, Stevenson, Francomacaro et al. 2016). These studies demonstrate the applicability of the prairie vole model for assessing potential pharmacotherapies for AUD in social models of alcohol consumption.

1.4 Pharmacotherapies for AUD

Evidence-based use of pharmacotherapies for AUD originated in 1948 when Hald and Jacobsen discovered that disulfiram taken prior to consuming alcohol resulted in unpleasant "hangover-like symptoms" (i.e. headache, nausea, vomiting, perspiration, etc.) and that these symptoms were the result of disulfiram's ability to inhibit the metabolism of alcohol- specifically, the conversion of acetaldehyde to acetic acid (Hald and Jacobsen 1948). Hald and Jacobsen concluded that disulfiram could be used to sensitize individuals to alcohol and thus be used as a potential pharmacological treatment for AUD. Disulfiram was subsequently approved by the FDA in 1949, becoming the first drug approved for treatment of alcohol dependence, and it has been used consistently to maintain abstinence from alcohol by preventing relapse. Yet, disulfiram's efficacy is debated. A meta-analysis by Skinner, Lahmek et al. 2014 assessing

disulfiram's efficacy as a treatment for alcohol dependence found it to be overall effective in supporting abstinence compared to un-treated controls. However, when comparing studies in which subjects were blind to treatment vs. open-label, the effect of disulfiram compared to controls was found only in the open-label studies, with no effect in blind treatment studies (compared to controls). This implies disulfiram may only be efficacious when individuals are aware they are taking it. While these results suggest a possible placebo effect, they also serve to highlight the importance of social context and pharmacotherapeutic efficacy. Participant expectations of effects given information from study administrators in open-label trials reasonably contribute to this discrepancy in efficacy compared to those in blind trials.

The next pharmacotherapy for AUD did not surface until 1992, when two randomized controlled clinical trials demonstrated efficacy for the non-selective opioid receptor antagonist naltrexone in the treatment of alcohol dependence. (Volpicelli, Alterman et al. 1992) demonstrated that when taken orally, naltrexone decreased mean drinking days, craving and relapse rates during the course of a 12-week period in a population of alcohol-dependent male Veterans of the U.S. Armed Forces. (O'Malley, Jaffe et al. 1992) replicated this in a population of individuals receiving weekly outpatient care. Based on these results, naltrexone was FDA-approved for the treatment of AUD in 1994. While naltrexone exhibits a modest effect in reducing risk of heavy drinking and thereby may be effective in overall harm reduction, it seems to be less effective in supporting abstinence which may be the desired outcome for some patients (Rösner, Leucht et al. 2008, Jonas, Amick et al. 2014). Moreover, naltrexone's effect appears to be dependent on population factors, such as genetics. For example, naltrexone was shown to be more effective in reducing alcohol drinking in individuals carrying the Asp40 allele of the mu-opioid receptor (OPRM1) gene (Oslin, Berrettini et al. 2003). A double-blind, placebo-controlled

randomized clinical trial comparing the efficacy of naltrexone in German (PREDICT study) and U.S. (COMBINE study) populations showed naltrexone to be effective in decreasing the number of heavy drinking days in the COMBINE study, but no such effect was observed in the PREDICT study (Anton, O'Malley et al. 2006, Mann, Lemenager et al. 2013) -- offering further evidence of population-dependent effects.

Finally, in 2004, acamprosate was approved by the FDA for the treatment of AUD based on three European randomized, double-blind studies demonstrating that patients receiving acamprosate displayed longer durations of abstinence and increased rates of total abstinence compared to those receiving the placebo (Paille, Guelfi et al. 1995, Sass, Soyka et al. 1996, Pelc, Verbanck et al. 1997). However, more recent studies in the U.S. and Germany contradict these findings and failed to find acamprosate more effective than placebo (Anton, O'Malley et al. 2006, Mason, Goodman et al. 2006, Mann, Lemenager et al. 2013). Regardless, acamprosate is considered to be more effective than naltrexone in the maintenance of abstinence from alcohol use and is more effective when abstinence has been achieved prior to onset of treatment (Maisel, Blodgett et al. 2013). Acamprosate is believed to function as a NMDA receptor antagonist and positive allosteric modulator of the gamma-aminobutyric acid (GABA)-A receptor ($GABA_A$) to correct imbalances between the inhibitory neurotransmitter GABA and excitatory neurotransmitter glutamate typical of AUD. Thus, differences in efficacy could stem from individual and population differences in various factors (e.g. genetic, societal, etc.), as well as severity of AUD.

While these currently approved pharmacotherapies for AUD have been demonstrated to be effective in decreasing alcohol consumption and other drug use in preclinical, rodent models of AUD/SUD (Griffiths, Wurster et al. 1975, Carroll and Lac 1992, Volpicelli, Alterman et al.

1992, Spanagel, Holter et al. 1996, He, Nebert et al. 1997, MacFadyen, Loveless et al. 2016), preclinical findings have largely failed to translate to clinical trials, often proving unsuccessful in treating SUD patients or only mildly more efficacious than placebo (Fuller, Branchey et al. 1986, Oslin, Berrettini et al. 2003, Haney and Spealman 2008, Mann, Lemenager et al. 2013). Thus, development of new pharmacotherapies is necessary.

1.5 Oxytocin as a pharmacotherapy for AUD

With its implicated roles in mediating the processes associated with drug use, pain and stress reduction, and emotional regulation (Tops, Koole et al. 2014, Leong, Cox et al. 2018), the OXT system seems a promising potential target for pharmacotherapies and particularly for AUD. Moreover, OXT's social effects (Marsh, Scheele et al. 2015, Jiang and Platt 2018) may serve to bolster abstinence as social support has been shown to be an important mediator of treatment outcomes (Dobkin, De et al. 2002, Stevens, Jason et al. 2015).

The relationship between OXT and alcohol requires further characterization on all levels; however, what is clear is that the relationship is complex. Alcohol has been shown to affect not only OXT levels, but underlying neurocircuitry (Fuchs 1966, Stevenson, Young et al. 2017, Walcott and Ryabinin 2017, Hansson, Koopmann et al. 2018, Walcott and Ryabinin 2019). Acutely, alcohol suppresses exogenously and endogenously-induced increases in OXT release into systemic circulation in both pre-clinical animal models (i.e. rabbit and rat; Fuchs and Wagner 1963, Fuchs 1966, Fuchs 1969) and clinical observations in humans (Wagner and Fuchs 1968, Cobo 1973, Fuchs, Husslein et al. 1982, Coiro, Alboni et al. 1992, Mennella, Pepino et al. 2005, Mennella and Pepino 2006). Decreases in OXT levels are likely caused by alcohol-induced decreases in the frequency of OXT release (Gibbens and Chard 1976). However, this may be dose-dependent as studies in ex-vivo hypothalamo-hypophyseal tissue from male rats show

decreased OXT release when incubated with alcohol at lower in vitro doses (35-50mM) and increased OXT release at higher doses (75-100mM; (Hashimoto, Noto et al. 1985). Alcohol's effect on basal OXT levels is less clear. Previous demonstrations show no effect of alcohol on basal OXT levels in both rodents (Blackburn, Stricker et al. 1994) and humans (Bershad, Kirkpatrick et al. 2015, Dolder, Holze et al. 2017), but also increases in basal levels at lower doses of ethanol (0.05-1g/kg, IP) in rodents (Uvnäs-Moberg, Lundeberg et al. 1993).

Effects of prolonged alcohol exposure are equally unclear. Three weeks of alcohol via liquid diet decreased alcohol-evoked inhibition of OXT release from ex-vivo hypophyseal terminals in male Sprague Dawley rats (Knott, Dayanithi et al. 2000). Conversely, 4 weeks of similar liquid diet alcohol exposure in male Wistar rats showed no differences in plasma OXT levels versus controls (Da Silva, Ruginsk et al. 2013). Six and 10 month liquid diet alcohol exposure in male Wistar rats resulted in loss of OXT neurons in the PVN and a compensatory increase in OXT mRNA expression in remaining OXT neurons (Silva, Madeira et al. 2002). After approximately 1 year of alcohol via liquid diet, loss of OXT neurons in the SON was observed in both male and female Wistar rats (Madeira, Sousa et al. 1993, Sousa, Madeira et al. 1995), with compensatory increase in size of the remaining OXT neurons. Importantly, these studies included proper controls for nutritional and hydration deficiencies from alcohol liquid diet in comparisons. Following an extensive withdrawal period (6 months), a reversal of OXT neuronal loss and enlargement has been reported (Sousa, Madeira et al. 1995).

A more intensive alcohol exposure protocol consisting of 7 weeks of intermittent alcohol vapor inhalation showed significant reduction in PVN and SON OXT and OXT mRNA expression levels in male Wistar rats (Hansson, Koopmann et al. 2018), which remained significant up to 21 days during alcohol withdrawal. Increases in OXT mRNA levels were also

observed in a number of brain areas including the NAcc, caudate putamen, amygdala and ventral hippocampus. The directionality of these changes in OXT mRNA expression in the NAcc varied over the course of withdrawal—with alcohol exposed animals displaying lower OXT mRNA levels than controls immediately following their last exposure session, as well as the day after, and higher levels than controls 3 weeks after their last session.

Specifically, in prairie voles: male and female prairie voles voluntarily consuming alcohol during a 24 hr cohabitation period, no changes in OXT-immunoreactivity (ir) were observed in the PVN or SON; however, a significant decrease was observed in the number of OXT-ir fibers in the laterodorsal tegmental area in both sexes (Anacker, Ahern et al. 2014). Longer periods of voluntary consumption have been shown to significantly decrease OXT-ir in the PVN of male prairie voles. A 1 week long period was shown to significantly decrease OXT-ir in the PVN (but not SON; Walcott and Ryabinin 2017, Walcott and Ryabinin 2019) and a 7 week long period was shown to significantly decrease OXT-ir in the anterior (but not posterior) PVN (Stevenson, Young et al. 2017).

In humans, no significant correlations between self-reported alcohol consumption and plasma OXT levels were found in young men volunteers (Betka, Gould et al. 2018). In post-mortem brain tissue from men with AUD, increased OXT and OXTR binding was observed in the ventral striatum, anterior cingulate, nucleus caudatus, and Brodmann area 9 (Hansson, Koopmann et al. 2018), decreased OXT-ir was detected in the SON (Sivukhina, Dolzhikov et al. 2006) and increased OXT mRNA expression levels were found in the PFC (Lee, Schwandt et al. 2017) compared to control subjects.

OXT has been implicated in the regulation of responses to alcohol such as tolerance, craving, abstinence and withdrawal-induced anxiety, as well as social engagement (Szabó,

Kovács et al. 1985, Lee and Weerts 2016, Koob 2021, Ryabinin and Fulenwider 2021). These observations suggest that stimulation of the OXT system may have the potential to diminish excessive alcohol consumption through modulation of the processes associated with multiple phases of the addiction cycle; specifically, OXT could moderate the effects of alcohol during intoxication, reduce the toxicity of alcohol during withdrawal/abstinence and/or reduce craving for alcohol during the anticipation/preoccupation phase (McGregor and Bowen 2012, Pedersen, Smedley et al. 2013, Leong, Cox et al. 2018, Che, Cai et al. 2021).

In particular, the ability of OXT to modulate activity within the mesocorticolimbic system, as well as the hypothalamic-pituitary-adrenal (HPA) axis (Koob 2003, Koob and Volkow 2016, Koob 2021), has been suggested as a possible mechanism by which OXT is able to exert its effect on alcohol drinking (King, Gano et al. 2020); whereby OXT promotes allostasis (Quintana and Guastella 2020) at the system level by regulating dysfunctional neurochemical signaling within circuits (Bowen and Neumann 2017, Pedersen 2017, Baracz, Everett et al. 2020). Within the dopaminergic mesolimbic reward pathways, OXT may act to directly or indirectly recover altered DA functioning and modify and/or recover reward processes altered by alcohol by enhancing and reinforcing the positive effects of natural rewards (e.g. social rewards) (Sanna and De Luca 2021). Within the HPA axis, OXT is thought to act both agonistically on adrenocorticotropin hormone (ACTH) release and facilitate corticotropin releasing factor (CRF)-mediated release of ACTH within the pituitary as evidenced by in vitro studies of OXT administration to superfused hemipituitary or pituitary cells (Antoni, Holmes et al. 1983, Gibbs 1984, Link, Dayanithi et al. 1992). OXT has implicated roles in both basal functioning and stress-induced activation of HPA activity (Neumann, Wigger et al. 2000). Plasma OXT levels have been shown to increase following physiological and psychological

stressors such as: restraint, forced swim, cold stress, shaker stress, hyperosmolarity, and social stress (Lang, Heil et al. 1983, Gibbs 1984, Jezova, Skultetyova et al. 1995, Hashiguchi, Ye et al. 1997, Engelmann, Ebner et al. 1999, Neumann, Wigger et al. 2000).

Interactions between OXT and CRF- a system also intricately involved in SUDs (Koob 2003, Koob and Volkow 2016, Koob 2021)- within the BSNT have been of particular interest in the context of OXT's effect on alcohol. The BNST is part of the extended amygdala and functions as a key relay site between structures of the limbic forebrain and hypothalamic and brain stem regions responsible for neuroendocrine and autonomic functions, as well as behavioral responses in rodents and humans (e.g. ingestive and sociosexual behaviors, stress response, fear, drug consumption) (Crestani, Alves et al. 2013, Avery, Clauss et al. 2016, Sanna, Bratzu et al. 2017, Ch'ng, Fu et al. 2018, Bratzu, Bharatiya et al. 2019, Jadzic, Bassareo et al. 2021). The BNST receives OXT projections from the PVN (Dumais, Alonso et al. 2016) which moderate the activity of several neurotransmitters (e.g. DA, glutamic acid, and nitric oxide) (Bratzu, Bharatiya et al. 2019) -- modulating connectivity and consequently, physiological and behavioral responses (Walker, Miles et al. 2009, Baumgartner, Schulkin et al. 2021). Reciprocal interactions between the PVN and BNST have been demonstrated with CRF type 2 receptors found on PVN OXT terminals in the BNST and PVN cell bodies, as well as OXTR mRNA expression in BNST and PVN CRF neurons -- this suggests a feedback loop whereby OXT can directly moderate excitability of CRF neurons (Dabrowska, Hazra et al. 2011).

The negative feelings, dysphoria and stress associated with abstinence from alcohol have been shown to be associated with long-term neuroplastic alterations induced by alcohol use within the BNST (Harris and Winder 2018). Moreover, chronic stimulation of the BNST CRF system has been proposed as part of the complex neuroplastic changes related to the stress and

anxiety associated with alcohol abstinence—a lead precipitating factor in relapse (Koob and Schulkin 2019). In addition, synaptic rearrangements within the BLA-CeA-BNST circuit during long-term abstinence from alcohol may augment saliency of alcohol-related cues and increase the likelihood of reinstatement and relapse (Francesconi, Berton et al. 2009). OXT may exert its effect on alcohol by interfering with these negative, CRF-mediated aspects of withdrawal and abstinence (i.e. distress, negative feelings, anxiety) and function to promote and/or rearrange these neuroplastic changes through long-term potentiation/ long-term depression, activation of MAPK/ERK pathways and regulation of immediate early genes, gene expression and epigenetic modifications (Leong, Freeman et al. 2017, Busnelli and Chini 2018, Jurek and Neumann 2018, Lin and Hsu 2018, Fan, Shi et al. 2019).

In preclinical studies, OXT administered peripherally has been shown to decrease voluntary alcohol consumption and preference in isolated and semi-socially housed rodents, including mice, rats and prairie voles (McGregor and Bowen 2012, Lee and Weerts 2016, King, Griffin et al. 2017, Pedersen 2017, Stevenson, Wenner et al. 2017, Leong, Cox et al. 2018, Che, Cai et al. 2021, Ferrer-Pérez, Reguilón et al. 2021, Ryabinin and Fulenwider 2021, Sanna and De Luca 2021). The Ryabinin laboratory has shown repeated IP OXT to be efficacious in decreasing alcohol consumption in socially-housed prairie voles in across cage (treatment and control animals in separate cages) and prairie voles and mice in mixed cage (treatment and control animals in the same cage) designs (Walcott and Ryabinin 2020, Caruso, Robins et al. 2021). The direct applicability of the mixed design in particular, more appropriately modeling social situations an individual with AUD may face during medication-assisted maintenance of abstinence, further support OXT as a promising target for pharmacotherapy for AUD. Intracranial administration of OXT has also been shown to be effective in modulating alcohol-

related behaviors, such as alcohol cue-reactivity, stressed-induced reinstatement of alcohol seeking, and alcohol consumption (Peters, Bowen et al. 2017, Hansson, Koopmann et al. 2018, King and Becker 2019), demonstrating directly the central effects of OXT on alcohol-related behaviors.

Moreover, a limited number of clinical studies suggest the potential of OXT as a treatment for AUD. Intranasal (IN) OXT has been shown to decrease cue-induced alcohol craving, withdrawal symptoms and consumption (Pedersen, Smedley et al. 2013, Mitchell, Arcuni et al. 2016) in women and men with AUD at various doses (i.e. 24 – 40 International Units, IUs). In "social drinkers" a 24IU dose of IN OXT has been shown to decrease NAcc connectivity during an alcohol cue-reactivity task (Bach, Reinhard et al. 2019) and this dose has been shown to be similarly effective in decreasing alcohol cue-reactivity in men with AUD (Hansson, Koopmann et al. 2018).

However, more recent studies have proved less promising. In women and men experiencing alcohol withdrawal, this 24 IU dose of IN OXT was shown to be ineffective in decreasing the dose of concurrent oxazepam required for patients to complete a 3 day alcohol detoxification course and withdrawal treatment (Melby, Gråwe et al. 2019). It was also shown to have no effect on motor activity or sleep during acute withdrawal in men (Melby, Fasmer et al. 2020). A 40IU dose of IN OXT (with 20IU booster) was shown to have no subjective (e.g. mood and drug effect ratings), physiological (e.g. cardiovascular measures) or behavioral (i.e. assessing impulsivity, cognition and motor dexterity) effects on women and men social drinkers consuming alcohol concurrently to administration during the laboratory session (Vena, King et al. 2018). In patients self-administering 8IU of IN OXT up to three times daily following the detoxification process, no significant differences in: daily alcohol intake, number of drinking

days, number of days until returning to use, or proportion of relapsing subjects was found between treated and placebo control groups (Melby, Gråwe et al. 2021).

1.6 Optimization of a translationally-relevant, preclinical animal model for assessing OXT for AUD

The varying degrees of efficacy observed in preclinical animal studies and human clinical trials highlight the need for more nuanced approaches to OXT treatment and translationally relevant models for assessment of pharmacotherapies. In line with human clinical trials, in the current investigation, OXT was administered intranasally to increase translational relevance. It is known that IN OXT produces short- and long-term behavioral effects in prairie voles. Chronic, daily treatment with IN oxt (0.08IU/kg, 0.8IU/kg or 8.0IU/kg) for 21days, starting from post-natal days 21, showed in initial increases in social contact with familiar partners following treatment at all doses; however, the low and medium doses also resulted in long-term impairments in the formation of partner preference as evidenced by consistently reduced contact with partners during PPT – highlighting the importance of dose considerations (Bales, Perkeybile et al. 2013). Previous studies in rats indicate more alcohol-selective effects of OXT in the intranasal route of administration (Tunstall, Kirson et al. 2019), which would have important translational implications as this is the preferred route of administration in human clinical trials.

It has been suggested that the complex interactions with social environments- which a majority of preclinical studies have neglected to examine- may contribute to the discrepancy in efficacy between preclinical models and human clinical trials as well (Heilig, Epstein et al. 2016, Ahmed, Badiani et al. 2018, Inagaki 2018). Thus, a medication may be effective in a clinical setting such as an inpatient treatment facility, but can lose efficacy as the patient faces social influences outside the clinic. Demonstrating a potential medication's efficacy in social settings of

alcohol consumption (i.e. presence of conspecifics consuming alcohol) may serve to be critically important. Therefore, in the current investigation, animals were socially-housed and precise, individualized measures of consumption were collected through the combined use of radiofrequency identification (RFID) implants and precision balances in the Herdsman (HM) cage system. Alcohol and water were consumed *ad libitum* in a continuous access two bottle choice (CA-2BC) design. In addition, a mixed- cage design (treated and control animals in the same cage) was employed to more appropriately recapitulate scenarios human patients encounter during medication-assisted maintenance of abstinence (Walcott and Ryabinin 2020, Caruso, Robins et al. 2021). Moreover, prairie voles have been demonstrated to have high predictive validity in identifying the role of OXT in human social affiliations; for example, variations in the OXTR gene are associated with variations in human partner bonding and prairie vole partner preference (Insel and Hulihan 1995, Lee, Macbeth et al. 2009, Walum, Lichtenstein et al. 2012, King, Walum et al. 2016).

1.7 Potential for optimization of OXT's effect on alcohol consumption through use of a small molecular OXTR agonist

Determining the behavioral and molecular effects of OXT on alcohol consumption is critically important for translational application and optimization of treatment efficacy. OXT is noted for its poor pharmacokinetic properties: it is not orally available (limiting administration to IN, IP, or intravenous, i.v.), displays low bioavailability, and is metabolically unstable- following a two compartment disposition model, with the predominant component exhibiting a brief (5.5 minute, min, distributional and 1.2 hr terminal) half-life in humans (Nielsen, Al-Saqi et al. 2017) and (6.97 – 35.7 min mean clearance time) rodents (Tanaka, Furubayashi et al. 2018). However, a wealth of preclinical and clinical trials continue to demonstrate its efficacy in exerting both

central and behavioral effects, including on alcohol consumption (reviewed above). Recent investigations have changed our understanding of the organization of the OXT system (Grinevich and Ludwig 2021) and demonstrated the penetrance of relatively small proportions of exogenous OXT into the brain when administered via various relevant routes (i.e. IN, i.v.; (Lee, Shnitko et al. 2020)— suggesting that further explication of the mechanism of OXT's central effects on behavior is required.

Specifically, with consideration of OXT's metabolic instability, low brain penetrance and potential for non-specific effects on the related AVPR1a, development of an OXTR agonist with a more desirable pharmacokinetic profile could increase the translational potential of targeting the OXT system (Che, Cai et al. 2021, Cid-Jofré, Moreno et al. 2021). Because the majority of developed and tested OXTR agonists are peptides, with limited penetrance across the blood-brain barrier (BBB), development of non-peptide, small molecule OXTR agonists could improve pharmacokinetics. While three small molecule OXTR agonists have been reported (Pitt, Batt et al. 2004, Frantz, Pellissier et al. 2018, Che, Cai et al. 2021, Cid-Jofré, Moreno et al. 2021), two also have moderate affinity for the AVPR1a (TC-OT-39 and WAY-267646; (Frantz, Pellissier et al. 2018, Moy, Teng et al. 2019). This lack of selectivity with regard to affinity at OXTR may obscure their effects when administered systemically. However, the most recently developed small molecule OXTR agonist, LIT-001, has not only a high affinity for the OXTR, but also has a several magnitudes lower affinity to AVRP1a and similar agonist activity on OXTR, thus allowing for targeted, systemic pharmacology of the OXTR. LIT-001 has been shown to be effective in restoring a number of social and behavioral measures in a mu opioid receptor (OPRM1) knock out (KO) animal model of Autism Spectrum Disorder (ASD; (Frantz, Pellissier et al. 2018), as well as inhibiting hyperalgesia caused by inflammation (Hilfiger, Zhao et al.

2020). In order to assess efficacy of LIT-001 in decreasing alcohol consumption similarly to IN OXT in socially-housed prairie voles, I utilized a similar CA-2BC paradigm and mixed-cage treatment design.

1.8 Assessment of the role of the Receptor for Advanced Glycation End-products in the transport of OXT

OXT is thought to cross the BBB (Ermisch, Ruhle et al. 1985) and recent studies in rodents and non-human primates (NHPs) show small proportions of exogenous OXT in the brain when administered peripherally, with minute amounts in various brain regions when administered IN, including the hypothalamus, amygdala, and NAcc (Pisansky, Hanson et al. 2017, Lee, Shnitko et al. 2020). However, the precise mechanisms of OXT's transport into the brain remain elusive, with scarce direct or indirect evidence of the transport process beyond reports of microdialysates in rodent hippocampal and amygdalar tissue and cerebrospinal fluid (CSF) in humans (Striepens, Kendrick et al. 2013, Lefevre, Mottolese et al. 2017).

Recent demonstration of the transport of OXT into the brain by the Receptor for Advanced Glycation End-products (RAGE) on brain capillary endothelial cells using genetically modified mice (Yamamoto, Liang et al. 2019, Yamamoto and Higashida 2020) suggests this as a possible mediator of transport. RAGE is a multi-ligand transmembrane receptor of the immunoglobulin super family and is the primary receptor for the heterogeneous glycated products of proteins, lipids and nucleotides called advanced glycation end products (AGEs). RAGE is expressed in the lungs, heart, kidneys, skeletal muscle, brain and a number of cells, including endothelial cells, macrophages/monocytes, neutrophils, and lymphocytes (Neeper, Schmidt et al. 1992, Brett, Schmidt et al. 1993, Ding and Keller 2005). Interactions between AGEs and their receptors have been shown to adjust cell function via the generation of free

radicals (Schmidt, Hasu et al. 1994, Schmidt, Hori et al. 1994) and RAGE has been implicated in the pathogenesis of a variety of diseases including neurological disorders, cancers, cardiovascular disorders, arthritis, and diabetes (Yan, Ramasamy et al. 2009).

RAGE is composed of an extracellular region, a transmembrane domain, and a short cytoplasmic domain. Due to the diversity of ligands and their diverse effects in various cell types, RAGE-mediated signaling events are complex and involve activation of a diverse array of signaling molecules and signaling modules- such as ERK1/2 and P38 (Lander, Tauras et al. 1997), CDC42/RAC (Bondeva, Wojciech et al. 2011), SPAK/JNK (Hu, Lai et al. 2012) and NF-κB (Liu, Liang et al. 2010)- depending on duration and intensity of RAGE ligation. In all, AGE/RAGE signaling has been reported to include 95 molecules, consisting of 14 protein-protein interactions, 55 catalysis (2 direct and 53 induced), 19 protein translocation and 27 spatiotemporal activation/inhibition events (Soman, Raju et al. 2013). Membrane-bound, full-length RAGE (mRAGE) is expressed on endothelial cell surfaces in various organs, including endothelial cells in the neurovasculature (Candela, Gosselet et al. 2010) and AGE/RAGE signaling in endothelial cells has been shown to moderate endothelial permeability and dysfunction, endothelial-mesenchymal-transition, apoptosis, autophagy, inflammation, and oxidative stress (Toma, Stancu et al. 2009, Ma, Liu et al. 2010, Xu, Wang et al. 2010, Del Turco, Navarra et al. 2011, Li, Li et al. 2011, Xie, You et al. 2011). A soluble form of RAGE (sRAGE) also exists in the plasma, consisting of a product of alternative splicing of the AGER gene called endogenous soluble RAGE (esRAGE) and a form of mRAGE which has shed its ectodomain (Yonekura, Yamamoto et al. 2003).

Despite a wealth of research on RAGE signaling in humans (Yonekura, Yamamoto et al. 2003, Cheng, Tsuneyama et al. 2005, Bongarzone, Savickas et al. 2017) and other rodents

(Harashima, Yamamoto et al. 2006, Yamamoto, Liang et al. 2019), the existence of RAGE has not been previously demonstrated in the prairie vole brain. Preliminary results from the Ryabinin laboratory detected RAGE in hypothalamic tissue using RT-PCR and inspection of the prairie vole genome predicts mRNA variants. (Yamamoto, Liang et al. 2019) used plasmon resonance in mice to show RAGE binds intranasally administered OXT and reconstituted BBB in culture to demonstrate RAGE transports OXT primarily from the blood into the brain; however, to date, no direct evidence of this transport following IN administration of OXT in prairie voles exists. Whether RAGE is present in the prairie vole brain and enriched in regions known to express OXTR, as well as comparison of expression patterns within the cerebrum to previous findings in humans and mice (Cheng, Tsuneyama et al. 2005, Harashima, Yamamoto et al. 2006), must first be established. Once confirmed, the role of RAGE in transport of IN OXT can be assessed using a RAGE antagonist to block RAGE function and treatment with labelled, exogenous OXT which can be quantified using liquid chromatography-tandem triple quadrupole mass spectrometry (LC-MS/MS). Elucidation of the mechanisms of OXT's transport into the brain may prove crucial to the understanding and optimizing OXT's central effects.

1.9 Goals of this dissertation

The current investigation sought to use a translationally relevant animal model, which included IN administration, a social environment with untreated peers and a rodent with demonstrated similarities in the neurocircuitry regulating social behaviors to characterize the behavioral effects of OXT on alcohol consumption. Specifically, I sought to test the hypothesis that IN OXT was efficacious in decreasing alcohol consumption in a translationally relevant rodent model of alcohol consumption. In addition, I assessed whether a small molecule OXTR agonist (LIT-001) with a more desirable pharmacokinetic profile could be similarly used to

optimize effects. Thus, I tested the hypothesis that a small molecule OXTR agonist could be as or more efficacious in decreasing alcohol consumption as OXT. With consideration of its implicated role in transport of systemic OXT into the brain, as well as the conserved signaling mechanisms throughout the mammalian species, the presence and role of RAGE was examined to elucidate the molecular mechanisms of OXT's effect. I tested the hypothesis that RAGE was involved in the transport of IN OXT using a selective RAGE antagonist, labeled OXT and liquid chromatography-tandem triple quadrupole mass spectrometry LC-MS/MS to quantify penetrance. With respect to the known sex differences in OXT functioning (Lee, Macbeth et al. 2009), as well as conflicting preclinical and clinical reports on effects of sex/gender, examination of sex differences throughout the investigation was imperative. Ultimately, this dissertation sought to provide clarity to the growing body of evidence implicating the OXT system as a promising pharmacotherapeutic target for AUD.

Chapter 2: Materials and Methods

2.1 Animals

Adult female and male prairie voles from our laboratory's colony at Oregon Health & Science University were assessed in these experiments. Animals were weaned at age 21 days (±1 day) and housed in same-sex groups (typically siblings, maximum 4 animals) in standard cages (27 x 27 x 13cm) with (1/8in) pelleted cellulose bedding (Biofresh™, Patterson, NY), cotton nestlets and Enviro-Dri for nest building/thermoregulation, and wood blocks and Manzanita sticks for chewing/enrichment. It should be noted that female prairie voles are induced ovulators and therefore do not display estrous cycles unless directly exposed to / housed with a male. Female and male animals were housed in separate rooms prior to experiments and separate cages throughout; preventing induction of ovulation and negating need for assessment or tracking of estrous stages. *Ad libitum* access to water and a diet of rabbit chow (Laboratory Rabbit Diet High Fiber, 5326; LabDiet®, St. Louis, MO) and Timothy hay (Hand-Selected Timothy Grass; Standlee Premium Products, Kimberly, ID) was provided throughout the course of experiments. Animals experienced a 14:10 Light : Dark cycle, with lights-on at 06:00 (note: L : D schedule not adjusted for Daylight Saving Time, DST). All experiments were approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University (OHSU), Portland, OR, USA and conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

2.2 Apparatus

The Herdsman-2 (HM-2; MBrose Faaborg, Denmark) cage system (Figure 1) was used in the experiments measuring alcohol consumption. The HM2 system is designed to allow socially-housed animals unrestrained access to and interaction with the other animal(s) in the

cage, while accounting for precise, individualized measures of fluid intake through the combined use of balances and radiofrequency identification (RFID) implants. Only previous studies from the Ryabinin laboratory and one other have used this cage system to assess potential pharmacotherapies for AUD in rodents (Thomsen, Dencker et al. 2017). Techniplast 1500U Eurostandard type IV S (48 x 37.5 x 21cm) rodent housing cages are equipped with two (11cm) channels (front and back) on one side and stairs specially designed to accommodate prairie voles. Each channel leads to a bottle (one H_2O, one 5% EtOH, v/v) below which is a precision balance with catch tray for spillage- which is automatically deducted from consumption calculations. Breakage of the photobeam at the entrance of the channel triggers the RFID reader to register the animal. A consumption event is initiated when the animal touches the drinking spout and a change of weight of 0.02 grams (g) is detected; the starting weight is saved and ending weight is registered only after system receives a stable reading for 5 seconds (s). A consumption event is ended 30s after the last touch of the drinking spout, regardless of whether the animal remains in the channel. The system collects a number of measures, including: weight of liquid consumed, number of channel entries and consumptive vs. non-nutritive visits (whether liquid is consumed or not). Bedding, nesting/thermoregulating materials and enrichment conditions were identical to home cage, with the addition of a 10in Habitrail OVO tube (Habitrail, Hagen Inc.) to provide a supplementary nesting option in consideration of substantial increase in cage size.

Figure 1: The Herdsman-2 cage system. Precise, individualized measures of fluid consumption measures in socially-housed animals were obtained through the combined use of radiofrequency identification (RFID) implants and precision balances. Specially designed prairie vole stairs lead to channels (front and back) on left side of cage. Bottles (one H2O, one 5% EtOH) rest on precision balances with catch tray to account for any spillage. A photobeam at the entrance of the channel triggers RFID reader and registers animal, and consumption is measured once the animal touches the drinking spout via displacement of liquid on the balance.

2.3 Experimental design and timeline

For experiments examining effects of pharmacologically targeting the OXT system on alcohol (and water) consumption, female and male prairie voles (OXT: *n* = 113, females = 56, males = 57; 75-177 days, d, of age at start of experiment; LIT-001: *n* = 60, females = 31, males = 29; 76-138d) were implanted with RFID microchips (UNO MICRO ID/8, ISO Transponder, 2.12 x 8mm) under mild isoflurane anesthesia and allowed to recover in their home-cages for 2d. Animals were then assigned with same-sex cagemates (max 4 animals/cage) to HM2 cages and allowed 2d to habituate to the cage, with access to the channels blocked and water provided via 25 milliliter (ml) glass tubes equipped with sipper tops through the cage top. This habituation period ensured animals did not build nests in channels and all animals were observed to build nests in the cage during this time. Following habituation, access to channels was opened- one bottle contained water and one bottle contained a 5% ethyl alcohol in water solution (95% EtOH diluted, v/v). Bottle position (front or back) was counterbalanced across cages and remained consistent throughout the course of the experiment. Access remained *ad libitum* in a continuous access two bottle choice (CA-2BC) procedure for 5d to allow for collection of baseline measures of consumption.

