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Cross-talk between the Oxytocin and Vasopressin Systems in the Brian: Roles in Social Behavior

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CROSS-TALK BETWEEN THE OXYTOCIN AND VASOPRESSIN SYSTEMS IN THE

BRAIN: ROLES IN SOCIAL BEHAVIOR

by

ZHIMIN SONG

Under the Direction of H. Elliott Albers, PhD

ABSTRACT

Oxytocin (OT) and arginine vasopressin (AVP) are closely related nine amino acid neuropeptides primarily produced in the hypothalamus and released both in the peripheral and central nervous systems. For over 100 years, OT and AVP have been known for their roles in uterine contraction/milk letdown and blood vessel constriction, respectively. Over the past few decades, the roles of OT and AVP in the central nervous system have been extensively investigated in the regulation of a variety of complex social behaviors including sex, parenting, pair bonding, social play, and aggression. High levels of structural similarities exist between OT and AVP and between the OT and AVP1a receptor. Interestingly, there is little data on whether cross-talk between the OT

and AVP systems occurs in the central nervous system. Therefore, the goal of this dissertation is to examine cross-activation of OT receptors by AVP and of AVP1a receptors by OT, and the functional significance of this cross-talk in the regulation of social behavior. Three specific aims are addressed using Syrian hamsters as animal models: Aim 1 was to test the hypothesis that central OT enhances social communicative behavior by acting on V1aRs; Aim 2 was to test the hypothesis that central OT and AVP prolong social recognition via activation of the same receptor, OTRs or V1aRs. Aim 3 was to test the hypothesis that AVP in the ventral tegmental area (VTA) enhances social reward via activation of OTRs. Our data showed intraccerebroventricular (ICV) injections of OT or AVP act on V1aRs to induce social communication; ICV injections of OT and AVP act on OTRs to prolong social recognition; OT and AVP in the VTA act on OTRs and not V1aRs to enhance social reward. These results demonstrate the ability of OT and AVP to facilitate three essential aspects of a social interaction: communication, recognition, and reward. Our findings also strongly suggest OT and AVP act on both OTRs and V1aRs to influence social behavior but that OTRs regulate some social behaviors while V1aRs regulate other social behaviors.

INDEX WORDS: Social communication, Social recognition, Social reward, Prosocial
        behavior, Syrian hamsters, Aggression

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DEDICATION

This dissertation is dedicated to my wife, Rebecca. I cannot even begin to express how much you mean to me. You have supported and encouraged me so much in the past years while I'm at GSU. None of this work would have been possible without you. I love you with all my heart.

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AH, anterior hypothalamus

AVP, arginine vasopressin

α-MSH, alpha-melanocyte-stimulating hormone

BNST, the bed nucleus of the stria terminalis

CeA, central amygdaloid nucleus

Fos, protein product of the immediate early gene *c-fos*

LS, lateral septum

ICV, intracerebroventricle

MeA, medial nucleus of the amygdala

MPOA, medial preoptic area

MPOA---AH, medial preoptic area---anterior hypothalamus continuum

NAcc, nucleus accumbens

OT, oxytocin

OTA, oxytocin receptor antagonist

OTAG, oxytocin receptor agonist

OTR, oxytocin receptor

PAG, periaqueductal gray

PVN, paraventricular nucleus of the hypothalamus

SON, supraoptic nucleus of the hypothalamus

T, Testosterone

V1aA, vasopressin 1a receptor antagonist

V1aAG, vasopressin 1a receptor agonist

V1aR, vasopressin 1a receptor

V1bR, vasopressin 1b receptor

V2R, vasopressin 2 receptor

VMH, ventromedial nucleus of the hypothalamus

VTA, ventral tegmental area

CHAPTER 1: INTRODUCTION

1.1   General Introduction

Oxytocin (OT) and arginine vasopressin (AVP) are two evolutionarily conserved nonapeptides that serve as neurotransmitters and neurohormones influencing a variety of complex social behaviors in mammalian species. Both peripheral and central effects of OT and AVP on social behaviors have been well documented in a wide range of species including humans in the past few decades (Choleris *et al.*, 2013; Albers, 2015). Less is known, however, about the neuronal responses produced by OT and AVP activation in the regulation of social behavior. OT and AVP are structurally similar neuropeptides differing by only two of nine amino acids sequences (Light & Du Vigneaud, 1958). Receptors for OT (OTR) and for AVP (V1aR and V1bR) in the central nervous system (CNS) belong to the G-protein coupled family and are also very similar in structure with greater than 85% homology in all the species examined (Neumann & Landgraf, 2012). Despite the structural similarities among these peptides and their receptors, few studies have systematically investigated cross-activation of OTRs by AVP and of AVP receptors by OT in the regulation of social behavior. This dissertation focuses on this cross-talk of the OT and AVP systems in three areas of social behavior: social communication, social recognition, and social reward. These three aspects of social behavior are essential for the establishment and maintenance of all forms of social relationships. Because OT and/or AVP are involved in many aspects of social behavior, this dissertation aims to determine the potential role for cross-activation of OT and AVP in the regulation of these specific and functionally important social behaviors. I will also

discuss the functional significance of this cross-talk in the signaling of OT and AVP in the physiological level.