To habituate animals to intranasal (IN) administration or IP injection procedures, animals were habituated with vehicle for 3d prior to treatment. In the OXT study, 25 microliters (µl) of saline was administered intranasally into the nasal mucosa via a Hamilton syringe connected to cannula tubing and blunt cannula needle (33 gauge, 2.8 mm length; Plastics One, Roanoke, VA). In the LIT-001 study, vehicle was administered IP at a volume of 10 ml/kg of body weight. Animals were assigned to treatment or control groups semi-randomly to ensure relatively equal levels of baseline consumption and genetic diversity across groups. On the treatment day,

treatment animals received drug (OXT, IN or LIT-001, IP), while controls received vehicle only. Importantly, consistent with our previous study (Walcott and Ryabinin 2020), a mixed cage design- with treatment and control animals in the same cage- was employed. Alcohol and water consumption was measured for a full 24 hours (hr) post-treatment to examine any residual effects. Animals were then euthanized via carbon dioxide (CO_2) inhalation (Figure 2).

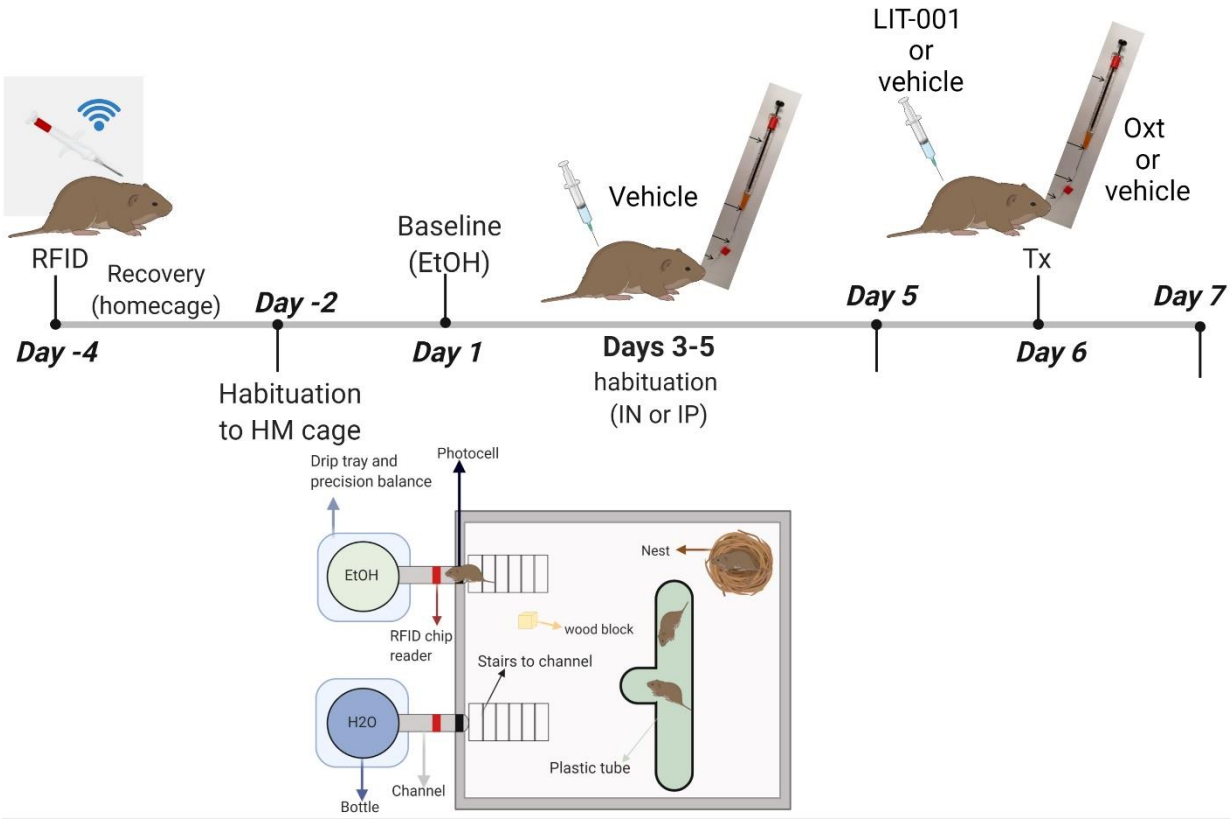


Figure 2: Schematic of experimental timeline for studies examining effects of pharmacologically targeting OXT system on alcohol consumption. Animals were implanted with RFID microchips (Day -4) and allowed to recover in home cages before being habituated to HM2 cages (Day -2), followed by baseline (Days 1-5) and habituation to intraperitoneal (IP) or intranasal (IN) administration procedures (Days 3-5). Animals were treated with (5mg/kg or 10mg/kg) IN OXT or (10mg/kg) IP LIT-001 or vehicle (Day 6) and consumption was measured for a full 24hrs (Day 7) to examine possible residual effects. Created with Biorender.com.

2.4 Drug

OXT acetate salt (Bachem, Torrence, CA, USA) dissolved in 0.9% saline to doses of 0.0 mg/kg (saline control), 5.0 mg/kg and 10.0 mg/kg (25µl, IN) was administered at 08: 30 on the treatment day in a between subjects design. Doses were based on those previously used in investigations of OXT's effects on central OXT systems in prairie voles (Bales, Perkeybile et al. 2013, Guoynes, Simmons et al. 2018) and our previous study (Walcott and Ryabinin 2020); dose calculations were based on average animal weight. Treatment time was based on previous observation of slightly higher consumption of alcohol during the light versus dark phase of circadian cycle (Anacker, Loftis et al. 2011, Anacker and Ryabinin 2013, Walcott and Ryabinin 2020) and occurrence of slightly higher drinking between 1-3hr after lights-on in pilot experiments. LIT-001 was obtained by its formulator, Dr. Marcel Hibert at the Laboratoire d'Innovation Thérapeutique at the University of Strasbourg, dissolved in 5% dimethyl sulfoxide (DMSO) in 0.9% saline, and administered at a dose of 10 mg/kg (IP). Similarly to OXT, LIT-001 was administered at 8:30 on treatment days and dose calculations were based on average animal weight. Dose was based on previous demonstrations of restorative effects on social behaviors in genetically modified mice (Frantz, Pellissier et al. 2018). Alcohol solutions were made by diluting 95% ethyl alcohol (EtOH) in water (H2O) to a concentration of 5% (v/v).

2.5 Immunohistochemistry

To examine RAGE expression within the prairie vole brain, female and male voles (*n* = 20, 10/sex 75- 179d) were cared for as described in Section 2.1 prior to being euthanized via CO_2 inhalation and perfused with 2% paraformaldehyde (PFA) in 0.1M phosphate buffer solution (PBS). Brains were extracted and fixed in 2% PFA/PBS for 24 – 48hr, then cryoprotected in increasing concentrations of (20%, 30%) sucrose with 0.1% sodium azide in PBS for 24hr.

Tissue was sliced in 40µm coronal sections and stored in (0.1%) sodium azide in PBS until assay. Sections containing hypothalamic regions were selected for analysis and regions of interest were determined using the Franklin and Paxinos 2008 mouse brain atlas. Areas of particular interest in examination were based on previous demonstrations of RAGE expression in the human and mouse brain for comparison (Cheng, Tsuneyama et al. 2005, Harashima, Yamamoto et al. 2006) and peripheral OXT penetrance (Lee, Shnitko et al. 2020). The anti-RAGE antibody abcam 3611 (1:1,000, Abcam, Waltham, MA) was used as the primary antibody, along with an anti-rabbit (produced in goat) secondary antibody (Vector Laboratories, Burlingame, CA) and signal was amplified using a VECTASTAIN® ABC-HRP kit (Vector Laboratories, Burlingame, CA). Finally, tissue was stained using a metal enhanced diaminobenzidine (DAB) substrate kit (ThermoFisher Scientific, Waltham, MA) and visualized using a Leica DM4000 bright-field microscope. Control tissue was processed identically with the exclusion of the primary anti-RAGE antibody and was assayed simultaneously.

2.6 Liquid chromatography-tandem triple quadrupole mass spectrometry

To test whether RAGE may have a role in the transport of IN OXT into the brain, female and male prairie voles (n = 29, 75- 207d) were treated as described in section 2.1 and assigned to control (n =14; females = 7) or treatment (n = 15; females = 8) groups semi-randomly to ensure genetic diversity across groups. Animals in the treatment group received the high affinity, BBB permeant RAGE antagonist, FPS ZM1 (1mg/kg; Tocris, Minneapolis, MN, USA) dissolved in 0.9% saline via IP injection and control animals received saline (IP) 30min prior to intranasal administration of 25µl of OXT-(*leucine*-5,5,5-d_3, *glycine*-2,2-d_2) trifluoroacetate salt (3µg/25µl in saline, d5 OXT; Sigma-Aldrich, St. Louis, MO, USA) to all animals. Dose of d5 OXT was based on previous assessments of OXT penetrance (Lee, Shnitko et al. 2020). After 10 min,

animals were deeply anaesthetized under 4% isoflurane before perfusion with heparinized saline. This time period was chosen with consideration of evidence in mice and rats showing that peripheral OXT treatment rapidly increases brain microdialysates and plasma levels during the first 30min following treatment, before returning to baseline (Neumann, Maloumby et al. 2013). Brains were extracted and snap-frozen in isopentane, then stored at -80°C until prepared for analysis via liquid chromatography-tandem triple quadrupole mass spectrometry (LC-MS/MS; Figure 3). Whole brain tissue samples were simultaneously analyzed for concentrations of exogenous d5-deuterated OXT and endogenous OXT and using a Shimadzu Nexera-LCMS-8060 (Shimadzu, Kyoto, Japan) LC-MS/MS instrument. This assay was a modification of an assay previously developed, validated, and utilized by the Endocrine Technologies Core (ETC) at Oregon National Primate Research Center (ONPRC) for analysis of OXT and d5 OXT in NHP samples (Lee, Shnitko et al. 2020). This work was performed in collaboration with Dr. David Erikson, Director of the ETC.

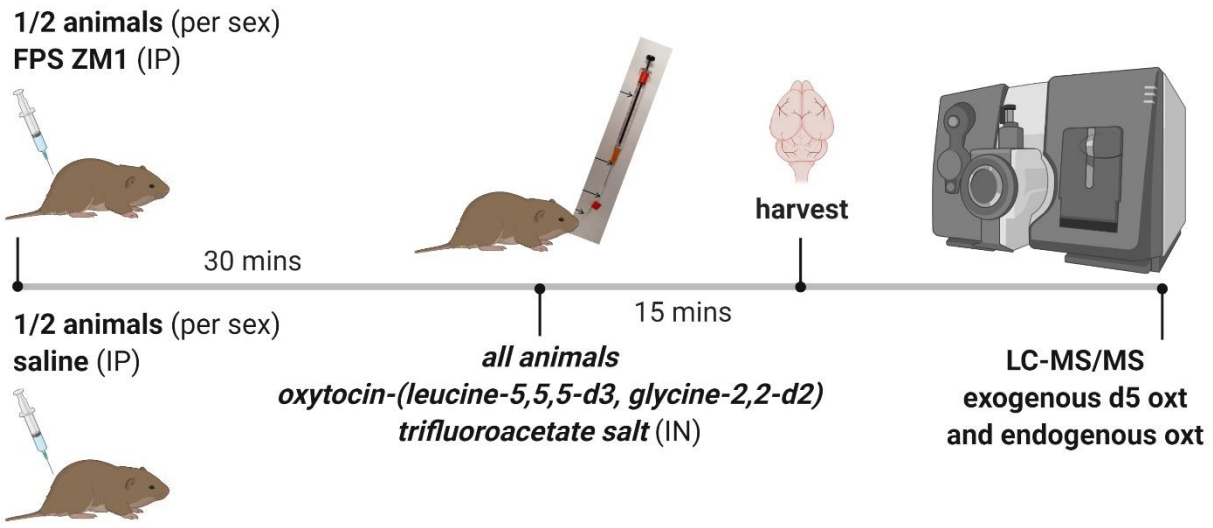


Figure 3: Schematic of experimental timeline for RAGE antagonist with labeled OXT. Half of the animals (per sex) were administered the selective RAGE antagonist FPS ZM1 (1 mg/kg) and the other half were administered saline before all animals were administered labeled d5 OXT. Brains were harvested and snap-frozen before LC-MS/MS analysis for concentrations of exogenous d5 OXT and endogenous OXT. Created with Biorender.com.

2.7 Statistical Analysis

All data were analyzed for normality of distribution and necessary assumptions for statistical test. Analyses were adjusted to appropriate non-parametric test when violations were noted. In consideration of the known sex differences in OXT functioning (Lee, Macbeth et al. 2009), sex was included in analyses. Data analysis was perform in IBM SPSS Statistics 27 and visualization was performed in Prism 9.3.1.

2.7.1 Pharmacological targeting of the oxytocin system

Alcohol and water intake were calculated for each animal by dividing grams of liquid consumed (adjusted per density of 5% concentration of EtOH and water) by kilogram of body weight. Alcohol and water drink size (DS) were determined by ml of liquid consumed. Consumptive visits (CV) were defined as the number of channel entries during which a consumption event was recorded and non-nutritive visits (NNV) were defined as channel entries which did not result in a consumption event (details of measures can be found in Section 2.2 Apparatus). Cumulative measures were analyzed at hourly intervals for 24hrs post-treatment to examine any possible residual effects and Kruskal-Wallis tests were used to examine group differences. Lack of baseline and pre-treatment (-1hr) consumption differences between groups were confirmed and cumulative consumption measures were analyzed from time of treatment (0hr, denoted Tx). When significant differences were found, significant pairwise comparisons were followed-up with Mann-Whitney U tests to confirm significant differences between groups.

2.7.2 Concentrations of exogenous and endogenous oxytocin

Concentrations of exogenously-administered and endogenous OXT (picogram, pg/mg) were obtained through LC-MS/MS analysis. In 3 (2 control male and 1 pre-treated male) out of 29 cases levels of d5 OXT were below lower limit of quantification (LLOQ). These samples

were removed from analysis. In cases when no d5 OXT was detected ("not detected" ND), the concentration was entered as zero (14 total). Seven (3 control female, 3 pre-treated female and 1 pre-treated male) out of 29 endogenous OXT values were determined to be a level of magnitude above the upper limit of quantification (ULOQ). This was most likely due to the contamination of the brain samples with fragments of the pituitary. These samples were thus removed from analysis. Two-way treatment (pre-treatment with RAGE antagonist, FPS ZM1, or saline control) x sex ANOVA was used to examine main effects or interactions on exogenous d5 OXT and endogenous OXT levels. Because no significant main effects or interactions of sex were observed, data were collapsed across sex and analyzed for effect of pre-treatment with RAGE antagonist. Endogenous OXT was analyzed using an unpaired t test; however, the considerable number of zero values (none detected) in exogenous OXT levels skewed distributions and thus a non-parametric Mann-Whitney U test was used to analyze exogenous OXT levels.

Chapter 3: Results

3.1 Effects of intranasal oxytocin on alcohol consumption

A previous study in the lab determined that intraperitoneal administration of OXT resulted in decreased alcohol consumption in socially housed prairie voles, but that this effect was not specific to alcohol and decreased water consumption as well (Walcott and Ryabinin 2020). To determine if OXT was also effective in decreasing alcohol consumption when administered intranasally (a translationally-relevant route), female and male socially-housed prairie voles were given OXT or saline in a mixed-cage design (treated and saline control animals housed in the same cage) and measures of consumption (alcohol/water intake, DS, CV and NNV) were collected.

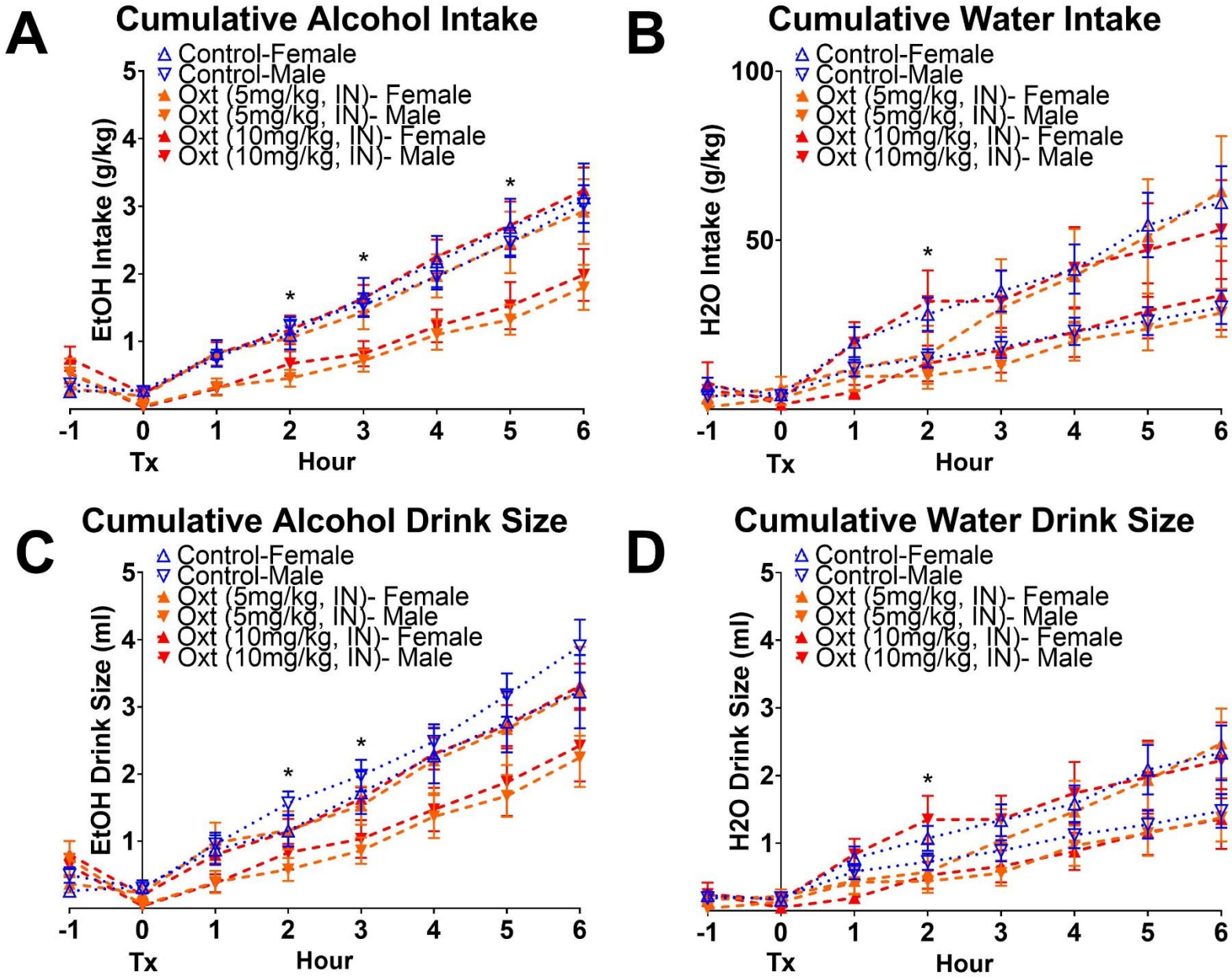
The effects of IN OXT on cumulative measures of consumption were examined at hourly intervals. The distribution of many of these measures was not normal. Therefore, non-parametric statistics were used. Specifically, Kruskal-Wallis tests were used to examine differences between subgroups (control female, control male, 5 mg/kg OXT-treated female, 5 mg/kg OXT-treated male, 10 mg/kg OXT-treated female and 10 mg/kg OXT-treated male) (Figure 4A-H). While group differences in consumption begin to appear at the 0hr time point, differences were confirmed non-significant (ps > 0.38). This is important to note because the 0hr time point includes the time of treatment (Tx) through the first hour post-treatment. Thus, any differences observed do not reflect potential pre-existing differences between groups prior to treatment as animals were assigned to groups semi-randomly to ensure relatively equal levels of baseline (5 days) drinking. The 1hr pre-treatment measures of consumption were also confirmed to be not significantly different between groups (ps > 0.18) and are included to further demonstrate lack of pre-existing differences in consumption between groups.

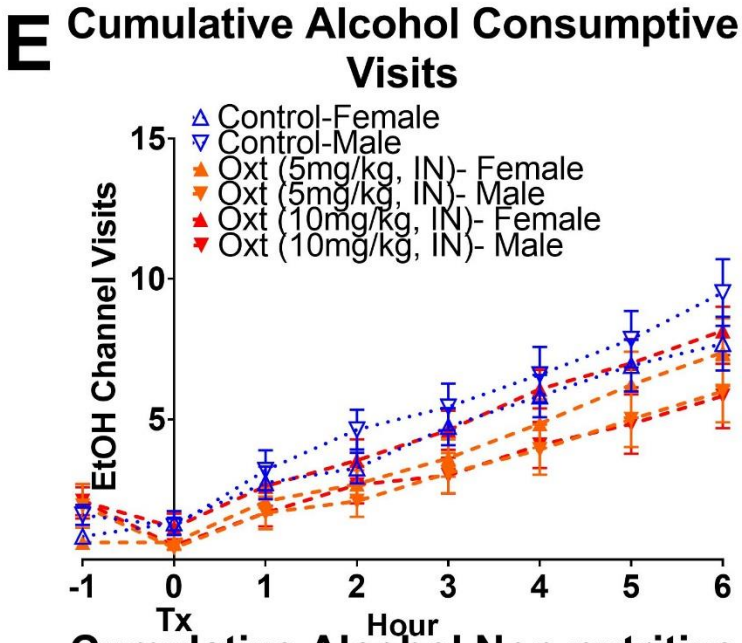
Differences in alcohol intake (Figure 4A) approached significance at the 1hr post-treatment time point ($H(5) = 9.92$, $p = 0.08$) and were found to be non-specific to alcohol at 2hrs post-treatment- affecting both alcohol and water intake (EtOH: $H(5) = 13.19$, $p = 0.02$; H2O: $H(5) = 11.78$, $p = 0.04$, Figure 4B). However, at 3hrs post-treatment, alcohol intake was significantly different between groups ($H(5) = 14.03$, $p = 0.02$), without differences in water intake ($H(5) = 9.04$, $p = 0.11$). A similar pattern of effects was observed in drink size (Figures 4C and D). Significant differences between groups were observed at both the 2- ($H(5) = 13.06$, $p = 0.02$) and 3hr ($H(5) = 11.46$, $p = 0.04$) time points, but alcohol specific effects were observed only at 3hrs (significant differences in water drink size at the 2hr, ($H(5) = 12.34$, $p = 0.03$). While differences in alcohol intake approached significance at 4hrs ($H(5) = 10.86$, $p = 0.054$) and were significant at 5hrs ($H(5) = 12.55$, $p = 0.03$), no differences in alcohol drink sizes were found at these time points ($p > 0.07$).

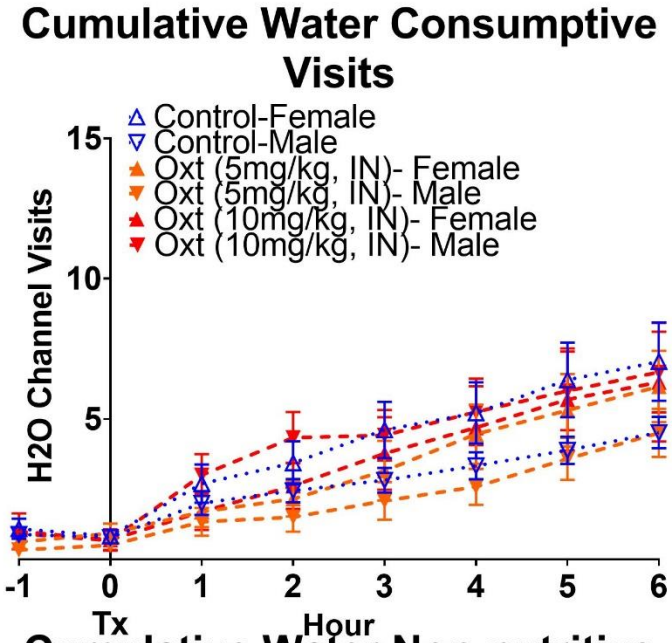
No significant differences were observed in alcohol or water consumptive (Figures 4E and F) or non-nutritive visits (Figures 4G and H) at any time point ($p > 0.94$). To confirm group differences noted in pairwise comparisons, Mann-Whitney U tests were used to examine differences in alcohol intake and drink size between groups at the 3hr time point (Figures 5A and C). Male prairie voles treated with 5 mg/kg- ($M = 0.72$, $\pm SEM = 0.16$; $U(N_{\text{control male}} = 27, N_{5\text{ mg/kg OXT male}} = 12) = 73.00$, $z = -2.71$, $p = 0.01$) and 10 mg/kg ($M = 0.82$, $\pm SEM = 0.19$; $U(N_{\text{control male}} = 27, N_{10\text{ mg/kg OXT male}} = 12) = 86.00$, $z = -2.31$, $p = 0.02$) IN OXT consumed significantly less alcohol than their saline control counterparts ($M = 1.54$, $\pm SEM = 0.17$; Figure 5A). This was also true for drink size, with 5 mg/kg- ($M = 0.85$, $\pm SEM = 0.20$; $U(N_{\text{control male}} = 27, N_{5\text{ mg/kg OXT male}} = 12) = 71.50$, $z = -2.75$, $p = 0.01$) and 10 mg/kg ($M = 1.03$, $\pm SEM = 0.28$; $U(N_{\text{control male}} = 27, N_{10}$

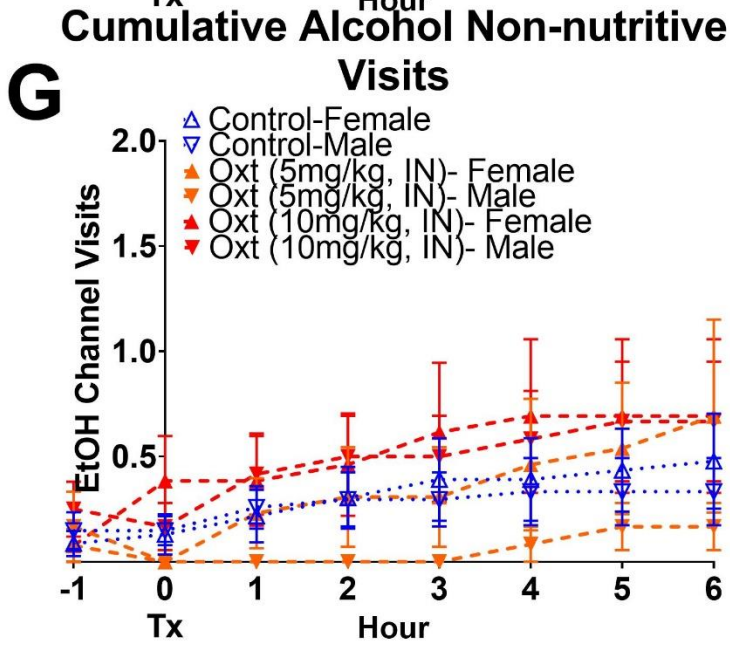
$_{\text{mg/kg OXT male}}$ = 12) = 85.50, z = -2.33, p = 0.02) displaying smaller drink sizes than controls (M = 1.98, ±SEM = 0.24; Figure 5C).

Interestingly, the effect of OXT on alcohol intake and drink sizes at the 3hr time point appeared to be sex-specific, with no effects of OXT observed on treated females and significant differences observed between treated males and females (Figures 5A and C). Males treated with 5 mg/kg IN OXT displayed lower levels of alcohol intake than both 5 mg/kg- (M = 1.44, ±SEM = 0.26; $U(N_{\text{5 mg/kg OXT female}}$ = 13, $N_{\text{5 mg/kg OXT male}}$ = 12) = 40.00, z = -2.07, p = 0.04) and 10 mg/kg (M = 1.64, ±SEM = 0.20; $U(N_{\text{10 mg/kg OXT female}}$ = 13, $N_{\text{5 mg/kg OXT male}}$ = 12) = 24.00, z = -2.94, p = 0.01) OXT-treated females and a similar effect was found on alcohol drink size (5 mg/kg OXT females: M = 1.53, ±SEM = 0.29; $U(N_{\text{5 mg/kg OXT female}}$ = 13, $N_{\text{5 mg/kg OXT male}}$ = 12) = 48.50, z = -1.61, p = 0.11; 10 mg/kg OXT females: M = 1.63, ±SEM = 0.17; $U(N_{\text{10 mg/kg OXT female}}$ = 13, $N_{\text{5 mg/kg OXT male}}$ = 12) = 33.00, z = -2.45, p = 0.01). Males treated with 10 mg/kg IN OXT also displayed lower levels of alcohol intake ($U(N_{\text{10 mg/kg OXT female}}$ = 13, $N_{\text{10 mg/kg OXT male}}$ = 12) = 27.00, z = -2.77, p = 0.01) and smaller drink sizes ($U(N_{\text{10 mg/kg OXT female}}$ = 13, $N_{\text{10 mg/kg OXT male}}$ = 12) = 43.50, z = -1.88, p = 0.06) than their female 10 mg/kg IN OXT counterparts. Taken together, these data identify a sex-dependent effect of IN OXT on consumption. OXT significantly decreased alcohol intake and DS in males, without affecting alcohol consumption in females. Analysis of drinking patterns suggest that OXT's effects on alcohol intake were primarily due to the size of alcohol drinking and not due to number of consumptive or non-consumptive visits to the alcohol spout.


A
Cumulative Alcohol Intake
Control-Female
Control-Male
Oxt (5mg/kg, IN)- Female
Oxt (5mg/kg, IN)- Male
Oxt (10mg/kg, IN)- Female
Oxt (10mg/kg, IN)- Male
EtOH Intake (g/kg)
Tx
Hour
B
Cumulative Water Intake
H2O Intake (g/kg)
C
Cumulative Alcohol Drink Size
EtOH Drink Size (ml)
D
Cumulative Water Drink Size
H2O Drink Size (ml)


E
Cumulative Alcohol Consumptive Visits
Control-Female
Control-Male
Oxt (5mg/kg, IN)- Female
Oxt (5mg/kg, IN)- Male
Oxt (10mg/kg, IN)- Female
Oxt (10mg/kg, IN)- Male
EtOH Channel Visits
15
10
5
-1
0
1
2
3
4
5
6
Tx
Hour


F
Cumulative Water Consumptive Visits
Control-Female
Control-Male
Oxt (5mg/kg, IN)- Female
Oxt (5mg/kg, IN)- Male
Oxt (10mg/kg, IN)- Female
Oxt (10mg/kg, IN)- Male
H2O Channel Visits
15
10
5
-1
0
1
2
3
4
5
6
Tx
Hour


G
Cumulative Alcohol Non-nutritive Visits
Control-Female
Control-Male
Oxt (5mg/kg, IN)- Female
Oxt (5mg/kg, IN)- Male
Oxt (10mg/kg, IN)- Female
Oxt (10mg/kg, IN)- Male
EtOH Channel Visits
2.0
1.5
1.0
0.5
-1
0
1
2
3
4
5
6
Tx
Hour

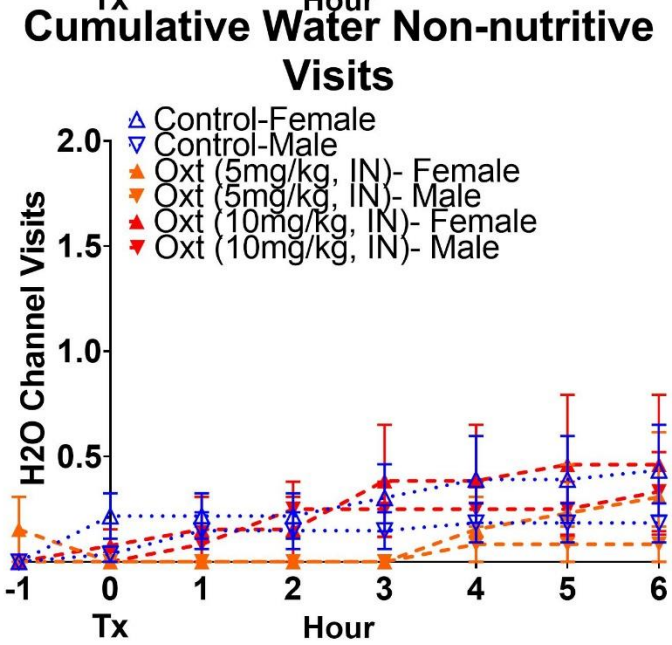
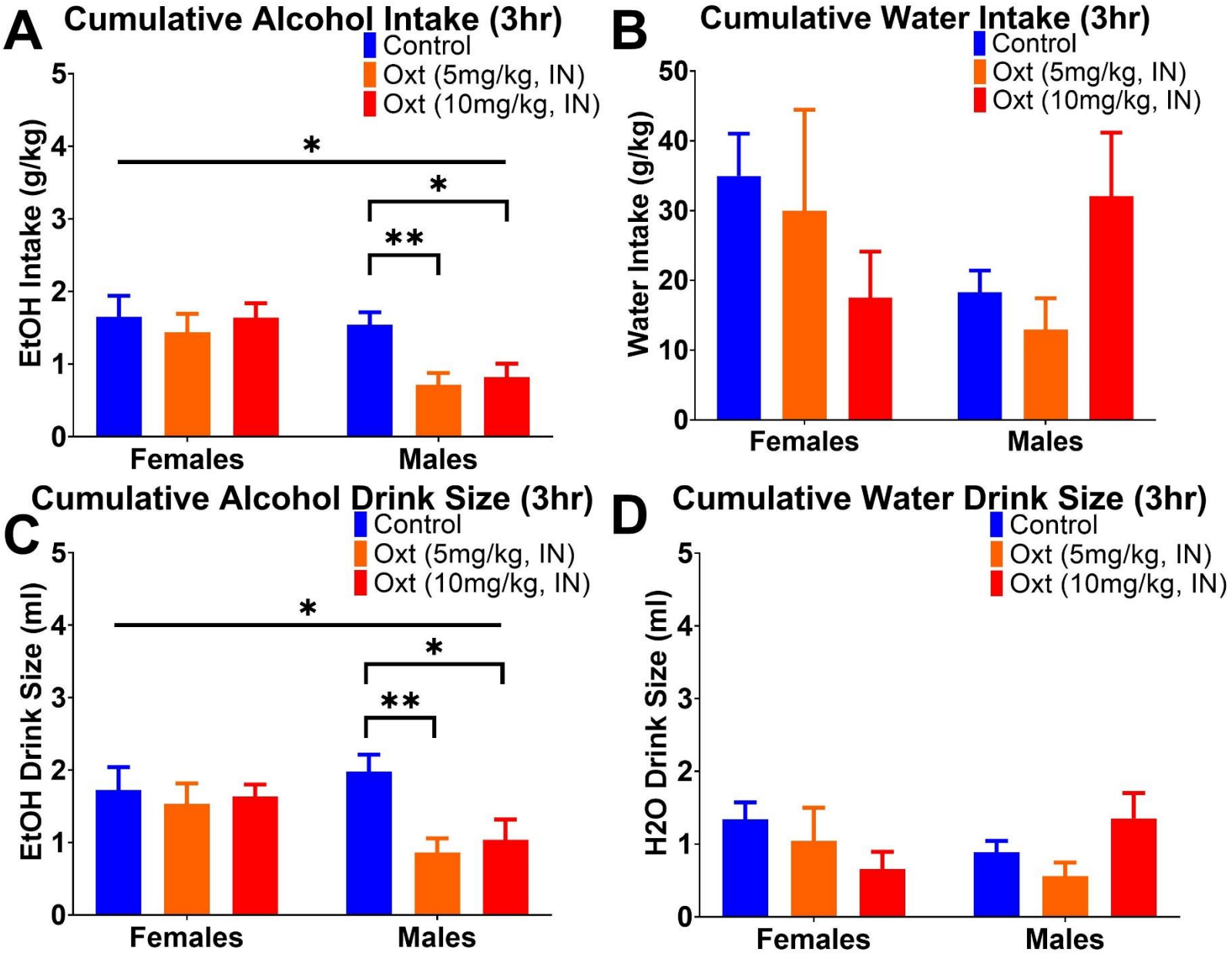

H
Cumulative Water Non-nutritive Visits
Control-Female
Control-Male
Oxt (5mg/kg, IN)- Female
Oxt (5mg/kg, IN)- Male
Oxt (10mg/kg, IN)- Female
Oxt (10mg/kg, IN)- Male
H2O Channel Visits
2.0
1.5
1.0
0.5
-1
0
1
2
3
4
5
6
Tx
Hour

Figure 4: Cumulative measures of alcohol and water consumption 1hr pre- and 6hrs post-intranasal oxytocin treatment. Significant differences were observed between groups in alcohol intake at 2, 3 and 5 hours post-treatment, while differences approached significance at 1 and 4hrs (A). Significant differences were also observed in alcohol drink size at the 2 and 3hr time points (C); however, significant differences in water intake (B) and drink size (D) at 2hrs suggest the effect of OXT not to be specific to alcohol at this time point. No significant differences in alcohol (E) or water (F) consumptive or non-nutritive visits (E and H) were observed at any time point. $*p < 0.05$, Kruskal-Wallis test. Error bars indicate mean ± standard error of mean (SEM), *n*'s: control female = 23, control male = 27, 5 mg/kg OXT female = 13, 5 mg/kg OXT male = 12, 10 mg/kg OXT female = 14, 10 mg/kg OXT male = 12. Tx denotes time of treatment. Note: -1hr time point included to demonstrate lack of differences between groups prior to treatment and is defined as the cumulative measure during the 1hr prior to treatment Cumulative measures post-treatment begin at the 0hr time point which encompasses the time of treatment (Tx) through the first hour.