OT and AVP

The nonapeptides OT and AVP are primarily synthesized in the magnocellular neurons of the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus, where they are transported to the posterior pituitary and released into the blood (Stoop, 2012). OT and AVP released in this peripheral pathway exert classic functions such as uterine contraction and milk ejection for OT, vasoconstriction and antidiuretic functions for AVP. OT and AVP are also synthesized in the parvocellular neurons in the PVN, which project to various brain regions (Stoop, 2012). Furthermore, AVP neurons also exist in brain regions outside of the hypothalamus, including medial amygdala (MeA) and the bed nucleus of the stria terminalis (BNST) (Rood *et al.*, 2013). OT and AVP act in multiple brain structures that form a decision-making neural network (O'Connell & Hofmann, 2011b) to influence many complex social behaviors (Lee *et al.*, 2009; Albers, 2012). OT and AVP are released in the CNS in response to physical and emotional stress (Nishioka *et al.*, 1998; Wotjak *et al.*, 2001) and OT is also known to be released upon positive social interactions including mating and affectionate physical contact (Uvna¨s-Moberg, 1998; Ross *et al.*, 2009).

Receptors for OT and AVP

To date, only one receptor (OTR) has been identified for oxytocin and three receptors for AVP: V1aR, V1bR, and V2. These receptors are all 7-transmembreane G-protein coupled receptors, consisting of seven transmembrane domains, an extracellular N-terminal and a cytoplasmic C-terminal domains (Gimpl & Fahrenholz, 2001). This dissertation work focuses on only OTRs and V1aRs because the V2 receptor is not

expressed centrally (Barberis *et al.*, 1998) and the expression of the V1b receptor is very restricted in the brain (Dhakar *et al.*, 2013).

Although the behavioral and physiological effects of OT and AVP have been well documented, our knowledge on the intra-cellular signaling pathways after activation of OTRs and V1aRs is still limited. Both OTRs and V1aRs are coupled to a class of GTP-binding proteins that stimulate phospholipase C and subsequent hydrolysis of phosphatidylinositol, which results in the formation of inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (Gimpl & Fahrenholz, 2001; Stoop, 2012). Little is known about the intra-cellular signaling mechanisms upon activation of OTRs and V1aRs that lead to their distinct functions in complex social behavior. Such distinct effects of OTRs and V1aRs are likely to be dependent on neuron phenotypes and brain regions involved.

1.2 Roles of OT and AVP in social behavior

The effects of OT and AVP in social behavior have been the intense focus of research in the past few decades. The two peptides have been shown to be involved in the regulation of social communication, aggression, sexual behavior, pair bonding, maternal behavior, social recognition, and social reward. In addition, OT and AVP influence stress responses as well in that OT attenuates stress responses and AVP exasperates the responses. This dissertation focuses on the roles of cross-activation of OT and AVP (see next section) in social communication, social recognition, and social reward, three aspects of social behaviors that are essential for establishing and

maintaining any social relationship and where the functions of OT and/or AVP have been relatively well established.

Social communication

Perhaps the most robust effect of central AVP is its ability to induce flank marking behavior in Syrian hamsters (Ferris *et al.*, 1984), a stereotypic behavior where hamsters rub their sebaceous glands on the dorso-lateral flanks against objects in the environment (Johnston & Lee, 1976). This behavior, by dispersing olfactory signals in the environment, serves functions of social communication including claiming territories, guarding mates, and establishing dominance status (Johnston & Lee, 1976). Microinjection of AVP in the anterior hypothalamus, the lateral septum, and the periaqueductal gray, stimulates vigorous flank marking behavior (Albers *et al.*, 1986; Irvin *et al.*, 1990; Hennessey *et al.*, 1992; Albers & Cooper, 1995). The flank-marking stimulating effect of AVP is mediated by V1aRs because selective V1aR agonists and antagonists block AVP-stimulated flank marking behavior (Albers *et al.*, 1986). While this flank marking behavior is somewhat unique to Syrian hamsters, studies of this behavior can potentially provide an important window into the mechanisms underlying the effects of AVP in the regulation of social behavior, particularly social communicative behavior, as many species engage in other forms of scent marking behavior.

Social recognition

Social recognition, the ability to distinguish a familiar individual from an unfamiliar one, is a fundamental ability for producing appropriate social behavior. Social recognition has been shown in many rodent species in the laboratory including mice, rats, hamsters, and gerbils (Choleris *et al.*, 2009; Gabor *et al.*, 2012). The habituation-dishabituation and the social discrimination paradigms are two commonly used and

well-established tests in studying social recognition. Both paradigms utilize the natural tendency of animals to decrease investigation time on familiar conspecifics compared to novel conspecifics, the social discrimination paradigm, however, is a more direct way of assessing social recognition in that it allows simultaneous assessment of the animals' ability to discriminate between a familiar stimulus and a novel stimulus in the same test (Choleris *et al.*, 2009).

Both central OT and AVP have been implicated in playing essential roles in social recognition (Gabor *et al.*, 2012). For instance, OT gene knockout induces social amnesia in mice (Ferguson *et al.*, 2000). Microdialysis administration of AVP into the septum improves social recognition in Brattleboro rats, a mutant rat strain that naturally lacks AVP (Engelmann & Landgraf, 1994). A majority of these studies tested social recognition after a short period time, ranging from 10min to 2hr; few studies, however, have examined social recognition after a relatively longer period of time. This is in part due to the fact that juvenile individuals or ovariectomized females were used as stimulus animals in most studies because they provide a relatively 'neutral' social stimulus. It is important, however, to use more ethologically relevant social stimuli such as adult conspecifics and test relatively long-term social recognition as animals in nature likely can recognize conspecifics for more than just a few hours. As a result, we developed a new model for studying social recognition employing odors from adult male hamsters.

Social reward

Social interaction is essential for emotional well-being, establishment and maintenance of social structure, and reproductive success in humans and animals. It has been revealed that sexual behavior, parental behavior, and social play have rewarding properties, by utilizing a variety of well-established paradigms including

conditioned place preference (CPP), operant lever pressing, and T-maze tests (Trezza *et al.,* 2011). There is also strong evidence that interactions with same-sex peers are rewarding among juveniles as well as adult individuals (Trezza *et al.*, 2011). Adult rats show preference for an environment paired with a socially active same sex partner (Van den Berg *et al.*, 1999). Adult rats in a T-maze task spend more time interacting with a same sex conspecific than time spent alone in the opposite arm (Taylor, 1981).

A large body of evidence indicates that OT and AVP play important roles in motivated social behavior by acting in multiple 'nodes' of the social behavior neural network (SBNN) including hypothalamus, amygdala, BNST, and lateral septum (Ferris *et al.*, 1984; Huhman & Albers, 1993; Bamshad & Albers, 1996; Martinez *et al.*, 2010; Gil *et al.*, 2011; Bredewold *et al.*, 2014), and there is growing evidence also shows OT and AVP act in multiple structures of the midbrain reward circuitry to influence behavior. For instance, AVP in the ventral pallidum, a structure within the basal ganglia, is important for pair bonding in male prairie voles as V1aR antagonism in this region disrupts this behavior (Lim & Young, 2004). OT in the VTA influences maternal behavior in female rats by interacting with the midbrain dopamine system (Baskerville & Douglas, 2010). Interestingly, even though the VTA is a key component of the midbrain reward circuitry, few studies have investigated the effects of OT or AVP in the VTA on social reward.

1.3   Cross-talk between OT and AVP: evolution, release patterns, and selectivity

Evolution of OT and AVP

OT and AVP are structurally very similar nonapeptides that only differ in two of the nine amino acids (Light & Du Vigneaud, 1958; Stoop, 2012). They both have a disulfide bridge between cysteine residues one and six, resulting a six-amino acid cyclic part and a three-residue tail (Light & Du Vigneaud, 1958). OT and AVP are believed to have evolved from one ancestral precursor via gene duplication and in all mammalian species OT and AVP genes are on the same chromosome locus but are transcribed in opposite directions (Gimpl & Fahrenholz, 2001). OT and AVP belong to a superfamily of OT- or AVP-like peptides found in a wide range of species including both vertebrates and invertebrates. Most invertebrates have only one OT- or AVP-like homolog system, such as annetocin in annelid worms, conopressin in snails, inotocin in insects (Donaldson & Young, 2008). These peptides in the invertebrates serve functions related to both reproduction (Fujino *et al.*, 1999) and learning and memory (Bardou *et al.*, 2010), similar to the functions of the OT and AVP systems in mammals. Taken together, these data suggest the intertwined relationship between OT and AVP and support the possibility that they may alter behavior via cross-activating each other's receptors.