A
Cumulative Alcohol Intake (3hr)
Control
Oxt (5mg/kg, IN)
Oxt (10mg/kg, IN)
EtOH Intake (g/kg)
0
1
2
3
4
5
*
*
**
Females
Males
B
Cumulative Water Intake (3hr)
Control
Oxt (5mg/kg, IN)
Oxt (10mg/kg, IN)
Water Intake (g/kg)
0
10
20
30
40
50
Females
Males
C
Cumulative Alcohol Drink Size (3hr)
Control
Oxt (5mg/kg, IN)
Oxt (10mg/kg, IN)
EtOH Drink Size (ml)
0
1
2
3
4
5
*
*
**
Females
Males
D
Cumulative Water Drink Size (3hr)
Control
Oxt (5mg/kg, IN)
Oxt (10mg/kg, IN)
H2O Drink Size (ml)
0
1
2
3
4
5
Females
Males

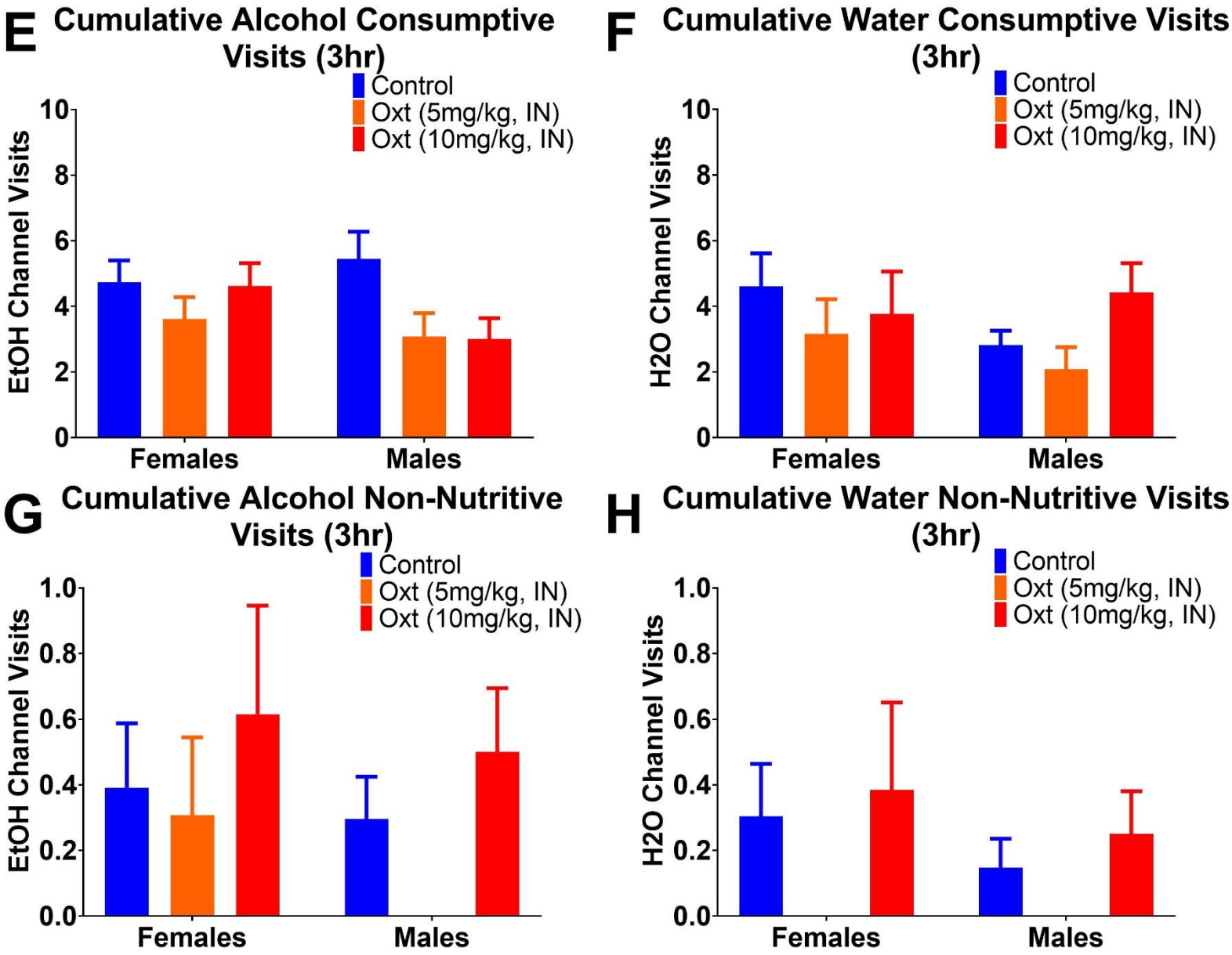

E
Cumulative Alcohol Consumptive Visits (3hr)
Control
Oxt (5mg/kg, IN)
Oxt (10mg/kg, IN)
EtOH Channel Visits
10
8
6
4
2
0
Females
Males
F
Cumulative Water Consumptive Visits (3hr)
Control
Oxt (5mg/kg, IN)
Oxt (10mg/kg, IN)
H2O Channel Visits
10
8
6
4
2
0
Females
Males
G
Cumulative Alcohol Non-Nutritive Visits (3hr)
Control
Oxt (5mg/kg, IN)
Oxt (10mg/kg, IN)
EtOH Channel Visits
1.0
0.8
0.6
0.4
0.2
0.0
Females
Males
H
Cumulative Water Non-Nutritive Visits (3hr)
Control
Oxt (5mg/kg, IN)
Oxt (10mg/kg, IN)
H2O Channel Visits
1.0
0.8
0.6
0.4
0.2
0.0
Females
Males

Figure 5: Cumulative measures of alcohol and water consumption 3hr following intranasal oxytocin treatment. OXT was observed to selectively decrease measures of alcohol consumption- without effecting water consumption- 3hrs post-treatment; thus, group comparisons were confirmed at this time point. Male prairie voles treated with 5 mg/kg- and 10 mg/kg IN OXT consumed significantly less alcohol and displayed smaller drinks sizes than their saline control counterparts (A and C). Note: While 5 mg/kg treated males also displayed lower levels of intake and drink size than 5 mg/kg and 10 mg/kg treated females and 10 mg/kg treated males displayed lower levels (intake and DS) than 10 mg/kg treated females, these comparisons are not included in the figure for ease of interpretation. No effect was observed on water intake or drink size (B and D) and no effects were observed in either alcohol (E) or water (F) consumptive or non-nutritive visits (G and H). *$p < 0.05$, ** $p < 0.01$, Mann-Whitney test. Error bars indicate mean ± standard error of mean (SEM), *n*'s: control female = 23, control male = 27, 5 mg/kg OXT female = 13, 5 mg/kg OXT male = 12, 10 mg/kg OXT female = 14, 10 mg/kg OXT male = 12.

3.2 Effects of oxytocin receptor agonist, LIT-001, on alcohol consumption

A question of clinical, translational relevance is whether a small molecule OXTR agonist could be designed with a more desirable pharmacokinetic profile, but similar or improved efficacy and selectivity (i.e. effect selective to alcohol and not water) in decreasing alcohol consumption. Thus, LIT-001- a small molecule OXTR agonist previously shown to restore social behaviors in a genetically modified mouse model of ASD (Frantz, Pellissier et al. 2018)- was adapted for this purpose and assessed similarly to OXT.

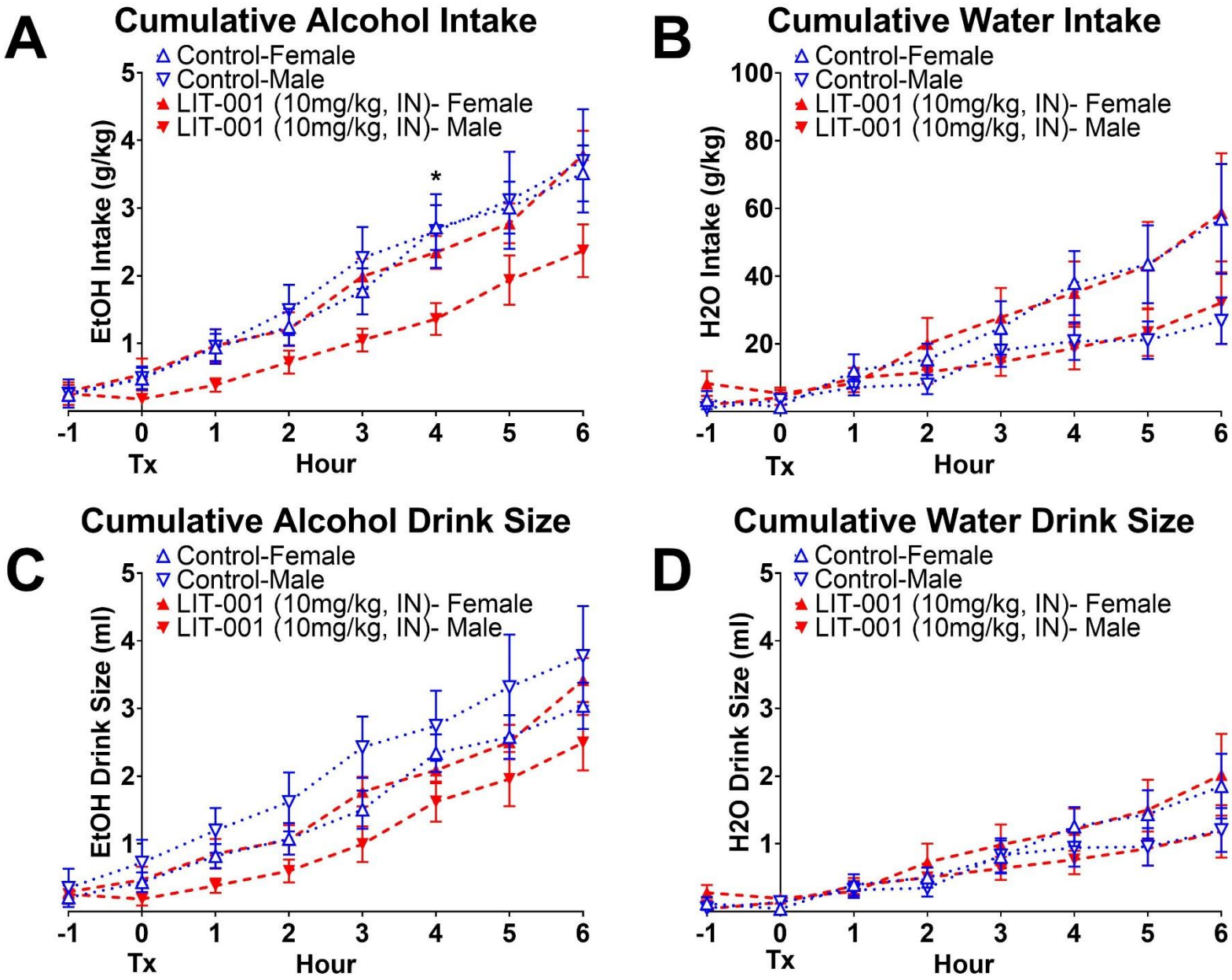
Female and male socially-housed prairie voles were given LIT-001 (IP) or vehicle (5% DMSO in saline) in a mixed-cage design (treated and control animals housed in the same cage) and measures of consumption (alcohol/water intake, DS, CV and NNV) were collected. The effects of LIT-001 on cumulative measures of consumption were similarly examined at hourly intervals (Figures 6A-H) and distributions of many of these measures were not normal; therefore, non-parametric statistics were used. Kruskal-Wallis tests were used to examine group (control female, control male, 10 mg/kg LIT-001-treated female and 10 mg/kg LIT-001-treated male) differences in cumulative hourly consumption measures (intake, drink size, CV, NNV) for 24hr post-IP LIT-001 treatment. LIT-001 was determined to be selectively effective in decreasing cumulative alcohol intake ($H(3) = 7.92$, $p = 0.05$) at 4hrs post-treatment (Figure 6A), without affecting water intake ($H(3) = 2.58$, $p = 0.46$; Figure 6B). Treatment with LIT-001 did not affect alcohol ($H(3) = 5.52$, $p = 0.14$) or water ($H(3) = 1.20$, $p = 0.75$) drink size (Figures 6C and D) or number of alcohol ($H(3) = 1.82$, $p = 0.61$) and water ($H(3) = 1.97$, $p = 0.58$) NNV (Figures 6G and H). However, group differences in alcohol CV were noted at this 4hr post-treatment time point ($H(3) = 9.35$, $p = 0.03$; Figure 6E), without differences in H2O CV ($H(3) = 2.08$, $p = 0.56$; Figure 6F).

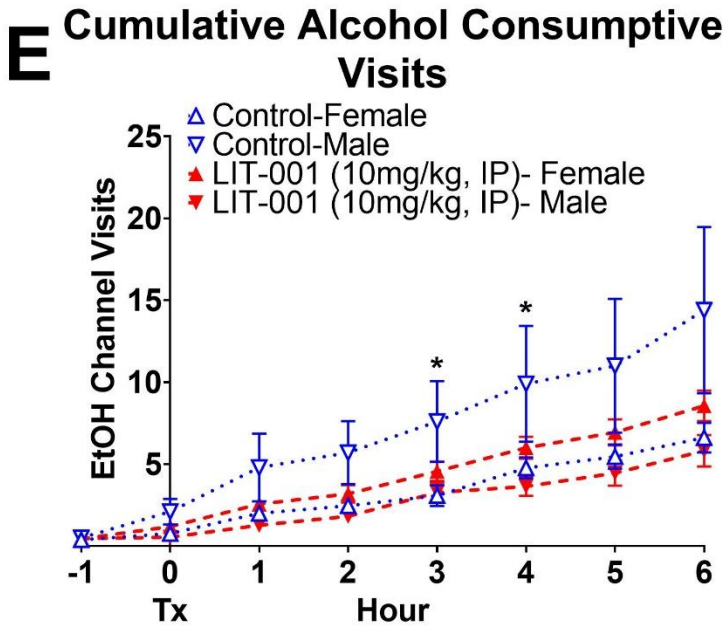
Group differences noted in pairwise comparison were confirmed with Mann-Whitney U tests examining differences in alcohol intake and CV between groups at the 4hr time point (Figures 7A and E). Despite notable differences, males treated with LIT-001 (M = 1.36, ±SEM = 0.24) did not consume significantly less than their male saline control counterparts (M = 2.66, ±SEM = 0.54; $U(N_{\text{control male}} = 10, N_{\text{10 mg/kg LIT-001 male}} = 11) = 31.00$, z = -1.69, p = 0.10). However, significant differences were found between LIT-001 treated males and female controls (M = 2.71, ±SEM = 0.33; $U(N_{\text{control female}} = 13, N_{\text{10 mg/kg LIT-001 male}} = 11) = 26.00$, z = -2.64, p = 0.01), as well as their LIT-001 treated female counterparts (M = 2.35, ±SEM = 0.24; $U(N_{\text{10 mg/kg LIT-001 female}} = 16, N_{\text{10 mg/kg LIT-001 male}} = 11) = 40.00$, z = -2.37, p = 0.02; Figure 7A). Treated males (M = 3.64, ±SEM = 0.58) were also observed to make fewer alcohol channel CV than both control males (M = 9.90, ±SEM = 3.54; $U(N_{\text{control male}} = 10, N_{\text{10 mg/kg LIT-001 male}} = 11) = 19.50$, z = -2.56, p = 0.01) and LIT-001 treated females (M = 6.00, ±SEM = 0.67; $U(N_{\text{10 mg/kg LIT-001 female}} = 16, N_{\text{10 mg/kg LIT-001 male}} = 11) = 43.00$, z = -2.26, p = 0.03; Figure 7E). No other significant differences between groups were noted in any of the other measures (Figure 7B-D, F-H).

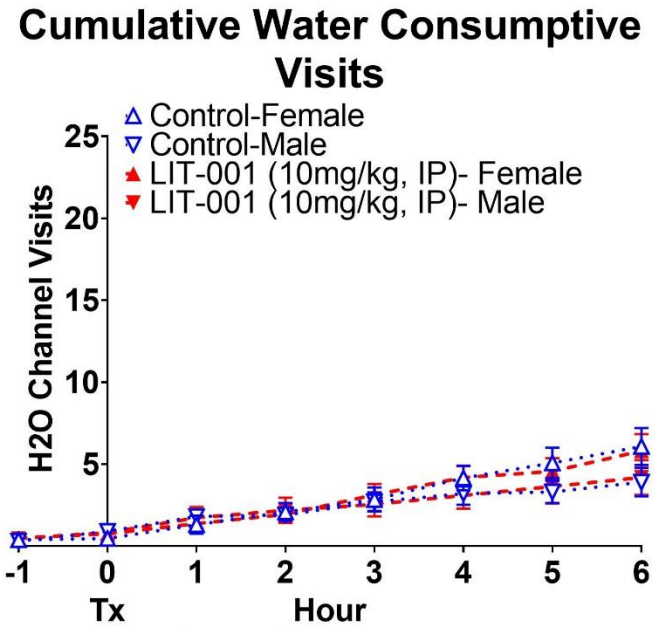
This data offers promising evidence that a small molecule OXTR agonist can be substituted for OXT to effect alcohol consumption. LIT-001 selectively decreased cumulative alcohol intake up to 4hrs post-treatment in males, without affecting consumption levels in females. While a significant difference was not observed between males treated with LIT-001 and saline treated male controls, alcohol intake levels in treated males (M = 1.36g/kg, ±SEM = 0.24) were nearly half those of male controls (M = 2.66g/kg, ±SEM = 0.54). This sizable decrease could signify considerable harm reduction. Moreover, alcohol intake levels in control males were only slightly lower than control females (M = 2.71g/kg, ±SEM = 0.33), which were found to be significantly different from LIT-001 treated males. Despite a slight reduction, LIT-

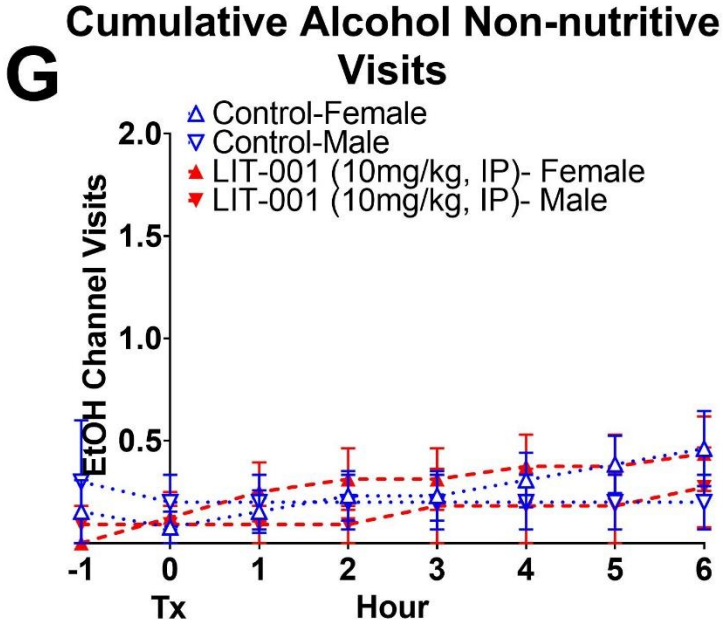
001 treated females (M = 2.35g/kg, ±SEM = 0.24) consumed at levels similar to female and male saline controls. This pattern of effects was similar to that observed with IN OXT.

Broadly, these results support the possibility a small molecule OXTR agonist with a more desirable pharmacokinetic profile can be designed with a similar, or improved, efficacy to OXT in selectively decreasing alcohol consumption, further augmenting clinical translatability. Given the greater affinity of LIT-001 for OXTR over the related AVPR1a, this data suggests that OXTR may be primarily mediating the observed decreases in alcohol consumption following agonism of the OXT system. This data also suggests that the effect of pharmacologically targeting the OXT system on alcohol intake may be sex-specific, with decreased consumption again observed in males but absent in females. This data provides exciting behavioral support for OXT's potential as a pharmacotherapy for AUD. However, given OXT's known metabolic instability the molecular mechanisms of OXT effect remain to be determined. I sought to elucidate aspects of these mechanisms beginning with whether RAGE could be involved in the transport of OXT into the brain following peripheral (IN) administration.


A
Cumulative Alcohol Intake
Control-Female
Control-Male
LIT-001 (10mg/kg, IN)- Female
LIT-001 (10mg/kg, IN)- Male
EtOH Intake (g/kg)
*
Tx
Hour
B
Cumulative Water Intake
H2O Intake (g/kg)
C
Cumulative Alcohol Drink Size
EtOH Drink Size (ml)
D
Cumulative Water Drink Size
H2O Drink Size (ml)


E
Cumulative Alcohol Consumptive Visits
Control-Female
Control-Male
LIT-001 (10mg/kg, IP)- Female
LIT-001 (10mg/kg, IP)- Male
EtOH Channel Visits
25
20
15
10
5
*
*
-1
0
1
2
3
4
5
6
Tx
Hour


F
Cumulative Water Consumptive Visits
Control-Female
Control-Male
LIT-001 (10mg/kg, IP)- Female
LIT-001 (10mg/kg, IP)- Male
H2O Channel Visits
25
20
15
10
5
-1
0
1
2
3
4
5
6
Tx
Hour


G
Cumulative Alcohol Non-nutritive Visits
Control-Female
Control-Male
LIT-001 (10mg/kg, IP)- Female
LIT-001 (10mg/kg, IP)- Male
EtOH Channel Visits
2.0
1.5
1.0
0.5
-1
0
1
2
3
4
5
6
Tx
Hour

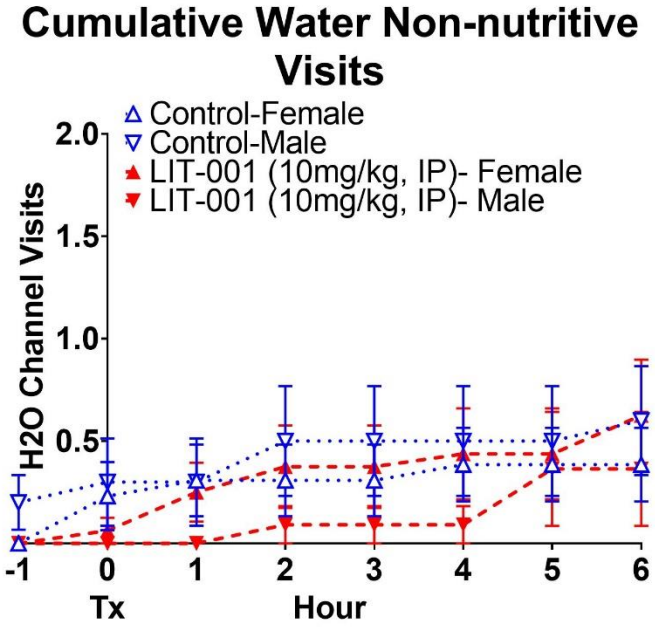
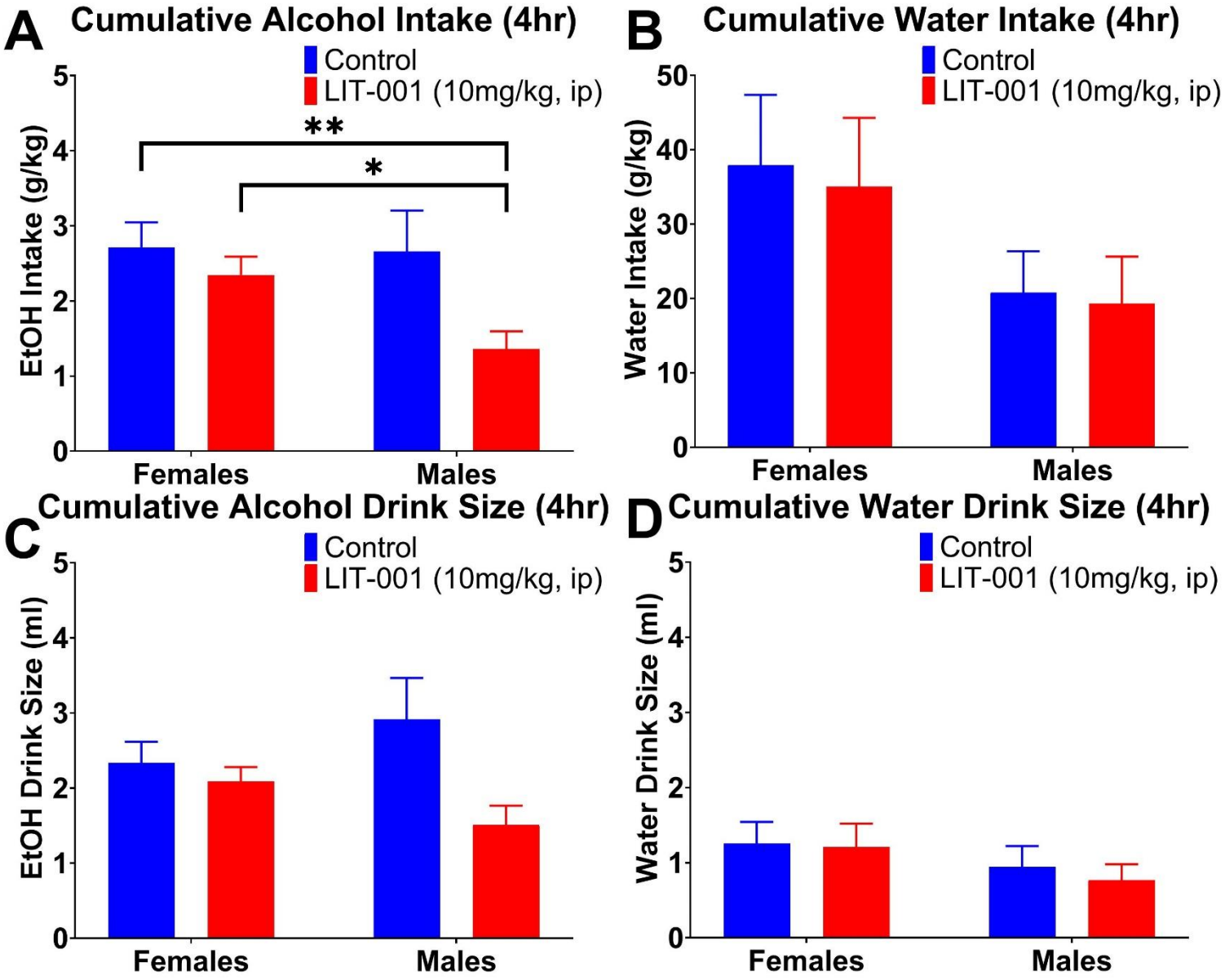

H
Cumulative Water Non-nutritive Visits
Control-Female
Control-Male
LIT-001 (10mg/kg, IP)- Female
LIT-001 (10mg/kg, IP)- Male
H2O Channel Visits
2.0
1.5
1.0
0.5
-1
0
1
2
3
4
5
6
Tx
Hour

Figure 6: Cumulative measures of alcohol and water consumption 1hr pre- and 6hrs post-LIT-001 treatment. Significant differences were observed between groups in alcohol intake at 4 hours post-treatment (A), without any effect on water intake (B). No differences were observed in alcohol (C) or water (D) drink size. Differences in alcohol CV were noted at 3- and 4hrs (E), but no differences in water CV (F) or alcohol G) or water (H) NNV were found. $*p < 0.05$, Kruskal-Wallis test. Error bars indicate mean ± SEM, *n*'s: control female = 13, control male = 10, 10 mg/kg LIT-001 female = 16, 10 mg/kg OXT male = 11. Tx denotes time of treatment. Note: -1hr time point included to demonstrate lack of differences between groups prior to treatment.