Synaptic and nonsynaptic transmissions of OT and AVP

OT and AVP can be released in a highly localized manner from synaptic terminals following the activation of voltage sensitive Ca^{2+} channels, a classic process termed as synaptic transmission (Albers, 2015). The peptides are released by this manner from hypothalamic parvocellular neuron axons that project to a variety of brain regions. In addition, OT and AVP can also be released diffusely from all parts of a neuron including soma and dendrites (i.e., non-synaptically) as the result of mobilization of intracellular stores of Ca^{2+}; a phenomenon called volume transmission (for review, see Trueta & De-Miguel, 2012). OT and AVP released from the hypothalamic magnocellular neurons via

this manner also contribute to the central effects elicited by these peptides. Volume transmission results in the diffuse release of OT or AVP that has been estimated to spread as far as 4-5 mm from its site of release (Engelmann *et al.*, 2000; Albers, 2015). Due to volume transmission, cross-talk seems highly likely because the release of OT or AVP can activate either OTRs or V1aRs or both within its relatively large diffuse range.

Selectivity of OT and AVP in binding OTRs and V1aRs

OT and AVP can activate each other's receptors due to the structural similarities between the peptides and their receptors. Species differences exist, however, in the selectivity of OT and AVP in binding their receptors (See Table 1.1). Most of the selectivity data of OT and AVP on OTRs and V1aRs are from rats, mice, and humans, and from studies on the peptide and non-peptide agonists and antagonists of OT and AVP as therapeutic agents or research tools (Manning *et al.*, 2012). Bioassays and receptor binding assays were applied in these studies. For bioassays, oxytocic and vasopressor effects of OT and AVP were determined for their affinity and selectivity in binding OTRs and V1aRs, respectively. For receptor binding assays, cells lines that express OTRs and V1aRs were applied and radiolabelled ligands were then added to the cell membrane preparations. Radio-signaling for bound ligand-receptor complex was assessed to determine affinity of the ligands in binding to the receptors.

Examples of cross-talk between OT & AVP in behavior

Although it is well known that OT and AVP are not selective in binding to OTRs and V1aRs in most species examined, the possibility of OT activating on V1aRs or AVP activating OTRs in influencing physiology and behavior had not been investigated until very recently. For instance, OT has been shown to affect non-social behavior such as analgesia by activating V1aRs (Schorscher-Petcu *et al.*, 2010). OT does not induce

analgesia in V1aR knock-out mice and OT-induced analgesia in wild-type mice can be blocked by pretreatment of V1aR but not OTR antagonists (Schorscher-Petcu et al., 2010). Another study also suggests that OT can produce behavioral effects by acting on V1aRs; OT rescues autistic-like deficits of OTR null mice in sociability, aggression, as well as cognitive flexibility by acting on V1aRs (Sala *et al.*, 2011).

1.4 Why Syrian hamsters?

Rodent species such as mice, rats, voles, and hamsters have been extensively used in studying the neurobiology underlying a variety of social behavior. We used Syrian hamsters in our studies because the behavioral models to study social behavior including social communication and social recognition are well established. In fact, one of the first studies that show the direct effects of AVP on social behavior was done in Syrian hamsters. AVP injection in the anterior hypothalamus of Syrian hamsters induces flank marking, a form of olfactory communication (Ferris et al., 1984). There are also numerous studies that have investigated social recognition using Syrian hamsters (Petrulis et al., 2004; Lai et al., 2005; Johnston & Peng, 2008). All these studies provide well-established behavioral models to examine the roles of OT and AVP and the possibility of cross-talk in the regulation of social behavior.

1.5 Goal of Dissertation

The overarching goal of this dissertation work is to examine the roles of cross-activation in the OT and AVP systems in the regulation of social behaviors. In order to address this goal, I ask the following questions: a) Do central OT and AVP induce social

communication by acting on V1aRs in Syrian hamsters? b) Do central OT and AVP

enhance social recognition by acting on either OTRs or V1aRs in Syrian hamsters? c) Do

OT and AVP in the VTA influence social reward by acting on OTRs in Syrian hamsters?

In asking these questions, I first examine the effects of OT and AVP in social

communication, social recognition, and social reward, three essential aspects of any

social relationship. I then examine the possibility of cross-talk between OT and AVP in

the measured effects on these social behaviors. I also test the functional significance of

cross-talk between OT and AVP and discuss how OT and AVP may function in a brain in

a physiological level. Taken together, these studies provide important information on

the roles of OT and AVP in the regulation of social behaviors and give great insights on

how OT and AVP in the central nervous system may cross-talk during the process.