A
Cumulative Alcohol Intake (4hr)
Control
LIT-001 (10mg/kg, ip)
EtOH Intake (g/kg)
**
*
Females
Males
B
Cumulative Water Intake (4hr)
Control
LIT-001 (10mg/kg, ip)
Water Intake (g/kg)
Females
Males
C
Cumulative Alcohol Drink Size (4hr)
Control
LIT-001 (10mg/kg, ip)
EtOH Drink Size (ml)
Females
Males
D
Cumulative Water Drink Size (4hr)
Control
LIT-001 (10mg/kg, ip)
Water Drink Size (ml)
Females
Males

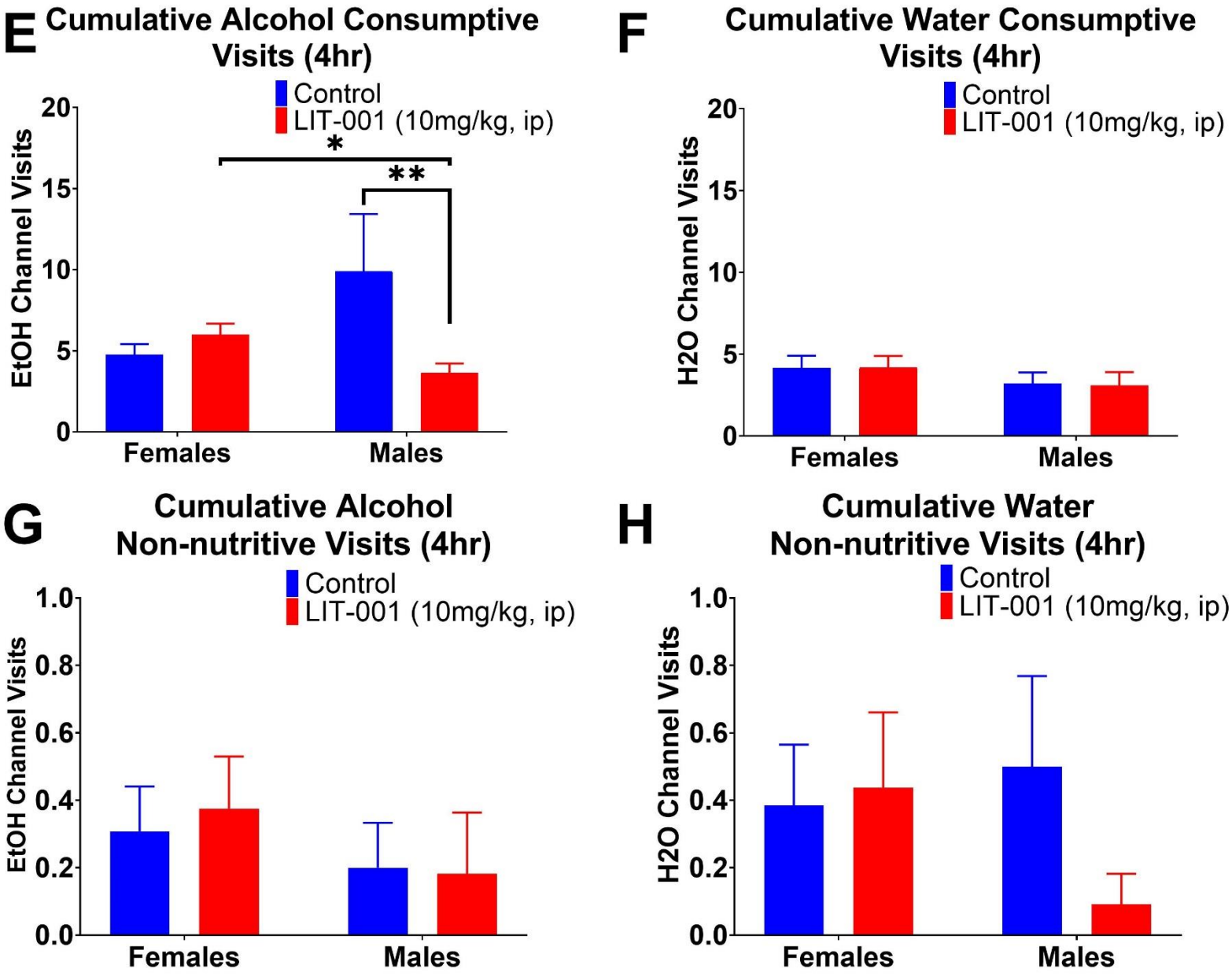
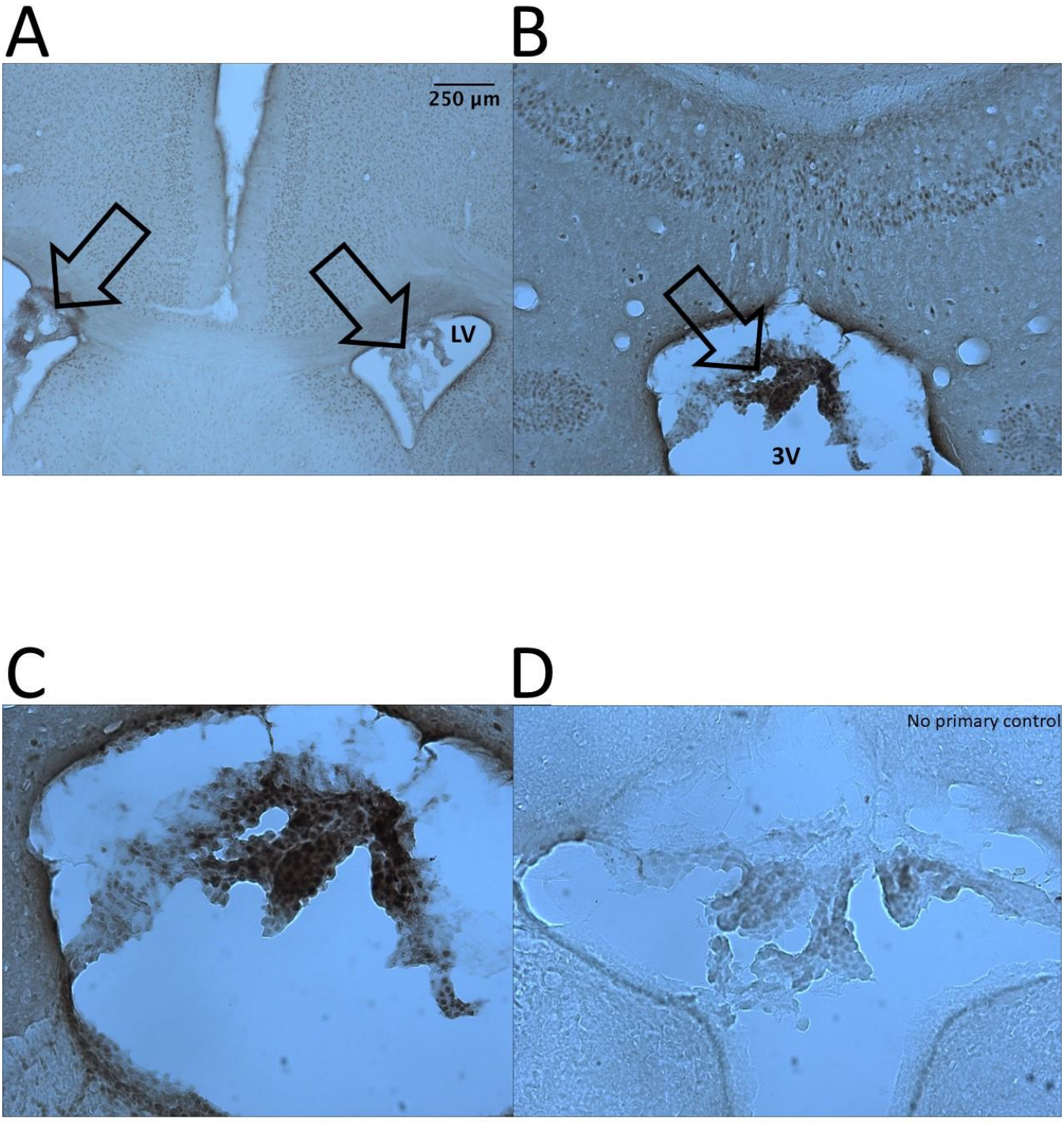

E
Cumulative Alcohol Consumptive Visits (4hr)
Control
LIT-001 (10mg/kg, ip)
EtOH Channel Visits
20
15
10
5
0
*
**
Females
Males
F
Cumulative Water Consumptive Visits (4hr)
Control
LIT-001 (10mg/kg, ip)
H2O Channel Visits
20
15
10
5
0
Females
Males
G
Cumulative Alcohol Non-nutritive Visits (4hr)
Control
LIT-001 (10mg/kg, ip)
EtOH Channel Visits
1.0
0.8
0.6
0.4
0.2
0.0
Females
Males
H
Cumulative Water Non-nutritive Visits (4hr)
Control
LIT-001 (10mg/kg, ip)
H2O Channel Visits
1.0
0.8
0.6
0.4
0.2
0.0
Females
Males

Figure 7: Cumulative measures of alcohol and water consumption 4hr following LIT-001 treatment. Despite observable differences, males treated with LIT-001 did not consume significantly less alcohol than male saline treated controls, but did consume less than both female controls and LIT-001 treated females (A), without any differences observed in water consumption (B). No differences were observed between groups in alcohol (C) or water (D) drink size; however, LIT-001 treated males were observed to make fewer CV in to alcohol channel (E) than control males and LIT-001 treated females, without differences in water CV (F), or alcohol (G) or water (H) NNV. *$p < 0.05$, ** $p < 0.01$, Mann-Whitney test. Error bars indicate mean ± SEM, *n*'s: control female = 13, control male = 10, 10 mg/kg LIT-001 female = 16, 10 mg/kg OXT male = 11.

3.3 The Receptor for Advanced Glycation End-products in the prairie vole brain

Recent evidence demonstrating transport of OXT into the brain by RAGE (Yamamoto, Liang et al. 2019) in mice suggests further examination of its role in transport of IN OXT may be warranted. Thus, I first sought to test the hypothesis that RAGE is present in the prairie vole brain and enriched in regions known to express OXTR, as well as compare expression patterns within the cerebrum to previous findings in humans and mice (Cheng, Tsuneyama et al. 2005, Harashima, Yamamoto et al. 2006).

To do this, RAGE expression was examined using immunohistochemistry (IHC). RAGE-ir was observed throughout the brain, with similar expression patterns observed in females and males (representative images provided below). Importantly, RAGE was detected surrounding the ventricles (lateral and third) and the choroid plexus (Figures 8A - E). RAGE was also found in hypothalamic regions including the PVN and VMH (Figures 8E) and the hippocampus (Figure 8G). No RAGE-ir was detected in control tissue in the absence of the primary anti-RAGE antibody (Figure 8D, F and H), confirming staining and wide distribution. These results demonstrate, for the first time, the presence and wide distribution of RAGE in the prairie vole brain, as well as confirm expression in brains areas also observed in humans and mice (Cheng, Tsuneyama et al. 2005, Harashima, Yamamoto et al. 2006). Moreover, detection of expression in key brain regions responsible for OXT production and modulation (PVN and other hypothalamic areas), as well as possible locations of central penetration (ventricles, choroid plexus), support a potential role for RAGE in the transport of peripheral OXT into the brain.


A
B
250 µm
LV
3V
C
D
No primary control

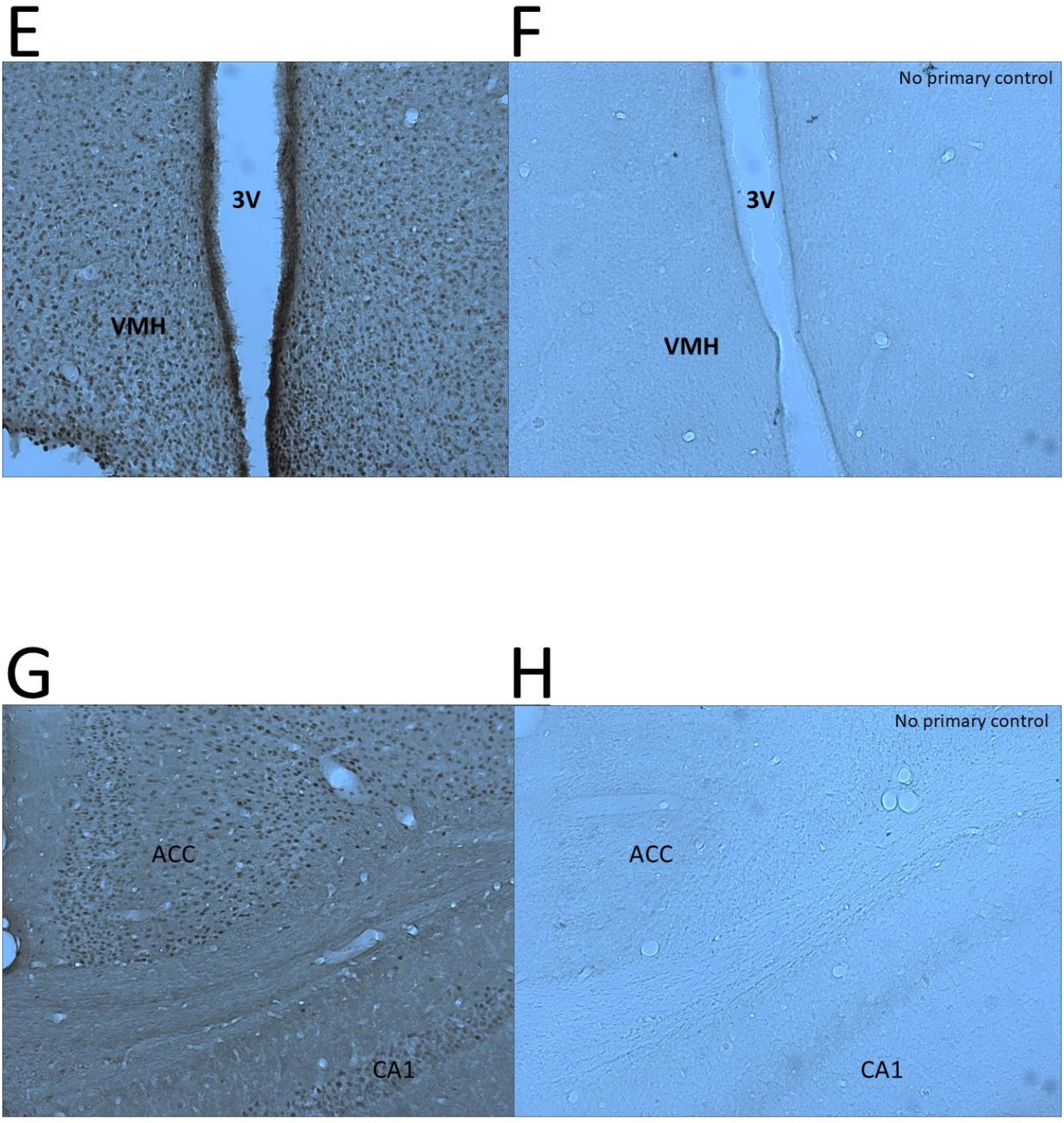

E
F
3V
VMH
No primary control
3V
VMH
G
H
ACC
CA1
No primary control
ACC
CA1

Figure 8: Representative photomicrographs of RAGE-immunoreactivity in the prairie vole brain. RAGE immunoreactivity (ir) was observed widely in the prairie vole in both female and male animals. RAGE-ir was detected surrounding the ventricles. RAGE-ir was detected surrounding the ventricles (lateral in A and third in B) and choroid plexus (as indicated by arrows in A and B). Increased magnification for detailed examination of staining is provided in (C) and comparison to no primary control in (D) confirms staining. RAGE-ir was also detected in hypothalamic regions including the PVN and VMH (E) and hippocampus (G) and staining was again confirmed when compared to no primary controls (F and H).3V = third ventricle; LV = lateral ventricle; VMH = ventromedial hypothalamus; ACC = anterior cingulate cortex; CA1 = CA1 region of the hippocampus.

3.4 RAGE-mediated transport of intranasal oxytocin: exogenous and endogenous concentrations of oxytocin

Despite demonstrations of its efficacy when administered peripherally, reports on central effects of systemic OXT remain conflicting (reviewed above). Recent studies in mice and NHPs using radio- and stable isotope-labeled OXT administered intranasally support acute penetration in various brain regions, including the hypothalamus, amygdala, and NAcc (Pisansky, Hanson et al. 2017, Lee, Shnitko et al. 2020). However, to date, no direct evidence of this transport following IN administration of OXT in prairie voles exists, despite well-established research on OXT in prairie voles. Thus, I sought to test directly whether exogenously administered labelled OXT penetrates into the brain. I also tested whether this penetrance is dependent on RAGE by inhibiting its activity using a selective RAGE antagonist.

As expected, exogenous d5 OXT concentrations were low, while endogenous OXT concentrations were observed to be high (Figure 9). A two-way treatment (pre-treatment with RAGE antagonist, FPS ZM1, or saline control) x sex ANOVA showed a significant main effect of treatment on exogenous OXT levels ($F(1, 21) = 5.48$, $p = 0.03$; Figure 9A), without a significant main effect of sex ($F(1, 21) = 2.18$, $p = 0.15$) or interaction ($F(1, 21) = 0.14$, $p = 0.71$). A similar two-way ANOVA showed no significant main effects of pre-treatment with RAGE antagonist ($F(1, 18) = 0.01$, $p = 0.95$) or sex ($F(1, 18) = 0.07$, $p = 0.79$), or interaction ($F(1, 18) = 0.11$, $p = 0.75$), on endogenous OXT levels (Figure 9B). When data was collapsed across sex and analyzed for effect of pre-treatment with RAGE antagonist, these results were confirmed. A Mann-Whitney U test comparing ranked distributions of exogenous d5 OXT levels between treatment groups confirmed differences (Mann-Whitney $U = 40$, $p = 0.02$; Figure 10A). Pre-treated animals had lower levels of exogenous d5 OXT (median = 0.000 pg/mg, $M = 0.003$

pg/mg, ±*SEM* = 0.002, *n* = 13) than controls (median = 0.01 pg/mg, *M* = 0.02 pg/mg, ±*SEM* = 0.006, *n* = 12). No difference was observed between pre-treated animals (*M* = 44.92 pg/mg, ±*SEM* = 6.90) and controls (*M* = 45.90 pg/mg, ±*SEM* = 5.49) in endogenous OXT levels ($t(20) = 0.11$, $p = 0.91$; Figure 10B). These results indicate that exogenously administered OXT, specifically via the translationally relevant IN route, penetrates the prairie vole brain -- a process at least partially dependent on RAGE-mediated transport. Exogenously administered labeled OXT were not observed to affect exogenous brain OXT levels, failing to provide evidence of a feed-forward mechanism by which exogenous OXT stimulates release of endogenous OXT.

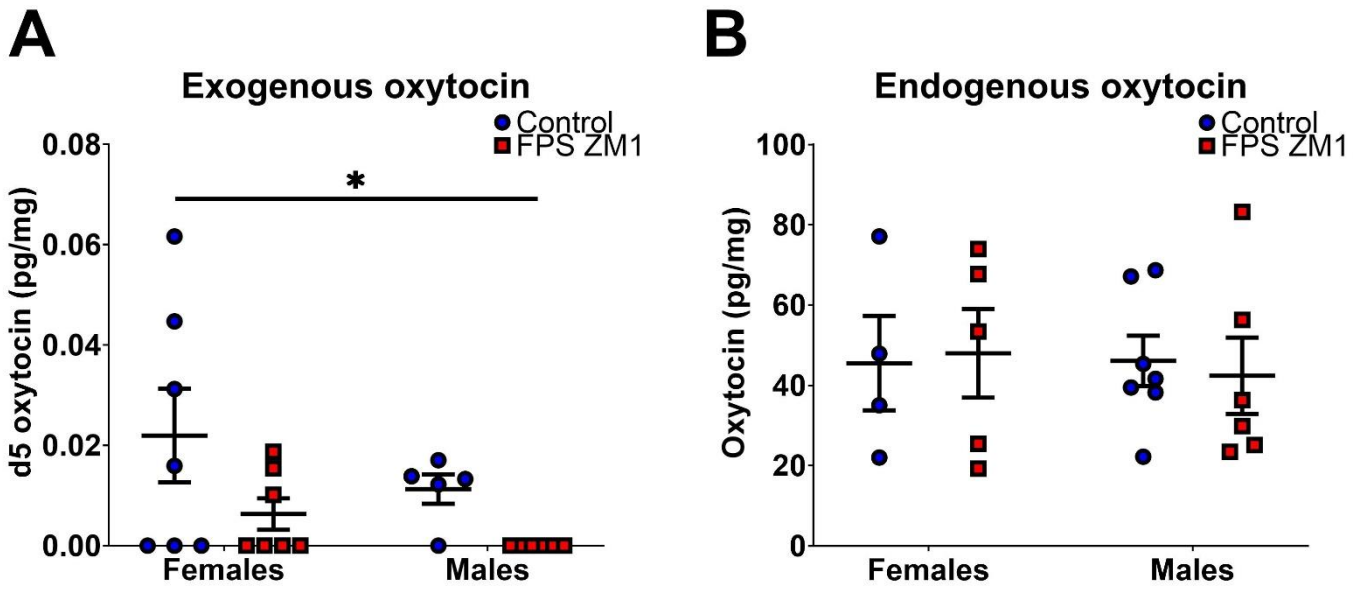


Figure 9: Exogenous d5- oxytocin and endogenous oxytocin levels following pre-treatment with RAGE antagonist. Pre-treatment with a RAGE antagonist significantly decreased exogenous d5 OXT levels (A) without affecting endogenous OXT levels (B). No significant main effects of sex or pre-treatment x sex interactions were observed in either endogenous or exogenous OXT levels. *$p < 0.05$, Two-way treatment (pre-treatment with RAGE antagonist, FPS ZM1, or saline control) x sex ANOVA. Error bars indicate mean ± SEM, *n*'s: control female = 7, control male = 7, pre-treated females = 8, pre-treated males = 7.

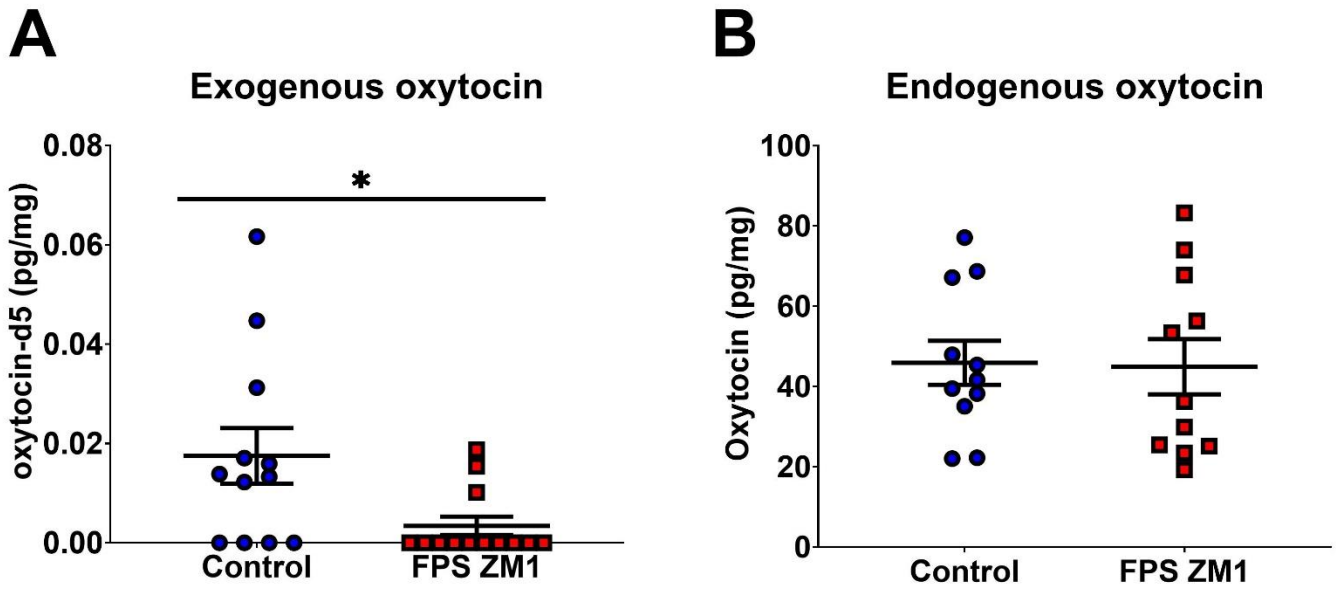


Figure 10: Exogenous d5- deuterated oxytocin and endogenous oxytocin levels following pre-treatment with RAGE antagonist collapsed across sex. When data were collapsed across sex, effects of pre-treatment with RAGE antagonist were further confirmed. Exogenous OXT levels were found to be significantly lower in pre-treated animals- with a large number of samples with no d5 OXT detected and thus, the distributions of the groups were observed to significantly differ (A). No difference was observed between groups in endogenous OXT levels (B). *$p < 0.05$, Mann-Whitney. Error bars indicate ± SEM, *n*'s: control = 14; pre-treated = 15.

Chapter 4: Discussion

The prevalence and negative impacts of alcohol use disorder (AUD) demand efficacious treatment options. Currently approved pharmacotherapies have shown limited success in treating AUD clinically (Fuller, Branchey et al. 1986, Oslin, Berrettini et al. 2003, Haney and Spealman 2008, Mann, Lemenager et al. 2013), despite demonstrated efficacy in preclinical, animal models (Griffiths, Wurster et al. 1975, Carroll and Lac 1992, Volpicelli, Alterman et al. 1992, Spanagel, Holter et al. 1996, He, Nebert et al. 1997, MacFadyen, Loveless et al. 2016). OXT, a hormone with essential roles in various social behaviors (Lee, Macbeth et al. 2009, Stoop 2012), has drawn interest as a pharmacotherapy for AUD. Previous pre-clinical and clinical research has indicated a role for OXT in mediating the processes associated with alcohol use (Leong, Cox et al. 2018). OXT's ability to modulate mesocorticolimbic DA systems and HPA axis functioning have suggested a larger role for OXT in processing of somatosensory and social stimuli and allostasis (reviewed above)—providing various interconnected mechanisms whereby OXT could be exerting its effect on alcohol-related behaviors, in particular consumption. In particular, OXT's social effects (Marsh, Scheele et al. 2015, Jiang and Platt 2018) may serve to bolster abstinence through social support- a key mediator of treatment outcomes (Dobkin, De et al. 2002, Stevens, Jason et al. 2015). This is in line with the Social Salience Hypothesis of OXT functioning, which proposes an overarching role of OXT in the regulation of social cue saliency via its actions in the mesocorticolimbic dopaminergic systems (Shamay-Tsoory and Abu-Akel 2016).

Optimization of animal models for testing pharmacotherapies for AUD may help to bridge the gap between preclinical and clinical efficacy. In these studies, I administer OXT intranasally- a translationally relevant route exclusively used in clinical trials examining

behavioral outcomes in human patients. Among the obstacles in development of efficacious therapies for AUD that current therapies have failed to adequately address are the complex interactions of alcohol-related behaviors with the social environment (Heilig, Epstein et al. 2016, Ahmed, Badiani et al. 2018, Inagaki 2018). I examined the effect of pharmacologically targeting the OXT system on socially-housed prairie voles given unrestricted access to the other animals in the cage and continuous access to alcohol in a two-bottle choice paradigm (CA-2BC). As a socially-monogamous rodent species with demonstrated translational validity to humans through common mechanisms regulating social behaviors, in which the OXT system has been extensively explored and which not only voluntarily consume alcohol, but also show social influences on alcohol consumption similar to humans, prairie voles serve as an ideal animal model for these studies. Finally, a mixed-cage design was also used, with treatment and control animals in the same cage; this better recapitulates scenarios human patients would experience during medication-assisted maintenance of abstinence. Both IN OXT and an OXTR agonist, LIT-001, were shown to be effective in decreasing cumulative alcohol consumption hours after treatment; demonstrating the efficacy and plausibility of targeting the OXT as a pharmacotherapy for AUD. However, despite these results – as well as previous demonstrations of OXT's efficacy in decreasing measures associated with AUD pre-clinically (McGregor and Bowen 2012, King, Griffin et al. 2017, Stevenson, Wenner et al. 2017) and clinically (Pedersen, Smedley et al. 2013, Mitchell, Arcuni et al. 2016, Hansson, Koopmann et al. 2018)- the molecular mechanisms of OXT's effects remain elusive, including its transport into the brain.

Therefore, I next sought to test whether brain penetrance of exogenous OXT could be facilitated by RAGE. First, the presence of RAGE in the prairie vole brain was confirmed using IHC. Subsequently, the role of RAGE in the transport of systemically administered (exogenous)

OXT was directly assessed using LC-MS/MS and a selective RAGE antagonist. Given the noted metabolic instability of OXT and brief half-life in both rodents and humans (Nielsen, Al-Saqi et al. 2017, Tanaka, Furubayashi et al. 2018), how it is able to exert effects on the observed time scale of hours remains to be determined. One possibility is a feed-forward mechanism whereby exogenous OXT stimulated endogenous OXT release from MONs (Richard, Moos et al. 1991, Bowen, Carson et al. 2011). Thus, endogenous levels following systemic administration were similarly assessed using LC-MS/MS. Exogenous levels of OXT were found to be significantly decreased following pre-treatment with a RAGE antagonist, suggesting this receptor may be at least partially responsible for transport of IN OXT into the brain. Endogenous levels were unaffected, failing to provide evidence in support of a feed-forward mechanism. With consideration of the known sex differences in OXT functioning (Lee, Macbeth et al. 2009), sex was examined throughout the studies. While sex-specific effects were found in the effects of targeting the OXT system on alcohol consumption (i.e. decreases only observed in males), no significant differences were observed in RAGE expression or exogenous and endogenous OXT levels following treatment with a RAGE antagonist and IN OXT. This suggests that while penetrance of peripherally administered OXT is similar between sexes, central effects may differ and ultimately result in various behavioral responses like the variable efficacy of treatment observed in these studies. This dissertation provides important insight into the behavioral and molecular mechanisms of OXT's demonstrated effect in decreasing alcohol consumption and offers support to OXT as a promising pharmacotherapy for AUD.

4.1 Intranasal oxytocin selectively decreases alcohol consumption in male prairie voles

Whether OXT was also effective in decreasing alcohol consumption when administered via the translationally relevant route of IN administration was assessed in female and male

socially-housed prairie voles in a mixed-cage, CA-2BC paradigm. Cumulative measures of consumption (alcohol/water intake, DS, CV and NNV) were examined at hourly intervals. While differences in alcohol intake and drink size between subgroups (vehicle, 5 mg/kg OXT, or 10 mg/kg OXT/sex) were noted 1 hr post-treatment, differences were non-significant until the 2hr time point. The effect of OXT was not specific to alcohol at this time point, however, as significant differences were also noted in water intake and drink size. At 3hrs post-treatment, the effect on water appeared to abate and OXT's effect was specific to alcohol- decreasing both intake and drink size. Significant differences in alcohol intake between groups remains through 5hrs post-treatment, though without complementary differences in alcohol drink size. No significant differences were observed in alcohol or water consumptive or non-nutritive visits at any time point, suggesting that OXT's effects on alcohol intake were primarily due to the size of alcohol drinking and not due to number of visits. This selective effect on alcohol consumption, without affecting water consumption, as well as lack of effect on consumptive or non-nutritive visits suggests a lack of sedative effects. However, this was not tested directly and could be a point of consideration for future studies.

Peak levels of OXT have been reported 30 – 60 min following IN delivery in mice, rats and humans (Neumann, Maloumby et al. 2013, Striepens, Kendrick et al. 2013, Yamamoto, Liang et al. 2019). With consideration of evolutionarily conserved mechanisms in mammals and the demonstrated translational validity of the prairie vole model reviewed in the Introduction, it is reasonable to assume a similar pattern is present in prairie voles. The timeline of effect observed here (differences emerging at 1 hr post-treatment and remaining through 5 hrs) could potentially be explained by a feed-forward mechanisms whereby exogenous OXT stimulates the release of endogenous OXT from MONs (Richard, Moos et al. 1991, Bowen, Carson et al.

2011), allowing for more prolonged effects. Previous studies in the Ryabinin lab utilizing a similar CA-2BC alcohol consumption paradigm in socially-housed prairie voles showed repeated treatment with 3 mg/kg OXT (IP) to be effective in decreasing alcohol consumption in both female and male animals during the first 6 hrs on the first day of treatment, as well as the first 3 hrs on the first 3 days of treatment. Given the similarities in experimental design between these studies, the most likely contributor to these discrepancies in time periods of effects following a single OXT treatment (i.e. 3 hrs vs. 6 hrs on first day of treatment), as well as possibly sex-specific effects, is route of OXT administration.

OXTRs are expressed throughout the body (e.g. the myometrium of the uterus, corpus cavernosum and epididymis of the penis, heart, intestines) and peripheral nervous systems (Viero, Shibuya et al. 2010). It is possible systemically administered OXT exerts its effects via action the periphery, including at the related AVPRs. It should be noted that while both AVP and AVPR1a are intricately linked to OXT, and the OXT and AVP system are often discussed in concert, this discussion is outside the scope of this dissertation; however, various excellent reviews exist (Landgraf and Neumann 2004, Landgraf 2005, Lim and Young 2006, Caldwell, Lee et al. 2008, Raggenbass 2008). For example, OXT could be acting within feedback loops between receptors in the intestine and central OXT systems to regulate satiety. Orally administered OXT has been demonstrated to be transported by RAGE on intestinal epithelial cells (Yamamoto and Higashida 2020) and OXT has been shown to regulate intake of food and various solutions (e.g. sucrose), generally suppressing intake. Dose-dependent decreases in food intake have been demonstrated with IP OXT, while an OXTR antagonist blocks this effect (Arletti, Benelli et al. 1989, Arletti, Benelli et al. 1990). Moreover, IP OXT has also been shown to dose-dependently decrease *ad libitum* water intake and stimulate thirst (Arletti, Benelli et al.

1990). In humans, IN OXT has been shown to decrease food consumption in healthy control subjects, but not patients with conditions such as anorexia nervosa, bulimia nervosa, binge eating disorder, and schizophrenia—suggesting possible altered functioning of OXT systems (Chen, Chiang et al. 2021).

OXT's role in regulation of satiety may explain the lack of specificity of its effect on alcohol observed in the previous (IP) study, as evidenced by the absence of decreased preference for alcohol (to water)—suggesting a concomitant decrease in water consumption following treatment. However, in the current study, OXT's effect was observed to be specific to alcohol at 3 hrs, without affecting water consumption. Route of administration may also account for this difference in OXT's effect on water consumption. IN administration can result in the penetration of OXT into the brain through a number of pathways, including: transmission along the olfactory and trigeminal neural bundles, absorption directly from basal epithelia in the nasal cavity into CSF, through circumventricular organs, and crossing the BBB (Grinevich and Neumann 2021, Yeomans, Hanson et al. 2021). This provides the possibility of penetrance into various brain regions responsible for various physiological and behavioral responses (reviewed above and below). Route of delivery has been shown to affect central penetrance levels, with higher levels observed with i.v. compared to IN OXT administration in primates (Lee, Scheidweiler et al. 2018). Route of delivery may determine pharmacokinetic properties as well. For example, differences in mean clearance time have been reported between i.v. and IN routes of administration in rodents, with nearly 5 fold increase in time observed with IN delivery (Tanaka, Furubayashi et al. 2018).

Interestingly, the effect of OXT on alcohol intake and drink sizes appears to be sex-specific, with no effects observed on treated females and significant differences observed

between treated males and females. Specifically, male prairie voles treated with 5 mg/kg IN OXT consumed significantly less alcohol and displayed significantly smaller drink sizes than their saline control counterparts, as well as both 5 mg/kg and 10 mg/kg treated females; while males treated with 10 mg/kg IN OXT consumed significantly less alcohol and displayed significantly smaller drink sizes than their saline control and 10 mg/kg OXT treated female counterparts. These results are supported by previous reports of differences in OXT levels and OXTR binding in the brains of female and male alcohol-dependent rats, and post-mortem tissue from human patients with AUD (women and men) (Hansson, Koopmann et al. 2018, Hansson and Spanagel 2021). Specifically, as previously reviewed in Section 1.5, male rats and men with AUD were shown to have decreased OXT-ir and increased OXTR binding in a number of key hypothalamic, striatal and prefrontal areas (Hansson, Koopmann et al. 2018). Conversely, a more recent investigation in female rats and women with AUD showed no changes in OXT in hypothalamic nuclei and no changes in OXTR binding in striatal areas such as the NAcc or prefrontal areas such as the infralimbic and prelimbic cortex (Hansson and Spanagel 2021).

It has been suggested sex/gender differences could be explained by sex hormone levels, in particular estrogen. As reviewed in Section 1.2 above, estrogen plays a key role in the regulation of OXT functioning. Estrogen levels fluctuate throughout the estrus cycle—progressively increasing during proestrus until reaching peak levels and declining into the start of estrus. However, as noted in Section 2.1, female prairie voles are induced ovulators and therefore do not display estrous cycles unless directly exposed to / housed with a male. Exposure to a male has been shown to increase circulating estrogen levels in females, before peaking during lordosis and rapidly decreasing (Dluzen and Carter 1979). Interactions, presumably including olfactory and other cues, appear to be crucial as simple injections of OXT (ICV or IP)

do not facilitate sexual receptivity in female prairie voles primed with estrogen (Witt, Carter et al. 1990), nor does an OXTR antagonist inhibit sexual behavior (Witt and Insel 1991). As female and male prairie voles were housed separately, females were not induced into estrus; thus, estrus cycle was not monitored during this study and estrogen level information is not available. Future studies could measure estrogen levels in OXT treated females (and males), as well as incorporate estrogen pre-treatment in females to assess whether this may account for discrepancies in efficacy, and possibly augment effects in females.

While the relatively equal levels of alcohol intake between saline controls and both IN OXT dose groups in females appears to indicate a lack of an effect, it is also possible this is signaling a different type of effect in females. According to the Social Salience Hypothesis of OXT functioning (Shamay-Tsoory and Abu-Akel 2016), the salience of social stimuli (i.e. conspecifics) may be augmented over alternative rewards, such as alcohol. Indeed, IN OXT has been demonstrated to have different effects on processing of social stimuli in women and men. For example, women have indicated more distress and anger in response to a social stress test and men have reported fewer negative effects (Bredewold and Veenema 2018). Given prior demonstrations of social facilitation and inhibition of alcohol consumption in co-housed same-sex animals (Anacker, Loftis et al. 2011, Anacker and Ryabinin 2013), OXT may be acting to enhance the saliency of social cues in females and thus result in animals matching consumption with one or more animals in the cage. Future investigations could examine social interactions and consumption patterns between animals to determine whether this may be contributing to observations in both females and males.

Moreover, with consideration of demonstrated effects of social influences on alcohol consumption in same-sex and opposite-sex housed prairie voles reviewed in Section 1.3

(Anacker, Loftis et al. 2011, Anacker and Ryabinin 2013, Walcott and Ryabinin 2017, Walcott and Ryabinin 2019), as well as possible effects of estrogen on OXT functioning, investigation of OXT's efficacy in opposite-sex pairs may yield different results. Though, given OXT's intimate role in pair-bonding via its interactions with the mesocorticolimbic dopaminergic system (Liu and Wang 2003) (Young, Gobrogge et al. 2011) reviewed in Section 1.3 above, interactions may become compounded and convoluted, making effects hard to discern. Nevertheless, determining the nature of OXT's effect on alcohol in social situations may prove critically important in its clinical application. The potential competitive effects on affiliative behaviors do not explain the selective decreases observed in alcohol intake, but rather suggest that clinical studies should take into account time points following OXT administration as selective effects on alcohol were only observed after non-specific effects (on alcohol and water) faded at approximately 3hrs post-treatment.

Deleterious effects of long-term OXT exposure on social behaviors, such as the pair bond, have been described in a number of studies (Bales and Perkeybile 2012, Bales, Perkeybile et al. 2013, Guoynes, Simmons et al. 2018)- particularly during critical periods in development such as peri-adolescence. Thus, dose is an important consideration, as well as length of exposure and age, as well as sex, in clinical applications. While the current study found a single treatment of IN OXT to be effective in decreasing alcohol consumption, a majority of clinical studies which assessed short-term outcomes of OXT treatment did not observe decreases in drinking despite decreases in associated measures such as craving or cue-induced responses to alcohol (Pedersen, Smedley et al. 2013, Hansson, Koopmann et al. 2018, Bach, Reinhard et al. 2019, Bach, Koopmann et al. 2021) and others found no effect of IN OXT on alcohol-related measures such as: daily intake, number of drinking days, number of days until returning to use, or

proportion of relapsing subjects between treatment and control groups (Melby, Gråwe et al. 2021). In fact, only one clinical study has reported decreased alcohol drinking following IN OXT in women and men with a diagnosis of alcohol dependence according to DSM-IV criteria and were "heavy drinkers" as defined by ≥35 drinks/week for men and ≥ 28 drinks/week for women (Pedersen 2017). However, it is important to note this study administered OXT over a longer time and decreases in alcohol drinking only began to emerge during the second week of treatment. Effects then began to fade during the last weeks of treatment- indicating a desensitization to repeated treatments of OXT.

The effects of 5 mg/kg and 10 mg/kg IN OXT on males appear to be equivalent, suggesting the possibility of saturation of the OXTR at these doses- another point for clinical consideration. In recent studies using lower doses of OXT (20 - 24 IU), results on alcohol drinking and associated measures such as craving have been less consistent, despite noted efficacy in decreasing other measures such as anxiety or stress (Flanagan, Allan et al. 2019, Melby, Gråwe et al. 2021). This suggests that doses required for alcohol specific effects may be different than those effective in decreasing other associated measures. Doses used in this study were based on and consistent with previously used doses demonstrating behavioral effects in prairie voles (e.g. (Bales, Perkeybile et al. 2013, Guoynes, Simmons et al. 2018). As reviewed in Sections 1.2 and 1.5 and above, OXT's effects in various brain regions and interactions with several systems (e.g. dopaminergic reward pathways, HPA axis) result in diverse behaviors, which could also account for differences in observed effects.

A final, but crucial, consideration in assessing OXT's effect on alcohol consumption is individual differences in the OXT system. For example, in humans, a small group of men carrying a (guanine to adenine allele homozygous in OXTR rs53376) polymorphism show weak

oxytocinergic functioning which is associated with alcohol use and prevalence of AUD at ages 15, 18 and 25 (Vaht, Kurrikoff et al. 2016, Yang, Wang et al. 2017). It is possible these carriers could also be differentially sensitive to treatment with OXT. In addition, OXT has been shown to reduce alcohol craving in subjects with high attachment anxiety, but increase craving in subjects with low attachment anxiety (Mitchell, Arcuni et al. 2016), providing further evidence OXT may be differentially effective across patient populations. Future investigations of OXT treatments in diverse patient populations, particularly in those with associated deficits in OXT functioning and associated social behavioral processes (e.g. Autism Spectrum Disorder, schizophrenia, SUD; (Baskerville and Douglas 2010, Lee and Weerts 2016) could prove informative to OXT's clinical application for AUD. Identifying characteristics of subpopulations may help inform specific targets of OXT treatment as we move toward the development of more individualized treatments for AUD.

4.2 A small molecule oxytocin receptor agonist selectively decreases alcohol consumption in male prairie voles

The above results support OXT's efficacy in decreasing alcohol consumption in males, while also invoking the question of whether a small molecule OXTR agonist could be designed with a more desirable pharmacokinetic profile, and similar or improved efficacy (e.g. across sexes) and selectivity (e.g. effect selective to alcohol and not water). LIT-001- a small molecule OXTR agonist previously shown to restore social behaviors in a genetically modified mice (Frantz, Pellissier et al. 2018) and inhibit inflammation-induced hyperalgesia (Hilfiger, Zhao et al. 2020) was adapted for this purpose, and measures of consumption were similarly assessed in CA-2BC mixed-cage design.

LIT-001 selectively decreased cumulative alcohol intake up to 4hrs post-treatment in males, without affecting consumption levels in females. While a significant difference was not

observed between males treated with LIT-001 and saline treated male controls, alcohol intake levels in treated males (M = 1.36g/kg, ±SEM = 0.24) were nearly half those of male controls (M = 2.66g/kg, ±SEM = 0.54). This sizable decrease could signify considerable harm reduction. Moreover, alcohol intake levels in control males were only slightly lower than control females (M = 2.71g/kg, ±SEM = 0.33), which were found to be significantly different from LIT-001 treated males. Despite a slight reduction, LIT-001 treated females (M = 2.35g/kg, ±SEM = 0.24) consumed at levels similar to female and male saline controls.

A complementary, selective decrease in alcohol CV was also observed in males. Treated males were observed to make fewer alcohol channel CV than both control males and LIT-001 treated females. LIT-001 treatment did not affect alcohol or water drink size, or number of alcohol or water NNV. This suggests the effects of LIT-001 on alcohol consumption were primarily due to number of visits and not the size of alcohol drinking. However, taken together, the reduction in alcohol intake and fewer visits signal an overall reduction in alcohol exposure, which translates to harm reduction clinically—a tenet of effective AUD treatment.

Importantly, the selectivity of LIT-001's effect on alcohol intake in males support the possibility that a small molecule OXTR agonist can be designed with a more desirable pharmacokinetic profile. Taking into account LIT-001's greater affinity for the OXTR (K_i = 226 ± 75nM) over the related AVPR1a (K_i = 1253 ± 169nM; (Frantz, Pellissier et al. 2018), these data suggests that OXTR may be mediating the observed decreases in alcohol drinking following agonism of the OXT system. Given the ability of OXT to act through both OXTR and the related AVPR1a, it is entirely possible OXT may be exerting its effects on alcohol drinking through AVPR1a. Moreover, given the region- and sex- specific differential expression and responsiveness of these receptors (Dumais and Veenema 2016), as well as their differential

importance in pair bonding (Numan and Young 2016), it is plausible these differences extend to alcohol reward as well. This may explain the differential effects observed between females and males. It is possible OXT also interacts with AVPR1a receptors in effects on alcohol drinking, but further research is needed to say definitively.

Moreover, previous reports indicate that exogenously administered and centrally active OXT could exert effects through actions not involving conventional OXTR activation (reviewed in Sections 1.2 and 1.5 above and (Bowen, Peters et al. 2015, Freeman and Young 2016, Grinevich and Ludwig 2021). Regardless, the consistency of the sex-specific effect in males observed with both OXT and LIT-001 treatment suggest effects may mediated by the OXTR. With consideration of the interconnectedness of the OXT and AVP systems, it is also possible neither of these receptors is solely responsible for the effects of OXT on alcohol consumption. Moreover, it is possible that alterations to one system will be complemented by the other, producing unwanted side effects. Conversely, this interconnectedness may also serve as a benefit and act to bolster efficacy.

This data offers promising evidence that a small molecule OXTR agonist can be substituted for OXT to effect alcohol consumption. Taken together, these studies suggests that the effect of pharmacologically targeting the OXT system on alcohol consumption may be sex-specific. Decreased alcohol consumption was observed in both IN OXT and LIT-001 (IP) treated males, but absent in females. In total, these studies provide translational, behavioral support for OXT's potential as a pharmacotherapy for AUD. Broadly, these results support the possibility a small molecule OXTR agonist with a more desirable pharmacokinetic profile can be designed with a similar, or improved, efficacy to OXT in selectively decreasing alcohol consumption, further augmenting clinical translatability. In addition to small molecule agonists, increasing

penetrance of IN OXT has been proposed, particularly through use of mucoadhesives, nanoemulsions, and liposomal formulations (Bharadwaj, Tzabazis et al. 2021, Grinevich and Neumann 2021). As described below, development of allosteric modulators of RAGE may also serve to increase penetrance of exogenous OXT (Ryabinin and Zhang 2022).

4.3 RAGE facilitates the transport of intranasal oxytocin in prairie voles

Despite demonstrations of its efficacy when administered peripherally, reports on central effects of systemic OXT remain conflicting. Moreover, given OXT's known metabolic instability, the molecular mechanisms of OXT's effect remain to be determined. I sought to elucidate aspects of these mechanisms beginning with whether RAGE could be involved in the transport of OXT into the brain following peripheral (IN) administration. Recent demonstration of transport of OXT into the brain by RAGE in genetically modified mice (Yamamoto, Liang et al. 2019) suggested this possibility; however, the presence of RAGE had not been demonstrated in the prairie vole brain. Thus, I first sought to test the hypothesis that RAGE is present in the prairie vole brain and enriched in regions known to express the OXTR, as well as compare expression patterns within the cerebrum to previous findings in humans and mice (Cheng, Tsuneyama et al. 2005, Harashima, Yamamoto et al. 2006), using immunohistochemistry (IHC).

RAGE-ir was found in a number of key brain areas. Importantly, RAGE-ir was detected in key brain regions responsible for OXT production and modulation—specifically, the PVN and VMH. OXTR is particularly sensitive to gonadal steroids within the VHM-- an area noted for its role in the regulation of sexual behaviors- in both females and males (De Kloet, Voorhuis et al. 1985, de Kloet, Voorhuis et al. 1986, Coirini, Johnson et al. 1989, Johnson, Coirini et al. 1991, Bale and Dorsa 1995, Bale, Dorsa et al. 1995, Quiñones-Jenab, Jenab et al. 1997). While the current investigation is noted as exploratory to confirm the presence of RAGE in the prairie vole

brain, but did not quantify expression levels, patterns were observed to be similar in females and males. Future investigations could examine these areas more closely for possible sex differences in RAGE expression and IN OXT penetrance.

RAGE-ir was also detected surrounding both the lateral and third ventricles, and the choroid plexus, as well as the hippocampus, confirming expression in possible locations of central penetration—further supporting a role for RAGE in the transport of peripheral OXT into the brain. Previous demonstrations of RAGE on the caveolae of the hippocampus and choroid plexus have been suggested as evidence that RAGE may act as a carrier of oxytocin in vesicles (via endocytosis) or for transcytosis (Andreone, Chow et al. 2017). Together with previous demonstrations of RAGE's role in the transport of OXT across the BBB (Yamamoto, Liang et al. 2019), these findings suggest a role for RAGE in the blood-CSF barrier as well. Specifically, transport of OXT to the CSF may be mediated by the choroid plexus.

Recent demonstrations of the acute penetration of OXT in various brain regions, including the hypothalamus, amygdala, and NAcc (Pisansky, Hanson et al. 2017, Lee, Shnitko et al. 2020) in mice and NHPs using radio- and stable isotope-labeled OXT administered intranasally provide support for the transport of peripheral OXT centrally. However, to date, no direct evidence of this transport following intranasal administration of OXT in prairie voles existed, despite well-established research on OXT in prairie voles. Therefore, I sought to test directly whether brain penetrance of exogenous OXT could be aided by RAGE using a selective RAGE antagonist and labelled OXT. As expected, exogenous d5 OXT concentrations were low, while endogenous OXT concentrations were high.

Pre-treatment with a RAGE antagonist was found to significantly decrease exogenous OXT levels, without effecting endogenous levels. While pre-treated males displayed no

detectable levels of exogenous OXT, minute amounts were detected in a few female samples. However, female saline control animals displayed greater variability in exogenous OXT levels than male controls—possibly suggesting greater variation in OXT penetrance in females in general. Regardless, no differences were observed in exogenous or endogenous OXT levels between sexes. The lack of differences observed in endogenous OXT suggest a feed-forward mechanism (whereby exogenous OXT stimulates endogenous OXT) is not present following IN administration of OXT. It is important to note that levels were measured in whole brain samples, which in some cases included olfactory bulbs and pituitary—possibly explaining observed variability in exogenous and endogenous levels.

Future studies could target specific brain regions to more precisely measure penetrance. As reviewed in various sections above (including Sections 1.2 and 1.5), OXT functions in various locations throughout the brain to modulate the processing of stimuli and effect various physiological and behavioral responses. Thus, determination of levels of penetrance to specific brain regions associated with AUD (e.g. NAcc, BNST, CeA) could prove informative in the development of targeted treatments according to symptom(s) or pathology. For example, as discussed in Section 1.5, the negative feelings, dysphoria and stress associated with abstinence from alcohol have been shown to be associated with long-term neuroplastic changes induced by alcohol use within the BNST (Harris and Winder 2018). Targeted OXT delivered to the BNST to restore or modulate these alterations could serve to reduce these negative feelings and prevent relapse. Moreover, OXT and OXTR expression in areas such as the VTA, amygdala, NAcc, PFC, hippocampus and BNST have been shown to be crucial in the regulation of motivated behaviors (Goto and Grace 2005). Delivery of OXT to these areas could serve to redirect

motivated behaviors through OXT's role in altering dopaminergic neurotransmission within mesocorticolimbic systems.

The decrease in exogenous OXT following pre-treatment with a RAGE antagonist found in this study indicates at least a partial role for RAGE in the transport of IN administered OXT. While the precise mechanism(s) by which RAGE is able to transport OXT into the brain remain to be determined (Yamamoto and Higashida 2020), these results offer important insight into the potential molecular mechanisms of peripheral OXT's central penetrance.

4.4 Conclusions and future directions

In total, these studies demonstrate: 1) IN OXT treatment sex-specifically decreases alcohol consumption in a translationally-relevant rodent model in settings which recapitulate scenarios humans patients may face during medication-assisted maintenance of abstinence (i.e. in the presence of non-treated peers drinking alcohol), 2) a small molecule OXTR agonist can be substituted for OXT with a similar, sex-specific effect in decreasing alcohol consumption, 3) RAGE is widely expressed throughout the prairie vole brain, including in areas important in oxytocinergic and dopaminergic signaling—allowing for the possibility of exogenous OXT penetrance to these areas via RAGE, and 4) that RAGE is at least involved in the transport of OXT into the brain following IN administration.

Importantly, these results offer further support to the potential of OXT as a pharmacotherapy for AUD. However, a number of questions remain which require further examination in future studies. First, understanding the mechanisms of OXT's effect on alcohol consumption could augment efficacy by targeting distinct phases of alcohol use and associated symptoms (e.g. preventing relapse by targeting the BNST to treat negative symptoms associated with withdrawal). Given OXT's anxiolytic properties (Yoshida, Takayanagi et al. 2009, Tops,

Koole et al. 2014), whether its effect on alcohol consumption is mediated by decreases in anxiety-like behaviors could be examined using a light dark box test or by measuring allo-grooming. While the current investigation provides some evidence- specifically, no differences in consumptive or non-nutritive visits following IN OXT treatment- to suggest OXT's sedative effects are not mediating the decrease in alcohol, this could also be tested directly through examination of locomotor patterns following treatment.

What is clear from investigations (presented throughout this dissertation) into OXT functioning is that it is complex, involving a number of brain areas and systems with roles in processing of stimuli, and can effect various physiological and behavioral responses. Moreover, OXT is able to modulate the activity of various neurotransmitters (e.g. glutamate, GABA), providing additional systems through which it may be exerting its effect on alcohol-related behaviors, including consumption. Though beyond the scope of this dissertation, it should be noted that alcohol exerts some of its effects in the CNS via the $GABA_A$ receptor. Peripherally administered (IP and IN) OXT has been shown to block alcohol's effects on GABA release, as well as the enhanced motivation for alcohol in alcohol-dependent, but not, non-dependent, male Sprague Dawley and Wistar rats (Tunstall, Kirson, et al. 2019). It is possible OXT acts through a number of related systems to exert its effect on alcohol consumption (e.g. altering reward/motivation via dopaminergic mesolimbic systems, moderating effects of stress and promoting allostasis via the HPA axis) and thus could be effective in treating various aspects of AUD (e.g. alcohol tolerance, craving, consumption, etc.).

Importantly, given OXT's known social effects, whether the decrease in alcohol consumption is mediated by increasing the salience of social reward over the alcohol reward (Lee, Macbeth et al. 2009, Stoop 2012, Leong, Cox et al. 2018) could be directly tested as well

by measuring affiliative behaviors, such as huddling. This would be a consequential finding as OXT possesses the distinct potential to harness its social effects to not only offer an alternative source of (social) reward, but also to bolster abstinence through increased social support- a key mediator of treatment outcomes (Dobkin, De et al. 2002, Stevens, Jason et al. 2015). This presents a unique opportunity for true combination therapy, with pharmacotherapy complementing evidence-based psychological approaches such as Cognitive Behavioral Therapy (CBT). Finally, whether the effects of OXT are specific to alcohol and do not extend to other palatable liquids such as a saccharine solution should be directly tested.

The molecular mechanisms of OXT's effect require further explication as well. For example, while LIT-001's efficacy in decreasing alcohol consumption and greater affinity for OXTR over AVPR1a suggest it is OXTR mediating the decrease, this could be directly tested in recently developed CRISPR/Cas9 generated OXTR knock out (KO) prairie voles (Horie, Inoue et al. 2019). It is also possible that endogenous OXT is involved in the regulation of alcohol consumption and therefore basal alcohol consumption could be low in KO animals; therefore, alternatively, a highly-selective, brain penetrant OXTR antagonist could also be used. Additionally, while it has been previously shown in both mice and prairie voles that OXTR binding is decreased in response to exogenous OXT (Bales, Perkeybile et al. 2013, Huang, Michetti et al. 2014)- presumably via phosphorylation-mediated desensitization (Gimpl, Reitz et al. 2008)- increased OXTR binding has also been suggested to cause sensitization, for example during labor (Blanks, Shmygol et al. 2007). Thus, the effects of long-term exposure on OXT system organization must be firmly established. In addition, sex differences in OXTR desensitization could be investigated as a possible cause of the differences in efficacy of OXT treatment observed here and would have important clinical implications.

As mentioned previously, the region-specific expression of OXTR affords region-specific responses (Busnelli and Chini 2018), thus it is important to further elucidate the molecular mechanisms of OXT's demonstrated efficacy in decreasing alcohol intake. Centrally mediated (vs. peripheral) effects could be directly verified with microinjections of antagonists and OXT into the PVN or lateral hypothalamus. Moreover, with consideration of the effects of long-term exposure and region-specific effects, stimulation of endogenous OXT may prove to be a more suitable approach. Recent demonstrations in male mice utilizing chemogenetics to activate OXT neurons in the PVN showed endogenous OXT activity to also be effect in decreasing alcohol consumption in a binge-drinking paradigm (King, Griffin et al. 2021). Of course, further research is needed to determine the feasibility of developing efficacious approaches for stimulation of endogenous OXT systems for AUD. Given the results of these investigations, as well as others which demonstrate OXT to be more effective across several indicators in male rodents and humans compared to females (Hansson and Spanagel 2021), it is possible targeting of the OXT system for treatment of AUD may only be viable in males/men. Further demonstrating why discerning the behavioral and molecular effects of OXT are important for clinical application.

Finally, although a translationally relevant route, only a portion of total administered OXT has been shown to penetrate centrally (here and (Pisansky, Hanson et al. 2017). While this can occur through several pathways, including transmission via the olfactory and trigeminal neural bundles, direct absorption into cerebrospinal fluid (CSF) from basal epithelia, and across circumventricular organs (Grinevich and Neumann 2021, Yeomans, Hanson et al. 2021), variations in the relative sizes of neural bundles as compared to the whole brain amongst species may impact penetration to specific brain regions in rodents and humans. As mentioned in Section 4.3 above, efforts to augment penetration are in progress, such as the use of mucoadhesives,

nanoemulsions and liposomal formulations (Bharadwaj, Tzabazis et al. 2021, Grinevich and Neumann 2021). Given the implicated role of RAGE in facilitation of the transport of OXT into the brain demonstrated here, development of allosteric modulators of this receptor could also prove effective in increasing penetrance and efficacy of intranasally administered OXT.

While further research is needed to fully explicate the behavioral and molecular mechanisms of OXT's effect on alcohol consumption, the demonstration of IN OXT's efficacy in a translationally-relevant experimental design, substitution of a small molecule agonist in this effect, and evidence to suggest at least a partial role for RAGE in the transport of OXT centrally presented here provide further fundamental support for the potential of OXT as a promising treatment for AUD.

Appendix

This appendix has been reformatted and minimally edited for inclusion in this dissertation from Potretzke, S. & Ryabinin, A.E. (2019) The prairie vole model of pair-bonding and its sensitivity to addictive substances. *Frontiers in Psychology*. 10:2477. doi: 10.3389/fpsyg.2019.02477

Introduction

Reproduction, whether asexual or sexual, is of the upmost importance to the survival of a species. Consequently, organisms have evolved various mating systems to ensure reproduction. Nevertheless, throughout the animal kingdom, promiscuity reigns supreme. Approximately 95–97% of mammals utilize this mating strategy, while the remaining 3–5% exhibit social monogamy (Lukas and Clutton-Brock, 2013; Johnson and Young, 2015). Because social monogamy does not require sexual exclusivity, this strategy can provide a valuable insight into biological aspects of social attachments. Research on the prairie vole (*Microtus ochrogaster*) – a socially monogamous rodent species – allowed for characterization of the neurobiological underpinnings of the pair-bond. Moreover, the effects of alternative rewards and addictive substances on pair-bonds can be investigated by utilizing these animals. This review focuses on the prairie vole model of pair-bonding, its translational value to human social attachments, and its sensitivity to the effects of alcohol and drugs of abuse.

The pair bond as the hallmark of social monogamy

Pair-bonds are commonly described as enduring, preferential associations between two sexually mature adults, characterized by selective affiliation, contact, and mating with the partner over a stranger, which is generally called partner preference (PP; Young et al., 2011). Pair-bonded animals also show aggression toward sexual competitors – called "mate-guarding" – and biparental care of offspring (Kleiman, 1977; Buss, 1988; Fraley et al., 2005). These are social behaviors also seen in humans. The occurrence of sociosexual attachments in nearly all human civilizations provides compelling evidence in support of these attachments being intrinsic to human social behavior (Young et al., 2011).

There are physiological and psychological advantages of pair-bonds in humans. Paired individuals live longer than unpaired individuals across all demographic groups (House et al., 1988; Lillard and Waite, 1995). Interestingly, the level of intimacy between two bonded individuals is positively correlated with immune function and cardiovascular health, while it is inversely correlated with depressed mood (Millard et al., 1988; Kiecolt-Glaser and Newton, 2001). Importantly, socially monogamous behaviors appear to be facilitated by distinct and evolutionary conserved neural mechanisms that mediate selective social attachments.

The neurobiology of pair bonding

Dopamine (DA) signaling is implicated in the formation, expression, and maintenance of pair-bonds. Prairie voles display higher densities of DA2 receptors (D2Rs) and decreased expression of DA1 receptors (D1Rs) in the medial prefrontal cortex (mPFC), as well as a lower density of D1Rs in the nucleus accumbens (NAcc), compared to promiscuous meadow voles (Aragona et al., 2006; Smeltzer et al., 2006). Mating increases DA activity and D1R:D2R signaling ratio in the NAcc, facilitating PP formation (Young et al., 2011; Resendez and Aragona, 2013). D2R activation is necessary and sufficient for PP formation in both male and female prairie voles (Gingrich et al., 2000; Aragona et al., 2006). Following formation, bond maintenance is ensured by increased D1R expression in the NAcc (Aragona et al., 2006; Resendez and Aragona, 2013). In addition, DA cells have been found in the bed nucleus of the stria terminalis (BNST) and the medial amygdala (MeA) in the prairie vole but not in the meadow vole (Northcutt et al., 2007). The larger implication of studies in diverse species such as zebra finch and coppery titi monkeys is support for an evolutionarily conserved contribution of these reward and learning pathways to pair-bonding (Bales et al., 2007; Banerjee et al., 2013). Indeed, recent imaging studies point to the associations between levels of D2/3Rs in the ventral

striatum and self-reported social attachment (Caravaggio et al., 2017), and to increased DA activity in the MeA during bonding in humans (Atzil et al., 2017).

Oxytocin (OXT) is a conserved nonapeptide mediating species-specific social and maternal behaviors (Pedersen and Prange, 1979; Ferris et al., 1984; Kendrick et al., 1987; Argiolas and Melis, 2005). The distribution of OXT receptors (OXTR varies within and across species (Anacker and Beery, 2013; Albers, 2015). Specifically, socially monogamous voles display higher densities of OXTR in the BNST, mPFC, and NAcc but lower levels of OXTR binding in the ventromedial hypothalamus, lateral septum, and anterior cortical amygdala (Insel and Shapiro, 1992; Young et al., 1996; Smeltzer et al., 2006). OXTR expression within mesolimbic pathways is critical for pair-bonding (Young et al., 2011). Furthermore, the OXT and DA systems interact in their functions related to pair-bonding (Liu and Wang, 2003). In humans, OXT and OXTR are also closely associated with social behaviors (Ebstein et al., 2009; Heinrichs et al., 2009; Meyer-Lindenberg et al., 2011). Perhaps most interestingly, OXTR gene variants are associated with relationship status (Walum et al., 2008, 2012), and OXT levels within blood plasma can predict success rates in romantic relationships (Schneiderman et al., 2012).

Arginine vasopressin (AVP), a peptide similar to OXT, is also implicated in the regulation of social bonding. AVP receptor 1a (AVPR1a) expression is higher in the ventral pallidum (VP) and LS in monogamous versus promiscuous vole species (Nair and Young, 2006), and AVP signaling in VP and LS is causally linked to PP (Liu et al., 2001; Lim et al., 2004; Donaldson et al., 2010). On the other hand, mate-guarding in prairie voles is dependent on AVPR1a signaling in the anterior hypothalamus (Gobrogge et al., 2009). AVPR1a in the retrosplenial cortex is important for the regulation of monogamous behaviors in wild prairie

voles (Okhovat et al., 2015; Ophir, 2017). In agreement with the translational value of these findings, AVPR1 polymorphisms are associated with effects of childhood adversity on social interactions in adulthood (Liu et al., 2015). Moreover, administration of AVP increased empathic concerns and risky cooperative behaviors in humans (Tabak et al., 2015; Brunnlieb et al., 2016).

Pair-bonding also involves the corticotropin releasing factor (CRF) receptor system. Monogamous voles display lesser levels of CRFR1 and greater levels of CRFR2 binding within the NAcc (Lim et al., 2005, 2006). Administration of CRF into either the cerebral ventricles or intra-NAcc promoted PP formation in male prairie voles, and effects are prevented by concurrent administration of either a CRFR1 or CRFR2 antagonist (DeVries et al., 2002; Lim et al., 2007). These effects involve either CRF or urocortin 1, since the latter peptide has higher affinity than CRF to these receptors. Indeed, urocortin 1 also shows higher levels of expression in the centrally projecting Edinger-Westphal nucleus (EWcp) in promiscuous versus monogamous vole species (Lim et al., 2005, 2006). The contributions of the CRF system to social attachment are translationally relevant as human polymorphisms in the CRHR1 gene (encoding CRFR1) moderate loneliness in older adults (Chou et al., 2014) and effects of early life stress on emotional empathy (Grimm et al., 2017). Thus, collective neuroplastic abilities of these evolutionarily conserved and connected systems are responsible for the formation and maintenance of the pair-bond.

Effects of addictive substances on social bonding in humans

Addictive substances profoundly affect human social behavior. Many addictive substances are taken in social circumstances and are often expected to promote social bonding. However, drug abuse is associated deleterious effects on social relationships; in fact, alcohol and drug abuse are the third most cited reason for divorce in the United States (Amato and Previti,

2003). Because of the difficulties in obtaining data on the use of illicit drugs, researchers often combine data from several drugs to increase the statistical power. These studies consistently point to the negative association between drug abuse and social bonding, relationship stability, and relationship satisfaction (Dull, 1984; Fals-Stewart et al., 1999). This association is much better followed for addictive substances that are used legally, like alcohol.

While confirming the negative effect of heavy alcohol use on various measures of social bonding, research also identified differences between modes of alcohol drinking within couples. Specifically, couples in which only one spouse drinks heavily (discordant) are less stable than couples in which both spouses drink heavily (concordant) or abstinent couples, while concordant couples are significantly more stable than discordant drinking couples and may be just as stable as abstinent couples (Marshal, 2003; Ostermann et al., 2005; Torvik et al., 2013; Leonard et al., 2014). Additionally, rates of marital dissatisfaction and separation are higher among couples when there is a difference in alcohol consumption between partners (Mudar et al., 2001; Homish and Leonard, 2007; Homish et al., 2009). Interestingly, while this difference in rates of separation is observed in relation to alcohol, neither concordant nor discordant tobacco or marijuana use is associated with increased divorce (Leonard et al., 2014). The latter data indicate that while addictive substances have strong negative effects on the stability of human bonds, there are differences between specific drugs that should be examined. Intriguingly, while socioeconomic factors impact the stability of a marriage, these factors do not moderate effects of addictive substances on marital stability, suggesting involvement of biological factors (Ribar and Kenkel, 1994; Leonard et al., 2014).

Prairie voles as a model of the effects of addictive substances on pair bonding

While epidemiological research on associations between the use of specific drugs of abuse and social effects is being increasingly performed, assessing causal relations between factors requires the use of animal models. Traditional laboratory animals (i.e., mice and rats) are not very suitable for these experiments because they do not display social monogamy. By contrast, prairie voles offer a well-established model of pair bonding and affiliative behaviors. In addition, prairie voles freely prefer alcohol solutions over water (Anacker et al., 2011) and can also consume solutions of methamphetamine (Hostetler et al., 2016).

Early work investigating the influence of social factors on rewarding properties of drugs showed that pair-bond formation reduces amphetamine (AMPH) seeking as evaluated by conditioned place preference (CPP; Liu et al., 2010, 2011). CPP pairs a context with a stimulus, in this case a drug, and assesses preference for the paired context through comparison of time spent in the paired versus alternative, non-paired context. CPP does not assess effects of voluntary exposure to a drug and is accompanied by stress of drug administration. Therefore, subsequent studies used voluntary modes of self-administration, focusing on alcohol consumption. These studies demonstrated existence of social facilitation and social inhibition of alcohol drinking, as well as effects of social hierarchies on alcohol drinking (Anacker et al., 2011, 2014b; Hostetler et al., 2012; Hostetler and Ryabinin, 2014) – both increasing and decreasing alcohol consumption dependent on a number of contextual variables. These first experiments were performed in same-sex pairs of prairie voles. More recent studies observed facilitation of alcohol consumption in pair-bonded male-female pairs of prairie voles (Walcott and Ryabinin, 2017, 2019). The social facilitation of drug intake was observed for alcohol, but not for methamphetamine (Hostetler et al., 2016), highlighting differences in the effects of social environment on actions of these addictive substances.

While the latter studies highlighted the effects of pair-bond formation on consumption of addictive substances, they did not explain the disruptive effects of substance abuse on social bonds. A different series of studies specifically tested whether such disruptive effects observed in humans could be replicated in prairie voles (**Figure 1**). An early report demonstrated that administration of morphine attenuated huddling of male-female pairs (Shapiro et al., 1989). This effect was observed with a relatively high dose of morphine (10 mg/kg) also decreasing locomotor activity. The study also did not assess behavior of males and females separately. Nevertheless, it suggested that drugs of abuse can have inhibitory effects on processes indicative of pair-bonding. Subsequent studies showed that injection of AMPH prior to cohabitation could enhance pair-bond formation in male prairie voles and that this effect is dependent on D1R activation (Curtis and Wang, 2007). On the other hand, repeated (three times) AMPH administration in male prairie voles resulted in increased aggression toward female voles, an effect dependent on AVPR1a in the anterior hypothalamus (Gobrogge et al., 2009). Such repeated treatment disrupted formation of PP in male prairie voles. Blocking D1 receptors in the NAcc in this study rescued PP (Liu et al., 2010). Repeated AMPH was also shown to disrupt PP formation in female prairie voles at doses lower than in males, and administration of Oxt into the mPFC restored PP in these females (Young et al., 2014). The apparent contradiction between the first studies showing AMPH inducing PP and the subsequent studies showing inhibition of PP could be due to the fact that in the early study, AMPH was administered acutely and immediately prior to cohabitation, whereas in the subsequent studies, cohabitation happened at least 24 h after the last of repeated injections.

In the studies described above, AMPH was administered by an experimenter. To alleviate the effect of experimenter-induced stress, studies in our laboratory implemented voluntary

consumption of drugs to assess their effects on pair-bonding. In these studies, alcohol produced paradoxical sex-dependent effects on pair-bond formation. Alcohol consumption during cohabitation disrupted PP formation in male prairie voles, but facilitated it in females (Anacker et al., 2014a). A number of neural correlates accompanied the differences in PP, including sex-specific changes in the arcuate nucleus, EWcp, MeA, and BNST, suggesting complexity of actions through which alcohol affects pair-bonds. However, their contribution to regulation of pair-bond formation was not causally evaluated. Subsequent experiments mimicked earlier studies on effects of AMPH, but used animals that were voluntarily drinking a solution of methamphetamine during 3 days of cohabitation 24 h before the PP. Similar to the AMPH injection studies, methamphetamine decreased PP formation in both males and females (Hostetler et al., 2016). This effect was accompanied by a decrease in Oxt immunoreactivity in the paraventricular nucleus of hypothalamus (PVN).

There is an obvious difference between most of the above described experiments testing effects of psychostimulants and alcohol on pair-bonding. Alcohol was self-administered just prior to the PP test, whereas in all but one experiment with psychostimulants, there was at least 24 h after the last drug exposure. The alcohol and psychostimulant studies could be comparing acute effects versus effects of withdrawal. Future studies should address this discrepancy. Nevertheless, it is worth noting that one study that tested effects of acute AMPH in male prairie voles found induction of PP (Curtis and Wang, 2007), whereas acute alcohol consumption inhibited PP in male prairie voles (Anacker et al., 2014a), indicating differential effects of these addictive substances on pair-bonding.

The studies above showed that different drugs can have varied effects on the formation of pair-bonds. However, while substance abuse may delay the formation of social bonds, it seems

more clinically important to assess its effects on the stability of already established bonds. Moreover, studies in prairie voles indicate that maintenance of the pair-bond requires additional mechanisms beyond those involved in pair-bond formation (e.g., aversion to non-partner animals; Aragona et al., 2006; Resendez and Aragona, 2013). Studies modeling the effects of drugs of abuse on pair-bond maintenance have only been performed recently and only tested the effects of alcohol. These studies show disruption of the established pair-bonds in male prairie voles – as evidenced by decreased PP – when only the male consumes alcohol, but no disruption when both male and female consume alcohol (Walcott and Ryabinin, 2017). Conversely, no disruption of the established pair-bond was seen in females – irrespective of whether the partner consumed alcohol (Walcott and Ryabinin, 2019). Alcohol consumption decreased Oxt in the PVN of males and females regardless of whether bond was disrupted by alcohol or not (Walcott and Ryabinin, 2019). Interestingly, only males demonstrated an increase in immunoreactivity of the activity marker FosB in the periaqueductal gray (PAG) following discordant drinking – suggesting this area may be involved in mediating the effects of discordant drinking on pair-bond maintenance or sensitive to the conditions of discordant drinking (Walcott and Ryabinin, 2017). The PAG is involved in defensive behaviors and romantic love, besides other functions (Depaulis et al., 1992; Acevedo et al., 2012), and needs to be explored in greater detail. We are not aware of studies testing effects of other drugs of abuse on pair-bond maintenance.

The results of these prairie vole studies complement results of the limited epidemiological studies showing that discordant, but not concordant, alcohol consumption is associated with instability of established social bonds. This is important, as the epidemiological studies only assess associations, but not causality of the effects of alcohol. On the other hand, these results also partly contradict epidemiological results in that discordant drinking in the

epidemiological studies was associated with instability of social bonds in both males and females. A number of possible explanations for this contradiction have been put forth (Walcott and Ryabinin, 2019). Perhaps most notably, the vole experiments did not assess the same behavior(s) as the human studies on separations; for example, they did not examine actions of the non-intoxicated subject in the PP test. The experimental design of the vole studies contrasts with the epidemiological situation where the initiator of the separation is most likely the low-consuming individual and not the heavy-drinking spouse. Further behavioral data from both preclinical and clinical studies are required to understand the effects of alcohol on pair-bonds; for instance, is the non-intoxicated partner not interacting with the partner consuming the drug, vice versa or mutual?

The involvement of similar neural substrates in pair bonding and addiction has led a number of researchers to suggest that pair-bonding, or even love, is a form of addiction (Insel, 2003; Burkett and Young, 2012). However, we have argued that this similarity could be superficial. Instead, different addictive drugs can "hijack" neurocircuits that are either involved or not involved in various specific social behaviors (Hostetler and Ryabinin, 2012). As a result, different addictive drugs, or even different phases of actions of the same drug (e.g., intoxication versus withdrawal) can have different directions of effects on pair-bonding. Examples of these effects provided in this review (**Figure 1**) serve as evidence confirming this idea.

Looking forward, what is clearly missing in this literature is a careful examination of effects of different drugs of abuse on maintenance of pair-bonds. So far, only effects of alcohol on this phenomenon have been assessed. Studies on the effects of other drugs of abuse on maintenance of established pair bonds could suggest strategies to help afflicted individuals. Importantly, the prairie vole model is an excellent animal model allowing such future studies.

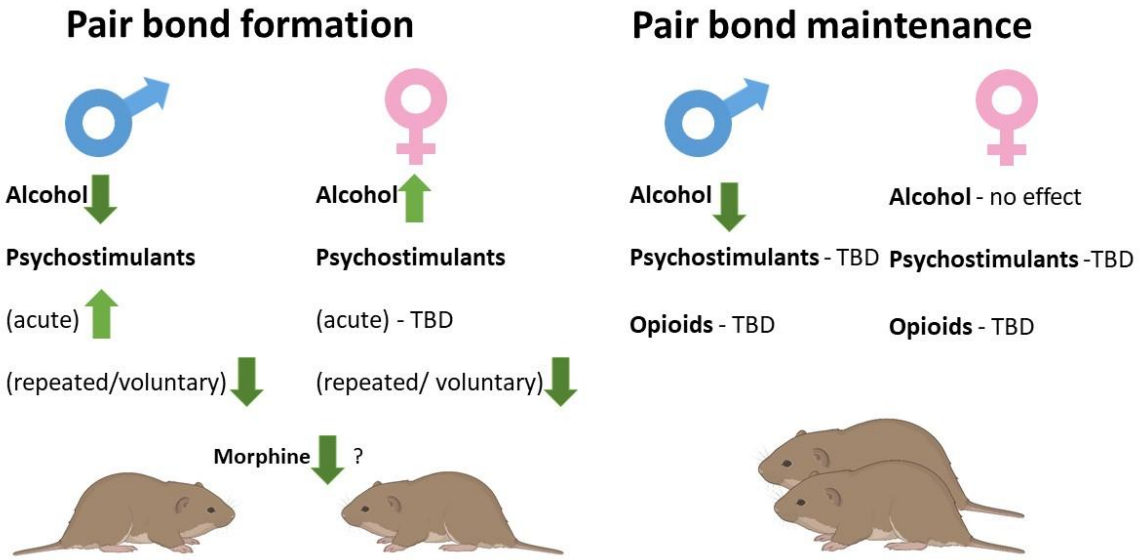


Figure 1: Effects of addictive substances on pair-bonding in prairie voles. Alcohol consumption inhibits pair-bond formation in males but facilitates it in females. Amphetamine administration can either enhance or inhibit pair-bond formation in males depending on timing of administration. Repeated amphetamine administration and methamphetamine drinking inhibit pair-bond formation in both males and females when the exposure occurs 24 h prior to testing partner preference. Morphine can inhibit huddling in male-female pairs. Depending on the partner's drinking status, alcohol consumption can inhibit pair-bond maintenance in males. Alcohol consumption does not have a significant effect on pair-bond maintenance in females.

References

(Appendix)

Acevedo, B. P., Aron, A., Fisher, H. E., and Brown, L. L. (2012). Neural correlates of long-term intense romantic love. *Soc. Cogn. Affect. Neurosci.* 7, 145–159. doi: 10.1093/scan/nsq092

Albers, H. E. (2015). Species, sex and individual differences in the vasotocin/ vasopressin system: relationship to neurochemical signaling in the social behavior neural network. *Front. Neuroendocrinol.* 36, 49–71. doi: 10.1016/j.yfrne.2014.07.001

Amato, P. R., and Previti, D. (2003). People's reasons for divorcing: gender, social class, the life course, and adjustment. *J. Fam. Issues* 24, 602–626. doi: 10.1177/0192513X03024005002

Anacker, A. M., Ahern, T. H., Hostetler, C. M., Dufour, B. D., Smith, M. L., Cocking, D. L., et al. (2014a). Drinking alcohol has sex-dependent effects on pair bond formation in prairie voles. *Proc. Natl. Acad. Sci. USA* 111, 6052–6057. doi: 10.1073/pnas.1320879111

Anacker, A. M., and Beery, A. K. (2013). Life in groups: the roles of oxytocin in mammalian sociality. *Front. Behav. Neurosci.* 7:185. doi: 10.3389/fnbeh.2013.00185

Anacker, A. M., Loftis, J. M., Kaur, S., and Ryabinin, A. E. (2011). Prairie voles as a novel model of socially facilitated excessive drinking. *Addict. Biol.* 16, 92–107. doi: 10.1111/j.1369-1600.2010.00234.x

Anacker, A. M., Smith, M. L., and Ryabinin, A. E. (2014b). Establishment of stable dominance interactions in prairie vole peers: relationships with alcohol drinking and activation of the paraventricular nucleus of the hypothalamus. *Soc. Neurosci.* 9, 484–494. doi: 10.1080/17470919.2014.931885

Aragona, B. J., Liu, Y., Yu, Y. J., Curtis, J. T., Detwiler, J. M., Insel, T. R., et al. (2006). Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nat. Neurosci.* 9, 133–139. doi: 10.1038/nn1613

Argiolas, A., and Melis, M. R. (2005). Central control of penile erection: role of the paraventricular nucleus of the hypothalamus. *Prog. Neurobiol.* 76, 1–21. doi: 10.1016/j.pneurobio.2005.06.002

Atzil, S., Touroutoglou, A., Rudy, T., Salcedo, S., Feldman, R., Hooker, J. M., et al. (2017). Dopamine in the medial amygdala network mediates human bonding. *Proc. Natl. Acad. Sci. USA* 114, 2361–2366. doi: 10.1073/pnas.1612233114

Bales, K., Mason, W., Catana, C., Cherry, S., and Mendoza, S. (2007). Neural correlates of pair-bonding in a monogamous primate. *Brain Res.* 1184, 245–253. doi: 10.1016/j.brainres.2007.09.087

Banerjee, S., Dias, B., Crews, D., and Adkins-Regan, E. (2013). Newly paired zebra finches have higher dopamine levels and immediate early gene Fos expression in dopaminergic neurons. *Eur. J. Neurosci.* 38, 3731–3739. doi: 10.1111/ejn.12378

Brunnlieb, C., Nave, G., Camerer, C. F., Schosser, S., Vogt, B., Münte, T. F., et al. (2016). Vasopressin increases human risky cooperative behavior. *PNAS* 113, 2051–2056. doi: 10.1073/pnas.1518825113

Burkett, J. P., and Young, L. J. (2012). The behavioral, anatomical and pharmacological parallels between social attachment, love and addiction. *Psychopharmacology* 224, 1–26. doi: 10.1007/s00213-012-2794-x

Buss, D. M. (1988). From vigilance to violence: tactics of mate retention in American undergraduates. *Ethol. Sociobiol.* 9, 291–317. doi: 10.1016/ 0162-3095(88)90010-6

Caravaggio, F., Chung, J. K., Gerretsen, P., Fervaha, G., Nakajima, S., Plitman, E., et al. (2017). Exploring the relationship between social attachment and dopamine D2/3 receptor availability in the brains of healthy humans using [11C]-(+)-PHNO. *Soc. Neurosci.* 12, 163–173. doi: 10.1080/ 17470919.2016.1152997

Chou, K. L., Cacioppo, J. T., Kumari, M., and Song, Y. Q. (2014). Influence of social environment on loneliness in older adults: moderation by polymorphism in the CRHR1. *Am. J. Geriatr. Psychiatry* 22, 510–518. doi: 10.1016/j.jagp.2012.11.002

Curtis, J. T., and Wang, Z. (2007). Amphetamine effects in microtine rodents: a comparative study using monogamous and promiscuous vole species. *Neuroscience* 21, 857–866. doi: 10.1016/j.neuroscience.2007.07.011

Depaulis, A., Keay, K. A., and Bandler, R. (1992). Longitudinal neuronal organization of defensive reactions in the midbrain periaqueductal gray region of the rat. *Exp. Brain Res.* 90, 307–318. doi: 10.1007/bf00227243

DeVries, A. C., Guptaa, T., Cardillo, S., Cho, M., and Carter, C. S. (2002). Corticotropin-releasing factor induces social preferences in male prairie voles. *Psychoneuroendocrinology* 27, 705–714. doi: 10.1016/S0306-4530(01)00073-7

Donaldson, Z. R., Spiegel, L., and Young, L. J. (2010). Central vasopressin V1a receptor activation is independently necessary for both partner preference formation and expression in socially monogamous male prairie voles. *Behav. Neurosci.* 124, 159–163. doi: 10.1037/a0018094

Dull, R. T. (1984). An empirical examination of the social bond theory of drug use. *Int. J. Addict.* 19, 265–286. doi: 10.3109/10826088409057181

Ebstein, R. P., Israel, S., Lerer, E., Uzefovsky, F., Shalev, I., Gritsenko, I., et al. (2009). Arginine vasopressin and oxytocin modulate human social behavior. *Ann. N. Y. Acad. Sci*. 1167, 87–102. doi: 10.1111/j.1749-6632.2009.04541.x

Fals-Stewart, W., Birchler, G. R., and O'Farrell, T. J. (1999). Drug-abusing patients and their intimate partners: dyadic adjustment, relationship stability, and substance use. *J. Abnorm. Psychol*. 108, 11–23. doi: 10.1037/0021-843X.108.1.11

Ferris, C. F., Albers, H. E., Wesolowski, S. M., Goldman, B. D., and Luman, S. E. (1984). Vasopressin injected into the hypothalamus triggers a stereotypic behavior in golden hamsters. *Science* 224, 521–523. doi: 10.1126/science.6538700

Fraley, R. C., Brumbaugh, C. C., and Marks, M. J. (2005). The evolution and function of adult attachment: a comparative and phylogenetic analysis. *J. Pers. Soc. Psychol*. 89, 731–746. doi: 10.1037/0022-3514.89.5.751

Gingrich, B., Liu, Y., Cascio, C., Wang, Z., and Insel, T. R. (2000). Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (*Microtus ochrogaster*). *Behav. Neurosci*. 114, 173–183. doi: 10.1037/0735-7044.114.1.173

Gobrogge, K. L., Liu, Y., Young, L. J., and Wang, Z. (2009). Anterior hypothalamic vasopressin regulates pair-bonding and drug-induced aggression in a monogamous rodent. *PNAS* 106, 19144–19149. doi: 10.1073/pnas.0908620106

Grimm, S., Wirth, K., Fan, Y., Weigand, A., Gartner, M., Feeser, M., et al. (2017). The interaction of corticotropin-releasing hormone receptor gene and early life stress on emotional empathy. *Behav. Brain Res.* 329, 180–185. doi: 10.1016/j.bbr.2017.04.047

Heinrichs, M., von Dawans, B., and Domes, G. (2009). Oxytocin, vasopressin, and human social behavior. *Front. Neuroendocrinol.* 30, 548–557. doi: 10.1016/j. yfrne.2009.05.005

Homish, G. G., and Leonard, K. E. (2007). The drinking partnership and marital satisfaction: the longitudinal influence of discrepant drinking. *J. Consult. Clin. Psychol.* 75, 43–51. doi: 10.1037/0022-006X.75.1.43

Homish, G. G., Leonard, K. E., Kozlowski, L. T., and Cornelius, J. R. (2009). The longitudinal association between multiple substance use discrepancies and marital satisfaction. *Addiction* 104, 1201–1209. doi: 10.1111/j.1360-0443.2009.02614.x

Hostetler, C. M., Anacker, A. M., Loftis, J. M., and Ryabinin, A. E. (2012). Social housing and alcohol drinking in male-female pairs of prairie voles (*Microtus ochrogaster*). *Psychopharmacology* 224, 121–132. doi: 10.1007/ s00213-012-2836-4

Hostetler, C. M., Phillips, T. J., and Ryabinin, A. E. (2016). Methamphetamine consumption inhibits pair bonding and hypothalamic oxytocin in prairie voles. *PLoS One* 11:e0158178. doi: 10.1371/journal.pone.0158178

Hostetler, C. M., and Ryabinin, A. E. (2012). Love and addiction: the devil is in the differences: a commentary on “the behavioral, anatomical and pharmacological parallels between social attachment, love and addiction”. *Psychopharmacology* 224, 27–29; discussion 31-22. doi: 10.1007/s00213-012-2858-y

Hostetler, C. M., and Ryabinin, A. E. (2014). Social partners prevent alcohol relapse behavior in prairie voles. *Psychoneuroendocrinology* 39, 152–157. doi: 10.1016/j.psyneuen.2013.10.006

House, J. S., Landis, K. R., and Umberson, D. (1988). Social relationships and health. *Science* 241, 540–545. doi: 10.1126/science.3399889

Insel, T. R. (2003). Is social attachment an addictive disorder? *Physiol. Behav.* 79, 351–357. doi: 10.1016/S0031-9384(03)00148-3

Insel, T. R., and Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc. Natl. Acad. Sci. USA* 89, 5981–5985. doi: 10.1073/pnas.89.13.5981

Johnson, Z. V., and Young, L. J. (2015). Neurobiological mechanisms of social attachment and pair bonding. *Curr. Opin. Behav. Sci.* 3, 38–44. doi: 10.1016/j. cobeha.2015.01.009

Kendrick, K. M., Keverne, E. B., and Baldwin, B. A. (1987). Intracerebroventricular oxytocin stimulates maternal behaviour in the sheep. *Neuroendocrinology* 46, 56–61. doi: 10.1159/000124796

Kiecolt-Glaser, J. K., and Newton, T. L. (2001). Marriage and health: his and hers. *Psychol. Bull.* 127, 472–503. doi: 10.1037/0033-2909.127.4.472

Kleiman, D. G. (1977). Monogamy in mammals. *Q. Rev. Biol.* 52, 39–69. doi: 10.1086/409721

Leonard, K. E., Smith, P. H., and Homish, G. G. (2014). Concordant and discordant alcohol, tobacco, and marijuana use as predictors of marital dissolution. *Psychol. Addict. Behav.* 28, 780–789. doi: 10.1037/a0034053

Lillard, L., and Waite, L. (1995). Til death do us part: marital disruption and mortality. *Am. J. Sociol*. 100, 1131–1156. doi: 10.1086/230634

Lim, M. M., Liu, Y., Ryabinin, A. E., Bai, Y., Wang, Z., and Young, L. J. (2007). CRF receptors in the nucleus accumbens modulate partner preference in prairie voles. *Horm. Behav*. 51, 508–515. doi: 10.1016/j.yhbeh.2007.01.006

Lim, M. M., Nair, H. P., and Young, L. J. (2005). Species and sex differences in brain distribution of corticotropin-releasing factor receptor subtypes 1 and 2 in monogamous and promiscuous vole species. *J. Comp. Neurol*. 487, 75–92. doi: 10.1002/cne.20532

Lim, M. M., Tsivkovskaia, N. O., Bai, Y., Young, L. J., and Ryabinin, A. E. (2006). Distribution of corticotropin-releasing factor and urocortin 1 in the vole brain. *Brain Behav. Evol*. 68, 229–240. doi: 10.1159/000094360

Lim, M. M., Wang, Z., Olazabal, D. E., Ren, X., Terwilliger, E. F., and Young, L. J. (2004). Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. *Nature* 429, 754–757. doi: 10.1038/nature02539

Liu, Y., Aragona, B. J., Young, K. A., Dietz, D. M., Kabbaj, M., Mazei-Robison, M., et al. (2010). Nucleus accumbens dopamine mediates amphetamine-induced impairment of social bonding in a monogamous rodent species. *PNAS* 107, 1217–1222. doi: 10.1073/pnas.0911998107

Liu, Y., Curtis, J. T., and Wang, Z. (2001). Vasopressin in the lateral septum regulates pair bond formation in male prairie voles (*Microtus ochrogaster*). *Behav. Neurosci*. 115, 910–919. doi: 10.1037/0735-7044.115.4.910

Liu, J. J., Lou, F., Lavebratt, C., and Forsell, Y. (2015). Impact of childhood adversity and vasopressin receptor 1a variation on social interaction in adulthood: a cross-sectional study. *PLoS One* 10:e0136436. doi: 10.1371/journal.pone.0136436

Liu, Y., and Wang, Z. X. (2003). Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience* 121, 537–544. doi: 10.1016/S0306-4522(03)00555-4

Liu, Y., Young, K. A., Curtis, J. T., Aragona, B. J., and Wang, Z. (2011). Social bonding decreases the rewarding properties of amphetamine through a dopamine D1 receptor-mediated mechanism. *J. Neurosci.* 31, 7960–7966. doi: 10.1523/JNEUROSCI.1006-11.2011

Lukas, D., and Clutton-Brock, T. H. (2013). The evolution of social monogamy in mammals. *Science* 341, 526–530. doi: 10.1126/science.1238677

Marshal, M. P. (2003). For better or for worse? The effects of alcohol use on marital functioning. *Clin. Psychol. Rev.* 23, 959–997. doi: 10.1016/j. cpr.2003.09.002

Meyer-Lindenberg, A., Domes, G., Kirsch, P., and Heinrichs, M. (2011). Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat. Rev. Neurosci.* 12, 524–538. doi: 10.1038/nrn3044

Millard, W., Bernhard, B., Holgar, P., and Thomas, S. (1988). Marriage and the psychological consequences of a heart attack: a longitudinal study of adaptation to chronic illness after 3 years. *Soc. Sci. Med.* 27, 149–158. doi: 10.1016/0277-9536(88)90323-1

Mudar, P., Leonard, K. E., and Soltysinski, K. (2001). Discrepant substance use and marital functioning in newlywed couples. *J. Consult. Clin. Psychol.* 69, 130–134. doi: 10.1037/0022-006X.69.1.130

Nair, H. P., and Young, L. J. (2006). Vasopressin and pair-bond formation: genes to brain to behavior. *Physiology* 21, 146–152. doi: 10.1152/ physiol.00049.2005

Northcutt, K. V., Wang, Z., and Lonstein, J. S. (2007). Sex and species differences in tyrosine hydroxylase-synthesizing cells of the rodent olfactory extended amygdala. *J. Comp. Neurol.* 500, 103–115. doi: 10.1002/cne.21148

Okhovat, M., Berrio, A., Wallace, G., Ophir, A. G., and Phelps, S. M. (2015). Sexual fidelity trade-offs promote regulatory variation in the prairie vole brain. *Science* 350, 1371–1374. doi: 10.1126/science.aac5791

Ophir, A. G. (2017). Navigating monogamy: nonapeptide sensitivity in a memory neural circuit may shape social behavior and mating decisions. *Front. Neurosci.* 11, 1–16. doi: 10.3389/fnins.2017.00397

Ostermann, J., Sloan, F. A., and Taylor, D. H. (2005). Heavy alcohol use and marital dissolution in the USA. *Soc. Sci. Med.* 61, 2304–2316. doi: 10.1016/j. socscimed.2005.07.021

Pedersen, C. A., and Prange, A. J. Jr. (1979). Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proc. Natl. Acad. Sci. USA* 76, 6661–6665. doi: 10.1073/pnas.76.12.6661

Resendez, S. L., and Aragona, B. J. (2013). Aversive motivation and the maintenance of monogamous pair bonding. *Rev. Neurosci.* 24, 51–60. doi: 10.1515/revneuro-2012-0068

Ribar, D., and Kenkel, D. (1994). Alcohol consumption and young adults' socioeconomic status. *Brook. Pap. Econ. Act.* 1994, 119–175.

Schneiderman, I., Zagoory-Sharon, O., Leckman, J. F., and Feldman, R. (2012). Oxytocin during the initial stages of romantic attachment: relations to couples' interactive reciprocity. *Psychoneuroendocrinology* 37, 1277–1285. doi: 10.1016/j. psyneuen.2011.12.021

Shapiro, L. E., Meyer, M. E., and Dewsbury, D. A. (1989). Affiliative behavior in voles: effects of morphine, naloxone, and cross-fostering. *Physiol. Behav.* 46, 719–723. doi: 10.1016/0031-9384(89)90357-0

Smeltzer, M. D., Curtis, J. T., Aragona, B. J., and Wang, Z. (2006). Dopamine, oxytocin, and vasopressin receptor binding in the medial prefrontal cortex of monogamous and promiscuous voles. *Neurosci. Lett.* 394, 146–151. doi: 10.1016/j.neulet.2005.10.019

Tabak, B. A., Meyer, M. L., Castle, E., Dutcher, J. M., Irwin, M. R., Han, J. H., et al. (2015). Vasopressin, but not oxytocin, increases empathic concern among individuals who received higher levels of paternal warmth: a randomized controlled trial. *Psychoneuroendocrinology* 51, 253–261. doi: 10.1016/j. psyneuen.2014.10.006

Torvik, F. A., Roysamb, E., Gustavson, K., Idstad, M., and Tambs, K. (2013). Discordant and concordant alcohol use in spouses as predictors of marital dissolution in the general population: results from the hunt study. *Alcohol. Clin. Exp. Res.* 37, 877–884. doi: 10.1111/acer.12029

Walcott, A. T., and Ryabinin, A. E. (2017). Alcohol's effects on pair-bond maintenance in male prairie voles. *Front. Psychol.* 8:226. doi: 10.3389/ fpsyt.2017.00226

Walcott, A. T., and Ryabinin, A. E. (2019). Effects of alcohol consumption on pair bond maintenance and potential neural substrates in female prairie voles. *Alcohol Alcohol*. 54, 353–360. doi: 10.1093/alcalc/agz041

Walum, H., Lichtenstein, P., Neiderhiser, J. M., Reiss, D., Ganiban, J. M., Spotts, E. L., et al. (2012). Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. *Biol. Psychiatry* 71, 419–426. doi: 10.1016/j.biopsych.2011.09.002

Walum, H., Westberg, L., Henningsson, S., Neiderhiser, J. M., Reiss, D., Igl, W., et al. (2008). Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. *Proc. Natl. Acad. Sci. USA* 105, 14153–14156. doi: 10.1073/pnas.0803081105

Young, K. A., Gobrogge, K. L., Liu, Y., and Wang, Z. (2011). The neurobiology of pair bonding: insights from a socially monogamous rodent. *Front. Neuroendocrinol*. 32, 53–69. doi: 10.1016/j.yfrne.2010.07.006

Young, L. J., Huot, B., Nilsen, R., Wang, Z., and Insel, T. R. (1996). Species differences in central oxytocin receptor gene expression: comparative analysis of promoter sequences. *J. Neuroendocrinol*. 8, 777–783. doi: 10.1046/ j.1365-2826.1996.05188.x

Young, K. A., Liu, Y., Gobrogge, K. L., Wang, H., and Wang, Z. (2014). Oxytocin reverses amphetamine-induced deficits in social bonding: evidence for an interaction with nucleus accumbens dopamine. *J. Neurosci*. 34, 8499–8506. doi: 10.1523/JNEUROSCI.4275-13.2014

References
(Dissertation)

Ahmed, S. H., A. Badiani, K. A. Miczek and C. P. Muller (2018). "Non-pharmacological factors that determine drug use and addiction." Neurosci Biobehav Rev.

Albers, H. E. (2015). "Species, sex and individual differences in the vasotocin/vasopressin system: relationship to neurochemical signaling in the social behavior neural network." Front Neuroendocrinol **36**: 49-71.

Althammer, F. and V. Grinevich (2017). "Diversity of oxytocin neurons: beyond magno- and parvocellular cell types?" Journal of neuroendocrinology.

Anacker, A. and A. Ryabinin (2010). "Biological contribution to social influences on alcohol drinking: evidence from animal models." Int J Environ Res Public Health **7**(2): 473-493.

Anacker, A. M., T. H. Ahern, C. M. Hostetler, B. D. Dufour, M. L. Smith, D. L. Cocking, J. Li, L. J. Young, J. M. Loftis and A. E. Ryabinin (2014). "Drinking alcohol has sex-dependent effects on pair bond formation in prairie voles." Proc Natl Acad Sci U S A **111**(16): 6052-6057.

Anacker, A. M. and A. K. Beery (2013). "Life in groups: the roles of oxytocin in mammalian sociality." Front Behav Neurosci **7**.

Anacker, A. M., J. M. Loftis, S. Kaur and A. E. Ryabinin (2011). "Prairie voles as a novel model of socially facilitated excessive drinking." Addict Biol **16**(1): 92-107.

Anacker, A. M., J. M. Loftis and A. E. Ryabinin (2011). "Alcohol intake in prairie voles is influenced by the drinking level of a peer." Alcohol Clin Exp Res **35**(10): 1884-1890.

Anacker, A. M., M. L. Smith and A. E. Ryabinin (2014). "Establishment of stable dominance interactions in prairie vole peers: relationships with alcohol drinking and activation of the paraventricular nucleus of the hypothalamus." Soc Neurosci **9**(5): 484-494.

Anacker, A. M. J. and A. E. Ryabinin (2013). "Identification of subpopulations of prairie voles differentially susceptible to peer influence to decrease high alcohol intake." Front Pharmacol **4**.

Andreone, B., B. Chow, A. Tata, B. Lacoste, A. Ben-Zvi, K. Bullock, A. Deik, D. Ginty, C. Clish and C. Gu (2017). "Blood-Brain Barrier Permeability Is Regulated by Lipid Transport-Dependent Suppression of Caveolae-Mediated Transcytosis." Neuron **94**(3).

Andrews, J. A., E. Tildesley, H. Hops and F. Li (2002). "The influence of peers on young adult substance use." Health Psychol **21**(4): 349-357.

Anton, R. F., S. S. O'Malley, D. a. Ciraulo, R. a. Cisler, D. Couper, D. M. Donovan, D. R. Gastfriend, J. D. Hosking, B. a. Johnson, J. S. LoCastro, R. Longabaugh, B. J. Mason, M. E. Mattson, W. R. Miller, H. M. Pettinati, C. L. Randall, R. Swift, R. D. Weiss, L. D. Williams and A. Zweben (2006). "Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence." Journal of American Medical Association **295**(17): 2003-2017.

Antoni, F., M. Holmes and M. Jones (1983). "Oxytocin as well as vasopressin potentiate ovine CRF in vitro." Peptides **4**(4).

Argiolas, A. and M. R. Melis (2005). "Central control of penile erection: role of the paraventricular nucleus of the hypothalamus." Prog Neurobiol **76**(1): 1-21.

Arletti, R., A. Benelli and A. Bertolini (1989). "Influence of oxytocin on feeding behavior in the rat." Peptides **10**(1).

Arletti, R., A. Benelli and A. Bertolini (1990). "Oxytocin inhibits food and fluid intake in rats." Physiology & behavior **48**(6).

Avery, S., J. Clauss and J. Blackford (2016). "The Human BNST: Functional Role in Anxiety and Addiction." Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology **41**(1).

Bach, P., A. Koopmann, J. M. Bumb, S. Zimmermann, S. Bühler, I. Reinhard, S. H. Witt, M. Rietschel, S. Vollstädt-Klein and F. Kiefer (2021). "Oxytocin attenuates neural response to emotional faces in social drinkers: an fMRI study." Eur Arch Psychiatry Clin Neurosci **271**(5): 873-882.

Bach, P., I. Reinhard, S. Bühler, S. Vollstädt-Klein, F. Kiefer and A. Koopmann (2019). "Oxytocin modulates alcohol-cue induced functional connectivity in the nucleus accumbens of social drinkers." Psychoneuroendocrinology **109**: 104385.

Bale, T. and D. Dorsa (1995). "Regulation of oxytocin receptor messenger ribonucleic acid in the ventromedial hypothalamus by testosterone and its metabolites." Endocrinology **136**(11).

Bale, T., D. Dorsa and C. Johnston (1995). "Oxytocin receptor mRNA expression in the ventromedial hypothalamus during the estrous cycle." The Journal of neuroscience : the official journal of the Society for Neuroscience **15**(7 Pt 1).

Bales, K. and A. Perkeybile (2012). "Developmental experiences and the oxytocin receptor system." Hormones and Behavior **61**: 313-319.

Bales, K., A. Perkeybile, O. Conley, M. Lee, C. Guoynes, G. Downing, C. Yun, M. Solomon, S. Jacob and S. Mendoza (2013). "Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles. ." Biological Psychology **74**: 180-188.

Baracz, S., N. Everett and J. Cornish (2020). "The impact of early life stress on the central oxytocin system and susceptibility for drug addiction: Applicability of oxytocin as a pharmacotherapy." Neuroscience and biobehavioral reviews **110**.

Baskerville, T. and A. Douglas (2010). "Dopamine and Oxytocin Interactions Underlying Behaviors: Potential Contributions to Behavioral Disorders - Baskerville - 2010 - CNS

Neuroscience & Therapeutics - Wiley Online Library." CNS Neuroscience & Therapeutics **16**: e92–e123.

Baumgartner, H., J. Schulkin and K. Berridge (2021). "Activating Corticotropin-Releasing Factor Systems in the Nucleus Accumbens, Amygdala, and Bed Nucleus of Stria Terminalis: Incentive Motivation or Aversive Motivation?" Biological psychiatry **89**(12).

Bershad, A., M. Kirkpatrick, J. Seiden and d. W. H (2015). "Effects of acute doses of prosocial drugs methamphetamine and alcohol on plasma oxytocin levels." Journal of clinical psychopharmacology **35**(3).

Betka, S., V. P. Gould, C, Y. Paloyelis, R. Bond, G. Pfeifer, H. Sequeira, T. Duka and H. Critchley (2018). "Impact of intranasal oxytocin on interoceptive accuracy in alcohol users: an attentional mechanism?" Social cognitive and affective neuroscience **13**(4).

Bharadwaj, V., A. Tzabazis, M. Klukinov, N. Manering and D. Yeomans (2021). "Intranasal Administration for Pain: Oxytocin and Other Polypeptides." Pharmaceutics **13**(7).

Blackburn, R., E. Stricker and J. Verbalis (1994). "Acute effects of ethanol on ingestive behavior in rats." Alcoholism, clinical and experimental research **18**(4).

Blanks, A., A. Shmygol and S. Thornton (2007). "Regulation of oxytocin receptors and oxytocin receptor signaling. - PubMed - NCBI." Seminars in Reproductive Medicine **25**: 52-59.

Blaustein, J. (2008). "Neuroendocrine regulation of feminine sexual behavior: lessons from rodent models and thoughts about humans." Annual review of psychology **59**.

Blevins, J., M. Schwartz and D. Baskin (2004). "Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size." American journal of physiology. Regulatory, integrative and comparative physiology **287**(1).

Bondeva, T., S. Wojciech and G. Wolf (2011). "Advanced glycation end products inhibit adhesion ability of differentiated podocytes in a neuropilin-1-dependent manner." American journal of physiology. Renal physiology **301**(4).

Bongarzone, S., V. Savickas, F. Luzi and A. D. Gee (2017). "Targeting the Receptor for Advanced Glycation Endproducts (RAGE): A Medicinal Chemistry Perspective." J Med Chem **60**(17): 7213-7232.

Borland, J., K. Grantham, L. Aiani, K. Frantz and H. Albers (2018). "Role of oxytocin in the ventral tegmental area in social reinforcement." Psychoneuroendocrinology **95**.

Bowen, M., D. Carson, A. Spiro, J. Arnold and I. McGregor (2011). "Adolescent oxytocin exposure causes persistent reductions in anxiety and alcohol consumption and enhances sociability in rats." PloS one **6**(11).

Bowen, M. and I. Neumann (2017). "Rebalancing the Addicted Brain: Oxytocin Interference with the Neural Substrates of Addiction." Trends in neurosciences **40**(12).

Bowen, M. T., S. T. Peters, N. Absalom, M. Chebib, I. D. Neumann and I. S. McGregor (2015). "Oxytocin prevents ethanol actions at δ subunit-containing GABAA receptors and attenuates ethanol-induced motor impairment in rats." Proc Natl Acad Sci U S A **112**(10): 3104-3109.

Bratzu, J., R. Bharatiya, E. Manca, C. Cocco, A. Argiolas, M. Melis and F. Sanna (2019). "Oxytocin induces penile erection and yawning when injected into the bed nucleus of the stria terminalis: A microdialysis and immunohistochemical study." Behavioural brain research **375**.

Bredewold, R. and A. Veenema (2018). "Sex differences in the regulation of social and anxiety-related behaviors: insights from vasopressin and oxytocin brain systems." Current opinion in neurobiology **49**.

Breton, C. and H. Zingg (1997). "Expression and region-specific regulation of the oxytocin receptor gene in rat brain." Endocrinology **138**(5).

Brett, J., A. Schmidt, S. Yan, Y. Zou, E. Weidman, D. Pinsky, R. Nowygrod, M. Neeper, C. Przysiecki and A. Shaw (1993). "Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues." The American journal of pathology **143**(6).

Brill-Maoz, N. and M. Maroun (2016). "Extinction of fear is facilitated by social presence: Synergism with prefrontal oxytocin." Psychoneuroendocrinology **66**: 75-81.

Brussaard, A., K. Kits and T. de Vlieger (1996). "Postsynaptic mechanism of depression of GABAergic synapses by oxytocin in the supraoptic nucleus of immature rat." The Journal of physiology **497 (Pt 2)**(Pt 2).

Burbach, J., S. Luckman, D. Murphy and H. Gainer (2001). "Gene regulation in the magnocellular hypothalamo-neurohypophysial system." Physiological reviews **81**(3).

Burkett, J. P. and L. J. Young (2012). "The behavioral, anatomical and pharmacological parallels between social attachment, love and addiction." Psychopharmacology **224**(1): 1-26.

Burns-Cusato, M., E. Scordalakes and E. Rissman (2004). "Of mice and missing data: what we know (and need to learn) about male sexual behavior." Physiology & behavior **83**(2): 217-232.

Busnelli, M. and B. Chini (2018). "Molecular Basis of Oxytocin Receptor Signalling in the Brain: What We Know and What We Need to Know. - PubMed - NCBI." Current Topics in Behavioral Neuroscience **35**: 3-29.

Buss, D. M. (1988). "From vigilance to violence: Tactics of mate retention in American undergraduates." Ethology and Sociobiology **9**(5): 291-317.

Caldwell, H., H. Lee, A. Macbeth and W. Young (2008). "Vasopressin: behavioral roles of an "original" neuropeptide." Progress in neurobiology **84**(1).

Candela, P., F. Gosselet, J. Saint-Pol, E. Sevin, M. Boucau, E. Boulanger, R. Cecchelli and L. Fenart (2010). "Apical-to-basolateral transport of amyloid-β peptides through blood-brain barrier cells is mediated by the receptor for advanced glycation end-products and is restricted by P-glycoprotein." Journal of Alzheimer's disease : JAD **22**(3).

Carlezon, W. and M. Thomas (2009). "Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis." Neuropharmacology **56 Suppl 1**(Suppl 1).

Carroll, M. E. and S. T. Lac (1992). "Effects of buprenorphine on self-administration of cocaine and a nondrug reinforcer in rats." Psychopharmacology (Berl) **106**(4): 439-446.

Carter, C. (2007). "Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders?" Behavioural brain research **176**(1).

Carter, C. and L. Getz (1993). "Monogamy and the prairie vole." Scientific American **268**(6).

Carter, C., W. Kenkel, E. MacLean, S. Wilson, A. Perkeybile, J. Yee, C. Ferris, H. Nazarloo, S. Porges, J. Davis, J. Connelly and M. Kingsbury (2020). "Is Oxytocin "Nature's Medicine"?" Pharmacological reviews **72**(4).

Caruso, M. A., M. T. Robins, H. D. Fulenwider and A. E. Ryabinin (2021). "Temporal analysis of individual ethanol consumption in socially housed mice and the effects of oxytocin." Psychopharmacology (Berl) **238**(3): 899-911.

Ch'ng, S., J. Fu, R. Brown, S. McDougall and A. Lawrence (2018). "The intersection of stress and reward: BNST modulation of aversive and appetitive states." Progress in neuro-psychopharmacology & biological psychiatry **87**(Pt A).

Chatterjee, O., K. Patil, A. Sahu, L. Gopalakrishnan, P. Mol, J. Advani, S. Mukherjee, R. Christopher and T. Prasad (2016). "An overview of the oxytocin-oxytocin receptor signaling network." Journal of cell communication and signaling **10**(4).

Che, X., J. Cai, Y. Liu, T. Xu, J. Yang and C. Wu (2021). "Oxytocin signaling in the treatment of drug addiction: Therapeutic opportunities and challenges." Pharmacology & therapeutics **223**.

Chen, C., Y. Chiang, T. Kuo, K. Tam and E. Loh (2021). "Effects of intranasal oxytocin in food intake and craving: A meta-analysis of clinical trials." Clinical nutrition (Edinburgh, Scotland) **40**(10).

Cheng, C., K. Tsuneyama, R. Kominami, H. Shinohara, S. Sakurai, H. Yonekura, T. Watanabe, Y. Takano, H. Yamamoto and Y. Yamamoto (2005). "Expression profiling of endogenous secretory receptor for advanced glycation end products in human organs." Mod Pathol **18**(10): 1385-1396.

Chini, B., M. Verhage and V. Grinevich (2017). "The Action Radius of Oxytocin Release in the Mammalian CNS: From Single Vesicles to Behavior." Trends in pharmacological sciences **38**(11).

Cid-Jofré, V., M. Moreno, M. Reyes-Parada and G. Renard (2021). "Role of Oxytocin and Vasopressin in Neuropsychiatric Disorders: Therapeutic Potential of Agonists and Antagonists." International journal of molecular sciences **22**(21).

Cobo, E. (1973). "Effect of different doses of ethanol on the milk-ejecting reflex in lactating women." American journal of obstetrics and gynecology **115**(6).

Coirini, H., A. Johnson and B. McEwen (1989). "Estradiol modulation of oxytocin binding in the ventromedial hypothalamic nucleus of male and female rats." Neuroendocrinology **50**(2).

Coiro, V., A. Alboni, D. Gramellini, C. Cigarini, L. Bianconi, D. Pignatti, R. Volpi and P. Chiodera (1992). "Inhibition by ethanol of the oxytocin response to breast stimulation in normal women and the role of endogenous opioids." Acta endocrinologica **126**(3).

Crestani, C., F. Alves, F. Gomes, L. Resstel, F. Correa and J. Herman (2013). "Mechanisms in the bed nucleus of the stria terminalis involved in control of autonomic and neuroendocrine functions: a review." Current neuropharmacology **11**(2).

Da Silva, A., S. Ruginsk, E. Uchoa, C. Crestani, A. Scopinho, F. Correa, B. De Martinis, L. Elias, L. Resstel and J. Antunes-Rodrigues (2013). "Time-course of neuroendocrine changes and its correlation with hypertension induced by ethanol consumption." Alcohol and alcoholism (Oxford, Oxfordshire) **48**(4).

Dabrowska, J., R. Hazra, T. Ahern, J. Guo, A. McDonald, F. Mascagni, J. Muller, L. Young and D. Rainnie (2011). "Neuroanatomical evidence for reciprocal regulation of the corticotrophin-releasing factor and oxytocin systems in the hypothalamus and the bed nucleus of the stria terminalis of the rat: Implications for balancing stress and affect." Psychoneuroendocrinology **36**(9).

de Castro, J. (1990). "Social, circadian, nutritional, and subjective correlates of the spontaneous pattern of moderate alcohol intake of normal humans." Pharmacology, biochemistry, and behavior **35**(4).

de Kloet, E., D. Voorhuis, Y. Boschma and J. Elands (1986). "Estradiol modulates density of putative 'oxytocin receptors' in discrete rat brain regions." Neuroendocrinology **44**(4).

De Kloet, E., T. Voorhuis and J. Elands (1985). "Estradiol induces oxytocin binding sites in rat hypothalamic ventromedial nucleus." European journal of pharmacology **118**(1-2).

De Luca, M. (2014). "Habituation of the responsiveness of mesolimbic and mesocortical dopamine transmission to taste stimuli." Frontiers in integrative neuroscience **8**.

Del Turco, S., T. Navarra, A. Gastaldelli and G. Basta (2011). "Protective role of adiponectin on endothelial dysfunction induced by AGEs: a clinical and experimental approach." Microvascular research **82**(1).

Delucchi, K. L., H. Matzger and C. Weisner (2008). "Alcohol in emerging adulthood: 7-year study of problem and dependent drinkers." Addict Behav **33**(1): 134-142.

DeVries, A. C., M. M. Cho, S. Cardillo and C. S. Carter (1997). "Oxytocin can suppress the HPA axis in prairie voles." Society for Neuroscience Abstract.

Ding, Q. and J. Keller (2005). "Splice variants of the receptor for advanced glycosylation end products (RAGE) in human brain." Neuroscience letters **373**(1).

Dluzen, D. and C. Carter (1979). "Ovarian hormones regulating sexual and social behaviors in female prairie voles, Microtus ochrogaster." Physiology & behavior **23**(3).

Dobkin, P. L., C. M. De, A. Paraherakis and K. Gill (2002). "The role of functional social support in treatment retention and outcomes among outpatient adult substance abusers." Addiction **97**(3): 347-356.

Dolder, P., F. Holze, E. Liakoni, S. Harder, Y. Schmid and M. Liechti (2017). "Alcohol acutely enhances decoding of positive emotions and emotional concern for positive stimuli and facilitates the viewing of sexual images." Psychopharmacology **234**(1).

Dölen, G., A. Darvishzadeh, K. Huang and R. Malenka (2013). "Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin." Nature **501**(7466).

Dumais, K., A. Alonso, M. Immormino, R. Bredewold and A. Veenema (2016). "Involvement of the oxytocin system in the bed nucleus of the stria terminalis in the sex-specific regulation of social recognition." Psychoneuroendocrinology **64**.

Dumais, K. M. and A. H. Veenema (2016). "Vasopressin and oxytocin receptor systems in the brain: sex differences and sex-specific regulation of social behavior." Front Neuroendocrinol **40**: 1-23.

Egli, M., G. Koob and S. Edwards (2012). "Alcohol dependence as a chronic pain disorder." Neuroscience and Biobehavioral Reviews **36**(10).

Eliava, M., M. Melchior, H. Knobloch-Bollmann, J. Wahis, M. da Silva Gouveia, Y. Tang, A. Ciobanu, R. Triana Del Rio, L. Roth, F. Althammer, V. Chavant, Y. Goumon, T. Gruber, N. Petit-Demoulière, M. Busnelli, B. Chini, L. Tan, M. Mitre, R. Froemke, M. Chao, G. Giese, R. Sprengel, R. Kuner, P. Poisbeau, P. Seeburg, R. Stoop, A. Charlet and V. Grinevich (2016). "A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing." Neuron **89**(6).

Engelmann, M., K. Ebner, R. Landgraf, F. Holsboer and C. Wotjak (1999). "Emotional stress triggers intrahypothalamic but not peripheral release of oxytocin in male rats." Journal of neuroendocrinology **11**(11).

Ermisch, A., H. J. Ruhle, R. Landgraf and J. Hess (1985). "Blood-brain barrier and peptides." J Cereb Blood Flow Metab **5**(3): 350-357.

Fan, X., G. Shi and P. Zhao (2019). "Reversal of oxycodone conditioned place preference by oxytocin: Promoting global DNA methylation in the hippocampus." Neuropharmacology **160**.

Ferrari, P., P. Palanza, S. Parmigiani, R. de Almeida and K. Miczek (2005). "Serotonin and aggressive behavior in rodents and nonhuman primates: predispositions and plasticity." European journal of pharmacology **526**(1-3).

Ferrer-Pérez, C., M. Reguilón, J. Miñarro and M. Rodríguez-Arias (2021). "Oxytocin Signaling as a Target to Block Social Defeat-Induced Increases in Drug Abuse Reward." International journal of molecular sciences **22**(5).

Ferretti, V., F. Maltese, G. Contarini, M. Nigro, A. Bonavia, H. Huang, V. Gigliucci, G. Morelli, D. Scheggia, F. Managò, G. Castellani, A. Lefevre, L. Cancedda, B. Chini, V. Grinevich and F. Papaleo (2019). "Oxytocin Signaling in the Central Amygdala Modulates Emotion Discrimination in Mice." Current biology : CB **29**(12).

Ferris, C. F., H. E. Albers, S. M. Wesolowski, B. D. Goldman and S. E. Luman (1984). "Vasopressin injected into the hypothalamus triggers a stereotypic behavior in golden hamsters." Science **224**(4648): 521-523.

Flanagan, J. C., N. P. Allan, C. D. Calhoun, C. L. Badour, M. Moran-Santa Maria, K. T. Brady and S. E. Back (2019). "Effects of oxytocin on stress reactivity and craving in veterans with co-occurring PTSD and alcohol use disorder." Exp Clin Psychopharmacol **27**(1): 45-54.

Forsling, M., I. Kalló, D. Hartley, L. Heinze, R. Ladek, C. Coen and S. File (2003). "Oestrogen receptor-beta and neurohypophysial hormones: functional interaction and neuroanatomical localisation." Pharmacology, biochemistry, and behavior **76**(3-4).

Fraley, R. C., C. C. Brumbaugh and M. J. Marks (2005). "The evolution and function of adult attachment: a comparative and phylogenetic analysis." J Pers Soc Psychol **89**(5): 731-746.

Francesconi, W., F. Berton, V. Repunte-Canonigo, K. Hagihara, D. Thurbon, D. Lekic, S. Specio, T. Greenwell, S. Chen, K. Rice, H. Richardson, L. O'Dell, E. Zorrilla, M. Morales, G. Koob and P. Sanna (2009). "Protracted withdrawal from alcohol and drugs of abuse impairs long-term potentiation of intrinsic excitability in the juxtacapsular bed nucleus of the stria

terminalis." The Journal of neuroscience : the official journal of the Society for Neuroscience **29**(17).

Franklin, K. B. and G. Paxinos (2008). "The Mouse Brain in Stereotaxic Coordinates." Academic Press(3).

Frantz, M. C., L. P. Pellissier, E. Pflimlin, S. Loison, J. Gandia, C. Marsol, T. Durroux, B. Mouillac, J. A. J. Becker, J. Le Merrer, C. Valencia, P. Villa, D. Bonnet and M. Hibert (2018). "LIT-001, the First Nonpeptide Oxytocin Receptor Agonist that Improves Social Interaction in a Mouse Model of Autism." J Med Chem **61**(19): 8670-8692.

Freeman, S. M. and L. J. Young (2016). "Comparative Perspectives on Oxytocin and Vasopressin Receptor Research in Rodents and Primates: Translational Implications." J Neuroendocrinol **28**(4).

Fuchs, A. (1966). "The inhibitory effect of ethanol on the release of oxytocin during parturition in the rabbit." The Journal of endocrinology **35**(2).

Fuchs, A. (1969). "Ethanol and the inhibition of oxytocin release in lactating rats." Acta endocrinologica **62**(3).

Fuchs, A., P. Husslein, L. Sumulong, J. Micha, M. Dawood and F. Fuchs (1982). "Plasma levels of oxytocin and 13, 14-dihydro-15-keto prostaglandin F2 alpha in preterm labor and the effect of ethanol and ritodrine." American journal of obstetrics and gynecology **144**(7).

Fuchs, A. and G. Wagner (1963). "Effect of alcohol on release of oxytocin." Nature **198**.

Fuller, R. K., L. Branchey, D. R. Brightwell, R. M. Derman, C. D. Emrick, F. L. Iber, K. E. James, R. B. Lacoursiere, K. K. Lee, I. Lowenstam and et al. (1986). "Disulfiram treatment of alcoholism. A Veterans Administration cooperative study." Jama **256**(11): 1449-1455.

Getz, L., C. Carter and L. Gavish (1981). "The mating system of the prairie vole, Microtus ochrogaster: Field and laboratory evidence for pair-bonding." Behavioral Ecology and Sociobiology **8**: 189-194.

Gibbens, G. and T. Chard (1976). "Observations on maternal oxytocin release during human labor and the effect of intravenous alcohol administration." American journal of obstetrics and gynecology **126**(2).

Gibbs, D. (1984). "Dissociation of oxytocin, vasopressin and corticotropin secretion during different types of stress." Life sciences **35**(5).

Gimpl, G., J. Reitz, S. Brauer and C. Trossen (2008). "Oxytocin receptors: ligand binding, signalling and cholesterol dependence. - PubMed - NCBI." Progress in Brain Research **170**: 193-204.

Gordon, I., A. Jack, C. M. Pretzsch, B. V. Wyk, J. F. Leckman, R. Feldman and K. A. Pelphrey (2016). "Intranasal Oxytocin Enhances Connectivity in the Neural Circuitry Supporting Social Motivation and Social Perception in Children with Autism." Scientific Reports **6**: 35054.

Goto, Y. and A. A. Grace (2005). "Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior." Nat Neurosci **8**(6): 805-812.

Griffiths, R. R., R. M. Wurster and J. V. Brady (1975). "Discrete-trial choice procedure: effects of naloxone and methadone on choice between food and heroin." Pharmacol Rev **27**(3): 357-365.

Grinevich, V. and M. Ludwig (2021). "The multiple faces of the oxytocin and vasopressin systems in the brain." J Neuroendocrinol **33**(11): e13004.

Grinevich, V. and I. Neumann (2021). "Brain oxytocin: how puzzle stones from animal studies translate into psychiatry." Molecular psychiatry **26**(1).

Groppe, S., A. Gossen, L. Rademacher, A. Hahn, L. Westphal, G. Gründer and K. Spreckelmeyer (2013). "Oxytocin Influences Processing of Socially Relevant Cues in the Ventral Tegmental Area of the Human Brain." Biological Psychiatry **74**(3): 172-179.

Guoynes, C., T. Simmons, G. Downing, S. Jacob, M. Solomon and K. Bales (2018). "Chronic Intranasal Oxytocin has Dose-dependent Effects on Central Oxytocin and Vasopressin Systems in Prairie Voles (Microtus ochrogaster). - PubMed - NCBI." Neuroscience **369**: 292-302.

Hald, J. and E. Jacobsen (1948). "A drug sensitizing the organism to ethyl alcohol." Lancet (London, England) **2**(6539).

Haney, M. and R. Spealman (2008). "Controversies in translational research: drug self-administration." Psychopharmacology (Berl) **199**(3): 403-419.

Hansson, A. C., A. Koopmann, S. Uhrig, S. Buhler, E. Domi, E. Kiessling, R. Ciccocioppo, R. C. Froemke, V. Grinevich, F. Kiefer, W. H. Sommer, S. Vollstadt-Klein and R. Spanagel (2018). "Oxytocin Reduces Alcohol Cue-Reactivity in Alcohol-Dependent Rats and Humans." Neuropsychopharmacology **43**(6): 1235-1246.

Hansson, A. C. and R. Spanagel (2021). "No changes in the oxytocin system in alcohol-dependent female rodents and humans: Towards a sex-specific psychopharmacology in alcoholism." Addict Biol **26**(2): e12945.

Harashima, A., Y. Yamamoto, C. Cheng, K. Tsuneyama, K. M. Myint, A. Takeuchi, K. Yoshimura, H. Li, T. Watanabe, S. Takasawa, H. Okamoto, H. Yonekura and H. Yamamoto (2006). "Identification of mouse orthologue of endogenous secretory receptor for advanced glycation end-products: structure, function and expression." Biochem J **396**(1): 109-115.

Harris, N. and D. Winder (2018). "Synaptic Plasticity in the Bed Nucleus of the Stria Terminalis: Underlying Mechanisms and Potential Ramifications for Reinstatement of Drug- and Alcohol-Seeking Behaviors." ACS chemical neuroscience **9**(9).

Hasan, M., F. Althammer, M. Silva da Gouveia, S. Goyon, M. Eliava, A. Lefevre, D. Kerspern, J. Schimmer, A. Raftogianni, J. Wahis, H. Knobloch-Bollmann, Y. Tang, X. Liu, A. Jain, V. Chavant, Y. Goumon, J. Weislogel, R. Hurlemann, S. Herpertz, C. Pitzer, P. Darbon, G. Dogbevia, I. Bertocchi, M. Larkum, R. Sprengel, H. Bading, A. Charlet and V. Grinevich (2019). "A Fear Memory Engram and Its Plasticity in the Hypothalamic Oxytocin System." Neuron **103**(1).

Hashiguchi, H., S. Ye, M. Morris and N. Alexander (1997). "Single and repeated environmental stress: effect on plasma oxytocin, corticosterone, catecholamines, and behavior." Physiology & behavior **61**(5).

Hashimoto, H., T. Noto, T. Nakajima and N. Kato (1985). "Effect of ethanol and acetaldehyde on the release of arginine-vasopressin and oxytocin from the isolated hypothalamo-hypophyseal system of rats." Endocrinologia japonica **32**(4).

Hasin, D. S., F. S. Stinson, E. Ogburn and B. F. Grant (2007). "Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions." Arch Gen Psychiatry **64**(7): 830-842.

Hatton, G. (1990). "Emerging concepts of structure-function dynamics in adult brain: the hypothalamo-neurohypophysial system." Progress in neurobiology **34**(6).

He, X. X., D. W. Nebert, V. Vasiliou, H. Zhu and H. G. Shertzer (1997). "Genetic differences in alcohol drinking preference between inbred strains of mice." Pharmacogenetics **7**(3): 223-233.

Heilig, M., D. H. Epstein, M. A. Nader and Y. Shaham (2016). "Time to connect: bringing social context into addiction neuroscience." Nat Rev Neurosci **17**(9): 592-599.

Hilfiger, L., Q. Zhao, D. Kerspern, P. Inquimbert, V. Andry, Y. Goumon, P. Darbon, M. Hibert and A. Charlet (2020). "A Nonpeptide Oxytocin Receptor Agonist for a Durable Relief of Inflammatory Pain." Scientific reports **10**(1).

Horie, K., K. Inoue, S. Suzuki, S. Adachi, S. Yada, T. Hirayama, S. Hidema, L. J. Young and K. Nishimori (2019). "Oxytocin receptor knockout prairie voles generated by CRISPR/Cas9 editing show reduced preference for social novelty and exaggerated repetitive behaviors." Horm Behav **111**: 60-69.

Hrabovszky, E., I. Kalló, A. Steinhauser, I. Merchenthaler, C. Coen, S. Petersen and Z. Liposits (2004). "Estrogen receptor-beta in oxytocin and vasopressin neurons of the rat and human hypothalamus: Immunocytochemical and in situ hybridization studies." The Journal of comparative neurology **473**(3).

Hrabovszky, E. and Z. Liposits (2008). "Novel aspects of glutamatergic signalling in the neuroendocrine system." Journal of neuroendocrinology **20**(6).

Hu, P., D. Lai, P. Lu, J. Gao and H. He (2012). "ERK and Akt signaling pathways are involved in advanced glycation end product-induced autophagy in rat vascular smooth muscle cells." International journal of molecular medicine **29**(4).

Huang, H., C. Michetti, M. Busnelli, F. Managò, S. Sannino, D. Scheggia, L. Giancardo, D. Sona, V. Murino, B. Chini, M. Scattoni and F. Papaleo (2014). "Chronic and acute intranasal oxytocin produce divergent social effects in mice. - PubMed - NCBI." Neuropsychopharmacology **5**: 1102-1114.

Hung, L., S. Neuner, J. Polepalli, K. Beier, M. Wright, J. Walsh, E. Lewis, L. Luo, K. Deisseroth, G. Dölen and R. Malenka (2017). "Gating of social reward by oxytocin in the ventral tegmental area." Science (New York, N.Y.) **357**(6358).

Inagaki, T. K. (2018). "Opioids and Social Connection:." Association for Psychological Science **27**(2): 85-90.

Insel, T., R. Gelhard and L. Shapiro (1991). "The comparative distribution of forebrain receptors for neurohypophyseal peptides in monogamous and polygamous mice." Neuroscience **43**(2-3).

Insel, T., L. Young, D. Witt and D. Crews (1993). "Gonadal steroids have paradoxical effects on brain oxytocin receptors." Journal of neuroendocrinology **5**(6).

Insel, T. R. and T. J. Hulihan (1995). "A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles." Behav Neurosci **109**(4): 782-789.

Insel, T. R., S. Preston and J. T. Winslow (1995). "Mating in the monogamous male: behavioral consequences." Physiol Behav **57**(4): 615-627.

Jadzic, D., V. Bassareo, A. Carta and E. Carboni (2021). "Nicotine, cocaine, amphetamine, morphine, and ethanol increase norepinephrine output in the bed nucleus of stria terminalis of freely moving rats." Addiction biology **26**(1).

Jezova, D., I. Skultetyova, D. Tokarev, P. Bakos and M. Vigas (1995). "Vasopressin and oxytocin in stress." Annals of the New York Academy of Sciences **771**.

Jiang, Y. and M. L. Platt (2018). "Oxytocin and vasopressin flatten dominance hierarchy and enhance behavioral synchrony in part via anterior cingulate cortex." Scientific Reports **8**(1): 8201.

Jochum, T., M. Boettger, C. Burkhardt, G. Juckel and K. Bär (2010). "Increased pain sensitivity in alcohol withdrawal syndrome." European journal of pain **14**(7).

Johnson, A., H. Coirini, T. Insel and B. McEwen (1991). "The regulation of oxytocin receptor binding in the ventromedial hypothalamic nucleus by testosterone and its metabolites." Endocrinology **128**(2).

Johnson, Z. V. and L. J. Young (2015). "Neurobiological mechanisms of social attachment and pair bonding." Curr Opin Behav Sci **3**: 38-44.

Jonas, D. E., H. R. Amick, C. Feltner, G. Bobashev, K. Thomas, R. Wines, M. M. Kim, E. Shanahan, C. E. Gass, C. J. Rowe and J. C. Garbutt (2014). "Pharmacotherapy for adults with alcohol use disorders in outpatient settings: a systematic review and meta-analysis." Jama **311**(18): 1889-1900.

Jurek, B. and I. D. Neumann (2018). "The Oxytocin Receptor: From Intracellular Signaling to Behavior." Physiol Rev **98**(3): 1805-1908.

Kendrick, K. M., E. B. Keverne and B. A. Baldwin (1987). "Intracerebroventricular oxytocin stimulates maternal behaviour in the sheep." Neuroendocrinology **46**(1): 56-61.

King, C. and H. Becker (2019). "Oxytocin attenuates stress-induced reinstatement of alcohol seeking behavior in male and female mice." Psychopharmacology **236**(9).

King, C., A. Gano and H. Becker (2020). "The role of oxytocin in alcohol and drug abuse." Brain research **1736**.

King, C. E., W. C. Griffin, M. F. Lopez and H. C. Becker (2021). "Activation of hypothalamic oxytocin neurons reduces binge-like alcohol drinking through signaling at central oxytocin receptors." Neuropsychopharmacology **46**(11): 1950-1957.

King, C. E., W. C. Griffin, L. N. Luderman, M. M. Kates, J. F. McGinty and H. C. Becker (2017). "Oxytocin Reduces Ethanol Self-Administration in Mice." Alcohol Clin Exp Res **41**(5): 955-964.

King, L., H. Walum, K. Inoue, N. Eyrich and L. Young (2016). "Variation in the Oxytocin Receptor Gene Predicts Brain Region-Specific Expression and Social Attachment." Biological psychiatry **80**(2).

Kleiman, D. G. (1977). "Monogamy in mammals." Q Rev Biol **52**(1): 39-69.

Knobloch, H., A. Charlet, L. Hoffmann, M. Eliava, S. Khrulev, A. Cetin, P. Osten, M. Schwarz, P. Seeburg, R. Stoop and V. Grinevich (2012). "Evoked axonal oxytocin release in the central amygdala attenuates fear response." Neuron **73**(3).

Knott, T., G. Dayanithi, V. Coccia, E. Custer, J. Lemos and S. Treistman (2000). "Tolerance to acute ethanol inhibition of peptide hormone release in the isolated neurohypophysis." Alcoholism, clinical and experimental research **24**(7).

Koob, G. (2021). "Drug Addiction: Hyperkatifeia/Negative Reinforcement as a Framework for Medications Development." Pharmacological reviews **73**(1).

Koob, G. and J. Schulkin (2019). "Addiction and stress: An allostatic view." Neuroscience and biobehavioral reviews **106**.

Koob, G. and N. Volkow (2016). "Neurobiology of addiction: a neurocircuitry analysis." The lancet. Psychiatry **3**(8).

Koob, G. F. (2003). "Alcoholism: allostasis and beyond." Alcohol Clin Exp Res **27**(2): 232-243.

Lander, H., J. Tauras, J. Ogiste, O. Hori, R. Moss and A. Schmidt (1997). "Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress." The Journal of biological chemistry **272**(28).

Landgraf, R. (2005). "Neuropeptides in anxiety modulation." Handbook of experimental pharmacology(169).

Landgraf, R. and I. Neumann (2004). "Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication." Frontiers in neuroendocrinology **25**(3-4).

Lang, R., J. Heil, D. Ganten, K. Hermann, T. Unger and W. Rascher (1983). "Oxytocin unlike vasopressin is a stress hormone in the rat." Neuroendocrinology **37**(4).

Larcher, A., J. Neculcea, C. Breton, A. Arslan, F. Rozen, C. Russo and H. Zingg (1995). "Oxytocin receptor gene expression in the rat uterus during pregnancy and the estrous cycle and in response to gonadal steroid treatment." Endocrinology **136**(12).

Leckman, J. and A. Herman (2002). "Maternal behavior and developmental psychopathology." Biological psychiatry **51**(1).

Lee, H. J., A. H. Macbeth, J. Pagani and W. S. Young (2009). "Oxytocin: the Great Facilitator of Life." Prog Neurobiol **88**(2): 127-151.

Lee, M., K. Scheidweiler, X. Diao, F. Akhlaghi, A. Cummins, M. Huestis, L. Leggio and B. Averbeck (2018). "Oxytocin by intranasal and intravenous routes reaches the cerebrospinal fluid in rhesus macaques: determination using a novel oxytocin assay." Molecular psychiatry **23**(1).

Lee, M., M. Schwandt, V. Sankar, P. Suchankova, H. Sun and L. Leggio (2017). "Effect of alcohol use disorder on oxytocin peptide and receptor mRNA expression in human brain: A post-mortem case-control study." Psychoneuroendocrinology **85**.

Lee, M. and E. Weerts (2016). "Oxytocin for the treatment of drug and alcohol use disorders." Behavioural pharmacology **27**(8).

Lee, M. R., T. A. Shnitko, S. W. Blue, A. V. Kaucher, A. J. Winchell, D. W. Erikson, K. A. Grant and L. Leggio (2020). "Labeled oxytocin administered via the intranasal route reaches the brain in rhesus macaques." Nat Commun **11**(1): 2783.

Lefevre, A., R. Mottolese, M. Dirheimer, C. Mottolese, J. Duhamel and A. Sirigu (2017). "A comparison of methods to measure central and peripheral oxytocin concentrations in human and non-human primates." Scientific reports **7**(1).

Lemoine, P., H. Harousseau, J. P. Borteyru and J. C. Menuet (2003). "Children of alcoholic parents--observed anomalies: discussion of 127 cases." Ther Drug Monit **25**(2): 132-136.

Leonard, K. E. and J. C. Rothbard (1999). "Alcohol and the marriage effect." J Stud Alcohol Suppl **13**: 139-146.

Leong, K., L. Freeman, C. Berini, S. Ghee, R. See and C. Reichel (2017). "Oxytocin Reduces Cocaine Cued Fos Activation in a Regionally Specific Manner." The international journal of neuropsychopharmacology **20**(10).

Leong, K. C., S. Cox, C. King, H. Becker and C. M. Reichel (2018). "Oxytocin and Rodent Models of Addiction." Int Rev Neurobiol **140**: 201-247.

Li, B., X. Li, H. Gao, J. Zhang, Q. Cai, M. Cheng and M. Lu (2011). "Grape seed procyanidin B2 inhibits advanced glycation end product-induced endothelial cell apoptosis through regulating GSK3β phosphorylation." Cell biology international **35**(7).

Lim, M. and L. Young (2006). "Neuropeptidergic regulation of affiliative behavior and social bonding in animals." Hormones and behavior **50**(4).

Lin, Y. and K. Hsu (2018). "Oxytocin receptor signaling in the hippocampus: Role in regulating neuronal excitability, network oscillatory activity, synaptic plasticity and social memory." Progress in neurobiology **171**.

Link, H., G. Dayanithi, K. Föhr and M. Gratzl (1992). "Oxytocin at physiological concentrations evokes adrenocorticotropin (ACTH) release from corticotrophs by increasing intracellular free calcium mobilized mainly from intracellular stores. Oxytocin displays synergistic or additive

effects on ACTH-releasing factor or arginine vasopressin-induced ACTH secretion, respectively." Endocrinology **130**(4).

Liu, Y., C. Liang, X. Liu, B. Liao, X. Pan, Y. Ren, M. Fan, M. Li, Z. He, J. Wu and Z. Wu (2010). "AGEs increased migration and inflammatory responses of adventitial fibroblasts via RAGE, MAPK and NF-kappaB pathways." Atherosclerosis **208**(1).

Liu, Y. and Z. X. Wang (2003). "Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles." Neuroscience **121**.

Loup, F., E. Tribollet, M. Dubois-Dauphin and J. Dreifuss (1991). "Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study." Brain research **555**(2).

Loup, F., E. Tribollet, M. Dubois-Dauphin, G. Pizzolato and J. Dreifuss (1989). "Localization of oxytocin binding sites in the human brainstem and upper spinal cord: an autoradiographic study." Brain research **500**(1-2).

Love, T. (2014). "Oxytocin, motivation and the role of dopamine." P **119**: 49-60.

Ludwig, M. and G. Leng (2006). "Dendritic peptide release and peptide-dependent behaviours." Nature reviews. Neuroscience **7**(2).

Lukas, D. and T. H. Clutton-Brock (2013). "The Evolution of Social Monogamy in Mammals."

Ma, J., T. Liu and X. Dong (2010). "Advanced glycation end products of bovine serum albumin-induced endothelial-to-mesenchymal transition in cultured human and monkey endothelial cells via protein kinase B signaling cascades." Molecular vision **16**.

MacFadyen, K., R. Loveless, B. DeLucca, K. Wardley, S. Deogan, C. Thomas and J. Peris (2016). "Peripheral oxytocin administration reduces ethanol consumption in rats." Pharmacol Biochem Behav **140**: 27-32.

MacGregor, D. and G. Leng (2013). "Spike triggered hormone secretion in vasopressin cells; a model investigation of mechanism and heterogeneous population function." PLoS computational biology **9**(8).

Mack, S., P. Kc, M. Wu, B. Coleman, F. Tolentino-Silva and M. Haxhiu (2002). "Paraventricular oxytocin neurons are involved in neural modulation of breathing." Journal of applied physiology (Bethesda, Md. : 1985) **92**(2).

Madeira, M., N. Sousa, A. Lieberman and M. Paula-Barbosa (1993). "Effects of chronic alcohol consumption and of dehydration on the supraoptic nucleus of adult male and female rats." Neuroscience **56**(3).

Maícas-Royo, J., G. Leng and D. MacGregor (2018). "A Predictive, Quantitative Model of Spiking Activity and Stimulus-Secretion Coupling in Oxytocin Neurons." Endocrinology **159**(3).

Mairesse, J., E. Gatta, M. Reynaert, J. Marrocco, S. Morley-Fletcher, M. Soichot, L. Deruyter, G. Camp, H. Bouwalerh, F. Fagioli, A. Pittaluga, D. Allorge, F. Nicoletti and S. Maccari (2015). "Activation of presynaptic oxytocin receptors enhances glutamate release in the ventral hippocampus of prenatally restraint stressed rats." Psychoneuroendocrinology **62**.

Maisel, N., J. Blodgett, P. Wilbourne, K. Humphreys and J. Finney (2013). "Meta-analysis of naltrexone and acamprosate for treating alcohol use disorders: when are these medications most helpful?" Addiction (Abingdon, England) **108**(2).

Mann, K., T. Lemenager, S. Hoffmann, I. Reinhard, D. Hermann, A. Batra, M. Berner, N. Wodarz, A. Heinz, M. N. Smolka, U. S. Zimmermann, S. Wellek, F. Kiefer and R. F. Anton (2013). "Results of a double-blind, placebo-controlled pharmacotherapy trial in alcoholism conducted in Germany and comparison with the US COMBINE study." Addict Biol **18**(6): 937-946.

Maroun, M. and S. Wagner (2016). "Oxytocin and Memory of Emotional Stimuli: Some Dance to Remember, Some Dance to Forget." Biological psychiatry **79**(3).

Marsh, N., D. Scheele, H. Gerhardt, S. Strang, L. Enax, B. Weber, W. Maier and R. Hurlemann (2015). "The Neuropeptide Oxytocin Induces a Social Altruism Bias." Journal of Neuroscience **47**.

Mason, B., A. Goodman, S. Chabac and P. Lehert (2006). "Effect of oral acamprosate on abstinence in patients with alcohol dependence in a double-blind, placebo-controlled trial: the role of patient motivation." Journal of psychiatric research **40**(5).

McGregor, I. S. and M. T. Bowen (2012). "Breaking the loop: oxytocin as a potential treatment for drug addiction." Horm Behav **61**(3): 331-339.

McLeod, J. D. (1993). "Spouse concordance for alcohol dependence and heavy drinking: evidence from a community sample." Alcohol Clin Exp Res **17**(6): 1146-1155.

Melby, K., O. B. Fasmer, T. E. Henriksen, R. W. Gråwe, T. O. Aamo and O. Spigset (2020). "Actigraphy assessment of motor activity and sleep in patients with alcohol withdrawal syndrome and the effects of intranasal oxytocin." PLoS One **15**(2): e0228700.

Melby, K., R. W. Gråwe, T. O. Aamo, Ø. Salvesen and O. Spigset (2019). "Effect of intranasal oxytocin on alcohol withdrawal syndrome: A randomized placebo-controlled double-blind clinical trial." Drug Alcohol Depend **197**: 95-101.

Melby, K., R. W. Gråwe, T. O. Aamo, E. Skovlund and O. Spigset (2021). "Efficacy of Self-Administered Intranasal Oxytocin on Alcohol Use and Craving After Detoxification in Patients With Alcohol Dependence. A Double-Blind Placebo-Controlled Trial." Alcohol Alcohol **56**(5): 565-572.

Melis, M., A. Argiolas and G. Gessa (1986). "Oxytocin-induced penile erection and yawning: site of action in the brain." Brain research **398**(2).

Melis, M., F. Sanna and A. Argiolas (2019). "Rats selectively bred for showing divergent behavioral traits in response to stress or novelty or spontaneous yawning with a divergent frequency show similar changes in sexual behavior: the role of dopamine." Reviews in the neurosciences **30**(4).

Mennella, J. and M. Pepino (2006). "Short-term effects of alcohol consumption on the hormonal milieu and mood states in nulliparous women." Alcohol (Fayetteville, N.Y.) **38**(1).

Mennella, J., M. Pepino and K. Teff (2005). "Acute alcohol consumption disrupts the hormonal milieu of lactating women." The Journal of clinical endocrinology and metabolism **90**(4).

Menon, R., T. Grund, I. Zoicas, F. Althammer, D. Fiedler, V. Biermeier, O. Bosch, Y. Hiraoka, K. Nishimori, M. Eliava, V. Grinevich and I. Neumann (2018). "Oxytocin Signaling in the Lateral Septum Prevents Social Fear during Lactation." Current biology : CB **28**(7).

Mitchell, J. M., P. A. Arcuni, D. Weinstein and J. D. Woolley (2016). "Intranasal Oxytocin Selectively Modulates Social Perception, Craving, and Approach Behavior in Subjects With Alcohol Use Disorder." J Addict Med **10**(3): 182-189.

Mitre, M., B. Marlin, J. Schiavo, E. Morina, S. Norden, T. Hackett, C. Aoki, M. Chao and R. Froemke (2016). "A Distributed Network for Social Cognition Enriched for Oxytocin Receptors." The Journal of neuroscience : the official journal of the Society for Neuroscience **36**(8).

Mohr, E. and E. Schmitz (1991). "Functional characterization of estrogen and glucocorticoid responsive elements in the rat oxytocin gene." Brain research. Molecular brain research **9**(4).

Mokdad, A. H., J. S. Marks, D. F. Stroup and J. L. Gerberding (2004). "Actual causes of death in the United States, 2000." JAMA **291**(10): 1238-1245.

Morris, J. (1976). "Hormone storage in individual neurosecretory granules of the pituitary gland: A quantitative ultrastructural approach to hormone storage in the neural lobe." The Journal of endocrinology **68**(02).

Moy, S. S., B. L. Teng, V. D. Nikolova, N. V. Riddick, C. D. Simpson, A. Van Deusen, W. P. Janzen, M. F. Sassano, C. A. Pedersen and M. B. Jarstfer (2019). "Prosocial effects of an oxytocin metabolite, but not synthetic oxytocin receptor agonists, in a mouse model of autism." Neuropharmacology **144**: 301-311.

Musher-Eizenman, D. R., S. C. Holub and M. Arnett (2003). "Attitude and peer influences on adolescent substance use: the moderating effect of age, sex, and substance." J Drug Educ **33**(1): 1-23.

Neeper, M., A. Schmidt, J. Brett, S. Yan, F. Wang, Y. Pan, K. Elliston, D. Stern and A. Shaw (1992). "Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins." The Journal of biological chemistry **267**(21).

Neumann, I., A. Wigger, L. Torner, F. Holsboer and R. Landgraf (2000). "Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus." Journal of neuroendocrinology **12**(3).

Neumann, I. D., R. Maloumby, D. I. Beiderbeck, M. Lukas and R. Landgraf (2013). "Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice." Psychoneuroendocrinology **38**(10): 1985-1993.

Nielsen, E. I., S. H. Al-Saqi, A. F. Jonasson and K. Uvnas-Moberg (2017). "Population Pharmacokinetic Analysis of Vaginally and Intravenously Administered Oxytocin in Postmenopausal Women." J Clin Pharmacol **57**(12): 1573-1581.

Nordmann, J. and J. Morris (1984). "Method for quantitating the molecular content of a subcellular organelle: hormone and neurophysin content of newly formed and aged neurosecretory granules." Proceedings of the National Academy of Sciences of the United States of America **81**(1).

Numan, M. and L. J. Young (2016). "Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications." Horm Behav **77**: 98-112.

O'Malley, S., A. Jaffe, G. Chang, R. Schottenfeld, R. Meyer and B. Rounsaville (1992). "Naltrexone and coping skills therapy for alcohol dependence. A controlled study." Archives of general psychiatry **49**(11).

Oliveira, V., M. Lukas, H. Wolf, E. Durante, A. Lorenz, A. Mayer, A. Bludau, O. Bosch, V. Grinevich, V. Egger, T. de Jong and I. Neumann (2021). "Oxytocin and vasopressin within the ventral and dorsal lateral septum modulate aggression in female rats." Nature communications **12**(1).

Oslin, D. W., W. Berrettini, H. R. Kranzler, H. Pettinati, J. Gelernter, J. R. Volpicelli and C. P. O'Brien (2003). "A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients." Neuropsychopharmacology **28**(8): 1546-1552.

Paille, F., J. Guelfi, A. Perkins, R. Royer, L. Steru and P. Parot (1995). "Double-blind randomized multicentre trial of acamprosate in maintaining abstinence from alcohol." Alcohol and alcoholism (Oxford, Oxfordshire) **30**(2).

Pedersen, C. A. (2017). "Oxytocin, Tolerance, and the Dark Side of Addiction." Int Rev Neurobiol **136**: 239-274.

Pedersen, C. A. and A. J. Prange, Jr. (1979). "Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin." Proc Natl Acad Sci U S A **76**(12): 6661-6665.

Pedersen, C. A., K. L. Smedley, J. Leserman, L. F. Jarskog, S. W. Rau, A. Kampov-Polevoi, R. L. Casey, T. Fender and J. C. Garbutt (2013). "Intranasal oxytocin blocks alcohol withdrawal in human subjects." Alcohol Clin Exp Res **37**(3): 484-489.

Pelc, I., P. Verbanck, O. Le Bon, M. Gavrilovic, K. Lion and P. Lehert (1997). "Efficacy and safety of acamprosate in the treatment of detoxified alcohol-dependent patients. A 90-day placebo-controlled dose-finding study." The British journal of psychiatry : the journal of mental science **171**.

Peris, J., K. MacFadyen, J. Smith, A. de Kloet, L. Wang and E. Krause (2017). "Oxytocin receptors are expressed on dopamine and glutamate neurons in the mouse ventral tegmental area that project to nucleus accumbens and other mesolimbic targets." The Journal of comparative neurology **525**(5).

Peters, S., M. Bowen, K. Bohrer, I. McGregor and I. Neumann (2017). "Oxytocin inhibits ethanol consumption and ethanol-induced dopamine release in the nucleus accumbens." Addiction biology **22**(3).

Petersson, M. (2002). "Cardiovascular effects of oxytocin." Progress in brain research **139**.

Pisansky, M., L. Hanson, I. Gottesman and J. Gewirtz (2017). "Oxytocin enhances observational fear in mice." Nature Communications **8**(1): 2102.

Pitkow, L. J., C. A. Sharer, X. Ren, T. R. Insel, E. F. Terwilliger and L. J. Young (2001). "Facilitation of affiliation and pair-bond formation by vasopressin receptor gene transfer into the ventral forebrain of a monogamous vole." J Neurosci **21**(18): 7392-7396.

Pitt, G., A. Batt, R. Haigh, A. Penson, P. Robson, D. Rooker, A. Tartar, J. Trim, C. Yea and M. Roe (2004). "Non-peptide oxytocin agonists." Bioorganic & medicinal chemistry letters **14**(17).

Potretzke, S. and A. E. Ryabinin (2019). "The Prairie Vole Model of Pair-Bonding and Its Sensitivity to Addictive Substances." Front Psychol **10**: 2477.

Prinstein, M. J., J. Boergers and A. Spirito (2001). "Adolescents' and their friends' health-risk behavior: factors that alter or add to peer influence." J Pediatr Psychol **26**(5): 287-298.

Quiñones-Jenab, V., S. Jenab, S. Ogawa, R. Adan, J. Burbach and D. Pfaff (1997). "Effects of estrogen on oxytocin receptor messenger ribonucleic acid expression in the uterus, pituitary, and forebrain of the female rat." Neuroendocrinology **65**(1).

Quintana, D. and A. Guastella (2020). "An Allostatic Theory of Oxytocin." Trends in cognitive sciences **24**(7).

Raggenbass, M. (2008). "Overview of cellular electrophysiological actions of vasopressin." European journal of pharmacology **583**(2-3).

Rash, J., A. Aguirre-Camacho and T. Campbell (2014). "Oxytocin and pain: a systematic review and synthesis of findings." The Clinical journal of pain **30**(5).

Rehm, J., C. Mathers, S. Popova, M. Thavorncharoensap, Y. Teerawattananon and J. Patra (2009). "Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders." Lancet **373**(9682): 2223-2233.

Richard, P., F. Moos and M. Freund-Mercier (1991). "Central effects of oxytocin." Physiological reviews **71**(2).

Richard, S. and H. Zingg (1990). "The human oxytocin gene promoter is regulated by estrogens." The Journal of biological chemistry **265**(11).

Rösner, S., S. Leucht, P. Lehert and M. Soyka (2008). "Acamprosate supports abstinence, naltrexone prevents excessive drinking: evidence from a meta-analysis with unreported outcomes." Journal of psychopharmacology (Oxford, England) **22**(1).

Ryabinin, A. and H. Fulenwider (2021). "Alcohol and oxytocin: Scrutinizing the relationship." Neuroscience and biobehavioral reviews **127**.

Ryabinin, A. and C. Hostetler (2016). "Prairie Voles as a Model to Screen Medications for the Treatment of Alcoholism and Addictions." International review of neurobiology **126**.

Ryabinin, A. and A. Walcott (2018). "Assessing Social Alcohol Drinking in Rodent Models: Are We There Yet? - PubMed - NCBI."

Ryabinin, A. E. and Y. Zhang (2022). "Barriers and Breakthroughs in Targeting the Oxytocin System to Treat Alcohol Use Disorder." Frontiers in Psychiatry **13**.

Sabatier, N., G. Leng and J. Menzies (2013). "Oxytocin, feeding, and satiety." Frontiers in endocrinology **4**.

SAMHSA (2021). Key substance use and mental health indicators in the United States: Results from the 2020 National Survey on Drug Use and Health. Rockville, MD, Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services.

Sanna, F., J. Bratzu, A. Argiolas and M. Melis (2017). "Oxytocin induces penile erection and yawning when injected into the bed nucleus of the stria terminalis: Involvement of glutamic acid, dopamine, and nitric oxide." Hormones and behavior **96**.

Sanna, F. and M. De Luca (2021). "The potential role of oxytocin in addiction: What is the target process?" Current opinion in pharmacology **58**.

Sass, H., M. Soyka, K. Mann and W. Zieglgänsberger (1996). "Relapse prevention by acamprosate. Results from a placebo-controlled study on alcohol dependence." Archives of general psychiatry **53**(8).

Sayette, M. A., K. G. Creswell, J. D. Dimoff, C. E. Fairbairn, J. F. Cohn, B. W. Heckman, T. R. Kirchner, J. M. Levine and R. L. Moreland (2012). "Alcohol and group formation: a multimodal investigation of the effects of alcohol on emotion and social bonding." Psychol Sci **23**(8): 869-878.

Schmidt, A., M. Hasu, D. Popov, J. Zhang, J. Chen, S. Yan, J. Brett, R. Cao, K. Kuwabara and G. Costache (1994). "Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins." Proceedings of the National Academy of Sciences of the United States of America **91**(19).

Schmidt, A., O. Hori, J. Brett, S. Yan, J. Wautier and D. Stern (1994). "Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions." Arteriosclerosis and thrombosis : a journal of vascular biology **14**(10).

Shamay-Tsoory, S. and A. Abu-Akel (2016). "The Social Salience Hypothesis of Oxytocin." Biological psychiatry **79**(3).

Silva, S. M., M. D. Madeira, C. Ruela and M. M. Paula-Barbosa (2002). "Prolonged alcohol intake leads to irreversible loss of vasopressin and oxytocin neurons in the paraventricular nucleus of the hypothalamus." Brain Res **925**(1): 76-88.

Sivukhina, E., A. Dolzhikov, I. Morozov, G. Jirikowski and V. Grinevich (2006). "Effects of chronic alcoholic disease on magnocellular and parvocellular hypothalamic neurons in men."

Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme **38**(6).

Skinner, M., P. Lahmek, H. Pham and H. Aubin (2014). "Disulfiram efficacy in the treatment of alcohol dependence: a meta-analysis." PloS one **9**(2).

Soman, S., R. Raju, V. Sandhya, J. Advani, A. Khan, H. Harsha, T. Prasad, P. Sudhakaran, A. Pandey and P. Adishesha (2013). "A multicellular signal transduction network of AGE/RAGE signaling." Journal of cell communication and signaling **7**(1).

Son, S., J. Filosa, E. Potapenko, V. Biancardi, H. Zheng, K. Patel, V. Tobin, M. Ludwig and J. Stern (2013). "Dendritic peptide release mediates interpopulation crosstalk between neurosecretory and preautonomic networks." Neuron **78**(6).

Sousa, N., M. Madeira, C. Ruela and M. Paula-Barbosa (1995). "Structural reorganization in the supraoptic nucleus of withdrawn rats following long-term alcohol consumption." Alcoholism, clinical and experimental research **19**(4).

Spanagel, R., S. M. Holter, K. Allingham, R. Landgraf and W. Zieglgansberger (1996). "Acamprosate and alcohol: I. Effects on alcohol intake following alcohol deprivation in the rat." Eur J Pharmacol **305**(1-3): 39-44.

Stedronsky, K., R. Telgmann, G. Tillmann, N. Walther and R. Ivell (2002). "The affinity and activity of the multiple hormone response element in the proximal promoter of the human oxytocin gene." Journal of neuroendocrinology **14**(6).

Stern, J. and W. Armstrong (1995). "Electrophysiological differences between oxytocin and vasopressin neurones recorded from female rats in vitro." The Journal of physiology **488 (Pt 3)**(Pt 3).

Stevens, E., L. A. Jason, D. Ram and J. Light (2015). "Investigating Social Support and Network Relationships in Substance Use Disorder Recovery." Subst Abus **36**(4): 396-399.

Stevenson, J., J. Buirkle, L. Buckley, K. Young, K. Albertini and A. Bohidar (2015). "GHS-R1A antagonism reduces alcohol but not sucrose preference in prairie voles." Physiology & behavior **147**.

Stevenson, J., L. Francomacaro, A. Bohidar, K. Young, B. Pesarchick, J. Buirkle, E. McMahon and C. O'Bryan (2016). "Ghrelin receptor (GHS-R1A) antagonism alters preference for ethanol and sucrose in a concentration-dependent manner in prairie voles." Physiology & behavior **155**.

Stevenson, J. R., S. M. Wenner, D. M. Freestone, C. C. Romaine, M. C. Parian, S. M. Christian, A. E. Bohidar, J. R. Ndem, I. R. Vogel and C. M. O'Kane (2017). "Oxytocin reduces alcohol consumption in prairie voles." Physiol Behav **179**: 411-421.

Stevenson, J. R., K. A. Young, A. E. Bohidar, L. M. Francomacaro, T. R. Fasold, J. M. Buirkle, J. R. Ndem and S. C. Christian (2017). "Alcohol Consumption Decreases Oxytocin Neurons in the Anterior Paraventricular Nucleus of the Hypothalamus in Prairie Voles." Alcohol Clin Exp Res **41**(8): 1444-1451.

Stevenson, K., P. Riley, H. Stewart, A. Flint and D. Wathes (1994). "Localization of oxytocin receptor mRNA in the ovine uterus during the oestrous cycle and early pregnancy." Journal of molecular endocrinology **12**(1).

Stoop, R. (2012). "Neuromodulation by oxytocin and vasopressin." Neuron **76**(1): 142-159.

Striepens, N., K. Kendrick, V. Hanking, R. Landgraf, U. Wüllner, W. Maier and R. Hurlemann (2013). "Elevated cerebrospinal fluid and blood concentrations of oxytocin following its intranasal administration in humans." Scientific reports **3**.

Swanson, L. and P. Sawchenko (1980). "Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms." Neuroendocrinology **31**(6).

Swanson, L. and P. Sawchenko (1983). "Hypothalamic integration: organization of the paraventricular and supraoptic nuclei." Annual review of neuroscience **6**.

Szabó, G., G. Kovács, S. Székeli and G. Telegdy (1985). "The effects of neurohypophyseal hormones on tolerance to the hypothermic effect of ethanol." Alcohol (Fayetteville, N.Y.) **2**(4).

Tanaka, A., T. Furubayashi, M. Arai, D. Inoue, S. Kimura, A. Kiriyama, K. Kusamori, H. Katsumi, R. Yutani, T. Sakane and A. Yamamoto (2018). "Delivery of Oxytocin to the Brain for the Treatment of Autism Spectrum Disorder by Nasal Application." Mol Pharm **15**(3): 1105-1111.

Tang, Y., D. Benusiglio, A. Lefevre, L. Hilfiger, F. Althammer, A. Bludau, D. Hagiwara, A. Baudon, P. Darbon, J. Schimmer, M. Kirchner, R. Roy, S. Wang, M. Eliava, S. Wagner, M. Oberhuber, K. Conzelmann, M. Schwarz, J. Stern, G. Leng, I. Neumann, A. Charlet and V. Grinevich (2020). "Social touch promotes interfemale communication via activation of parvocellular oxytocin neurons." Nature neuroscience **23**(9).

Thomas, J. and E. Birney (1979). "Parental care and mating system of the prairie vole, Microtus ochrogaster." Behavioral Ecology and Sociobiology **5**(2): 171-186.

Thomsen, M., D. Dencker, G. Wortwein, P. Weikop, E. Egecioglu, E. Jerlhag, A. Fink-Jensen and A. Molander (2017). "The glucagon-like peptide 1 receptor agonist Exendin-4 decreases relapse-like drinking in socially housed mice. - PubMed - NCBI." Pharmacology Biochemistry and Behavior **160**: 14-20.

Toma, L., C. Stancu, G. Botez, A. Sima and M. Simionescu (2009). "Irreversibly glycated LDL induce oxidative and inflammatory state in human endothelial cells; added effect of high glucose." Biochemical and biophysical research communications **390**(3).

Tops, M., S. L. Koole, I. J. H and F. T. Buisman-Pijlman (2014). "Why social attachment and oxytocin protect against addiction and stress: Insights from the dynamics between ventral and dorsal corticostriatal systems." Pharmacol Biochem Behav **119**: 39-48.

Tribollet, E., S. Audigier, M. Dubois-Dauphin and J. Dreifuss (1990). "Gonadal steroids regulate oxytocin receptors but not vasopressin receptors in the brain of male and female rats. An autoradiographical study." Brain research **511**(1).

Tribollet, E., C. Barberis and Y. Arsenijevic (1997). "Distribution of vasopressin and oxytocin receptors in the rat spinal cord: sex-related differences and effect of castration in pudendal motor nuclei." Neuroscience **78**(2).

Tribollet, E., M. Dubois-Dauphin, J. Dreifuss, C. Barberis and S. Jard (1992). "Oxytocin receptors in the central nervous system. Distribution, development, and species differences." Annals of the New York Academy of Sciences **652**.

Tunstall, B. J., D. Kirson, L. J. Zallar, S. A. McConnell, J. C. M. Vendruscolo, C. P. Ho, C. S. Oleata, S. Khom, M. Manning, M. R. Lee, L. Leggio, G. F. Koob, M. Roberto and L. F. Vendruscolo (2019). "Oxytocin blocks enhanced motivation for alcohol in alcohol dependence and blocks alcohol effects on GABAergic transmission in the central amygdala." PLoS Biol **17**(4): e2006421.

Uhl-Bronner, S., E. Waltisperger, G. Martínez-Lorenzana, L. M. Condes and M. Freund-Mercier (2005). "Sexually dimorphic expression of oxytocin binding sites in forebrain and spinal cord of the rat." Neuroscience **135**(1).

Uvnäs-Moberg, K., T. Lundeberg, G. Bruzelius and A. P (1993). "Low doses of ethanol may induce anti-nociceptive effects via an oxytocinergic mechanism." Acta physiologica Scandinavica **149**(1).

Vaht, M., T. Kurrikoff, K. Laas, T. Veidebaum and J. Harro (2016). "Oxytocin receptor gene variation rs53576 and alcohol abuse in a longitudinal population representative study." Psychoneuroendocrinology **74**: 333-341.

Vena, A., A. King, R. Lee and H. de Wit (2018). "Intranasal Oxytocin Does Not Modulate Responses to Alcohol in Social Drinkers." Alcohol Clin Exp Res **42**(9): 1725-1734.

Viero, C., I. Shibuya, N. Kitamura, A. Verkhratsky, H. Fujihara, A. Katoh, Y. Ueta, H. Zingg, A. Chvatal, E. Sykova and G. Dayanithi (2010). "Oxytocin: Crossing the bridge between basic science and pharmacotherapy." CNS neuroscience & therapeutics **16**(5).

Volpicelli, J. R., A. I. Alterman, M. Hayashida and C. P. O'Brien (1992). "Naltrexone in the treatment of alcohol dependence." Arch Gen Psychiatry **49**(11): 876-880.

Wagner, G. and A. Fuchs (1968). "Effect of ethanol on uterine activity during suckling in post-partum women." Acta endocrinologica **58**(1).

Walcott, A., M. Smith, J. Loftis and A. Ryabinin (2018). "Social transfer of alcohol withdrawal-induced hyperalgesia in female prairie voles." Social neuroscience **13**(6).

Walcott, A. T. and A. E. Ryabinin (2017). "Alcohol's Effects on Pair-Bond Maintenance in Male Prairie Voles." Frontiers in Psychiatry **8**(226).

Walcott, A. T. and A. E. Ryabinin (2019). "Effects of Alcohol Consumption on Pair Bond Maintenance and Potential Neural Substrates in Female Prairie Voles." Alcohol and Alcoholism.

Walcott, A. T. and A. E. Ryabinin (2020). "Assessing effects of oxytocin on alcohol consumption in socially housed prairie voles using radio frequency tracking." Addict Biol: e12893.

Walker, D., L. Miles and M. Davis (2009). "Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses." Progress in neuro-psychopharmacology & biological psychiatry **33**(8).

Walum, H., P. Lichtenstein, J. M. Neiderhiser, D. Reiss, J. M. Ganiban, E. L. Spotts, N. L. Pedersen, H. Anckarsater, H. Larsson and L. Westberg (2012). "Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior." Biol Psychiatry **71**(5): 419-426.

Wang, Z., G. Yu, C. Cascio, Y. Liu, B. Gingrich and T. R. Insel (1999). "Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles (Microtus ochrogaster): a mechanism for pair bonding?" Behav Neurosci **113**(3): 602-611.

Williams, J., T. Insel, C. Harbaugh and C. Carter (1994). "Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster)." Journal of neuroendocrinology **6**(3).

Williams, J. R., K. C. Catania and C. S. Carter (1992). "Development of partner preferences in female prairie voles (Microtus ochrogaster): the role of social and sexual experience." Horm Behav **26**(3): 339-349.

Witt, D., C. Carter and D. Walton (1990). "Central and peripheral effects of oxytocin administration in prairie voles (Microtus ochrogaster)." Pharmacology, biochemistry, and behavior **37**(1).

Witt, D. and T. Insel (1991). "A selective oxytocin antagonist attenuates progesterone facilitation of female sexual behavior." Endocrinology **128**(6).

Xie, Y., S. You, Y. Zhang, Q. Han, Y. Cao, X. Xu, Y. Yang, J. Li and C. Liu (2011). "Protective role of autophagy in AGE-induced early injury of human vascular endothelial cells." Molecular medicine reports **4**(3).

Xu, Y., S. Wang, L. Feng, Q. Zhu, P. Xiang and B. He (2010). "Blockade of PKC-beta protects HUVEC from advanced glycation end products induced inflammation." International immunopharmacology **10**(12).

Yamamoto, Y. and H. Higashida (2020). "RAGE regulates oxytocin transport into the brain." Communications biology **3**(1).

Yamamoto, Y., M. Liang, S. Munesue, K. Deguchi, A. Harashima, K. Furuhara, T. Yuhi, J. Zhong, S. Akther, H. Goto, Y. Eguchi, Y. Kitao, O. Hori, Y. Shiraishi, N. Ozaki, Y. Shimizu, T. Kamide, A. Yoshikawa, Y. Hayashi, M. Nakada, O. Lopatina, M. Gerasimenko, Y. Komleva, N. Malinovskaya, A. B. Salmina, M. Asano, K. Nishimori, S. E. Shoelson, H. Yamamoto and H. Higashida (2019). "Vascular RAGE transports oxytocin into the brain to elicit its maternal bonding behaviour in mice." Communications Biology **2**(1): 76.

Yan, S., R. Ramasamy and A. Schmidt (2009). "Receptor for AGE (RAGE) and its ligands-cast into leading roles in diabetes and the inflammatory response." Journal of molecular medicine (Berlin, Germany) **87**(3).

Yang, L., F. Wang, M. Wang, M. Han, L. Hu, M. Zheng, J. Ma, Y. Kang, P. Wang, H. Sun, W. Zuo, L. Xie, A. Wang, D. Yu and Y. Liu (2017). "Association between oxytocin and receptor genetic polymorphisms and aggression in a northern Chinese Han population with alcohol dependence." Neurosci Lett **636**: 140-144.

Yeomans, D., L. Hanson, D. Carson, B. Tunstall, M. Lee, A. Tzabazis, D. Jacobs and W. Frey (2021). "Nasal oxytocin for the treatment of psychiatric disorders and pain: achieving meaningful brain concentrations." Translational psychiatry **11**(1).

Yonekura, H., Y. Yamamoto, S. Sakurai, R. Petrova, M. Abedin, H. Li, K. Yasui, M. Takeuchi, Z. Makita, S. Takasawa, H. Okamoto, T. Watanabe and H. Yamamoto (2003). "Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury." The Biochemical journal **370**(Pt 3).

Yoshida, M., Y. Takayanagi, K. Inoue, T. Kimura, L. J. Young, T. Onaka and K. Nishimori (2009). "Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice." J Neurosci **29**(7): 2259-2271.

Young, K. A., K. L. Gobrogge, Y. Liu and Z. Wang (2011). "The neurobiology of pair bonding: insights from a socially monogamous rodent." Front Neuroendocrinol **32**(1): 53-69.

Zhang, B., L. Qiu, W. Xiao, H. Ni, L. Chen, F. Wang, W. Mai, J. Wu, A. Bao, H. Hu, H. Gong, S. Duan, A. Li and Z. Gao (2021). "Reconstruction of the Hypothalamo-Neurohypophysial System and Functional Dissection of Magnocellular Oxytocin Neurons in the Brain." Neuron **109**(2).

Zingg, H. and S. Laporte (2003). "The oxytocin receptor." Trends in endocrinology and metabolism: TEM **14**(5).