Table 1.1 Species differences in binding affinities (Ki) of OT and AVP to OTRs and V1aRs

Affinity values (in nM) were obtained on cells expressing the oxytocin receptor (OTR), V1a, V1b and V2 receptor subtypes. Ki = (Conc. Ligand) x (Conc. Receptor) / (Conc. of Ligand Receptor Complex); so a smaller number of Ki indicates a higher affinity. Modified from (Manning *et al.*, 2012). References: (Elands *et al.*, 1988; Chini *et al.*, 1995; Mouillac *et al.*, 1995; Thibonnier *et al.*, 1997; Derick *et al.*, 2002; Terrillon *et al.*, 2002; Serradeil-Le Gal *et al.*, 2007)

	Human			Rat			Mouse		
	OTR	V1aR	V1b	OTR	V1aR	V1b	OTR	V1aR	V1b
OT	0.8	120	>1000	1.0	71	294	0.6	46.1	494
AVP	1.7	1.1	0.7	1.7	2.6	0.3	1.8	1.3	0.3

CHAPTER 2: OXYTOCIN INDUCES SOCIAL COMMUNICATION BY ACTIVATING ARGININE-VASOPRESSIN V1A RECEPTORS AND NOT OXYTOCIN RECEPTORS

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2.1   Abstract

Arginine-vasopressin (AVP) and oxytocin (OT) and their receptors are very similar in structure. As a result, at least some of the effects of these peptides may be the result of crosstalk between their canonical receptors. The present study investigated this hypothesis by determining whether the induction of flank marking, a form of social communication in Syrian hamsters, by OT is mediated by the OT receptor or the AVP V1a receptor. Intracerebroventricular (ICV) injections of OT or AVP induced flank marking in a dose-dependent manner although the effects of AVP were approximately 100 times greater than those of OT. Injections of highly selective V1a receptor agonists but not OT receptor agonists induced flank marking, and V1a receptor antagonists but not OT receptor antagonists significantly inhibited the ability of OT to induce flank

marking. Lastly, injection of alpha-melanocyte-stimulating hormone (α-MSH), a peptide that stimulates OT but not AVP release, significantly increased odor-induced flank marking, and these effects were blocked by a V1a receptor antagonist. These data demonstrate that OT induces flank marking by activating AVP V1a and not OT receptors, suggesting that the V1a receptor should be considered to be an OT receptor as well as an AVP receptor.

Keywords: flank marking, aggression, prosocial behavior, α-MSH, social behavior, social recognition

2.2 Introduction

Oxytocin (OT) and arginine-vasopressin (AVP) are evolutionarily conserved mammalian neuropeptides that play a major role in many complex social behaviors (Lee *et al.*, 2009; Albers, 2012). OT and AVP are structurally very similar, differing only in two of nine amino acids (Light & Du Vigneaud, 1958; Caldwell & Young, 2006; Stoop, 2012). Receptors for OT (OTR) and for AVP (V1aR and V1bR) in the central nervous system (CNS) belong to the G-protein coupled receptor family (Gimpl & Fahrenholz, 2001), and they are also very similar in structure with approximately 85% homology (Sala *et al.*, 2011; Neumann & Landgraf, 2012). Because of the structural similarities among these peptides and their receptors, it is not surprising that OT and AVP can reciprocally activate each other's receptors. In fact, in rats and mice OT and AVP display little selectivity for OT, V1a or V1b receptors (Manning *et al.*, 2012). Taken

together, these data suggest the hypothesis that the central effects of both OT and AVP can be mediated by OT, V1a and/or V1b receptors.

Perhaps the most robust effect of AVP on behavior is its ability to induce high levels of flank marking in Syrian hamsters following its injection into the brain (Ferris *et al.*, 1984; Bamshad & Albers, 1996). Flank marking is a form of social communication in which hamsters deposit flank gland secretions by rubbing their flank glands against objects in the environment (Johnston & Lee, 1976; Johnston & Brenner, 1982). Studies of flank marking have not only provided an important window into the mechanisms underlying the control of social behavior by AVP (Ferris *et al.*, 2013), they have also proven to be a powerful bioassay for the behavioral effects of AVP/OT agonists and antagonists in the brain (Albers *et al.*, 1986; Ferris *et al.*, 1988). In the following studies we tested the hypothesis that OT can influence flank marking by acting on V1a receptors.

2.3 Materials and Methods

2.3.1 Animals

Adult male Syrian hamsters (Harlan Laboratories Inc., Prattville, AL, and Charles River Laboratories Inc., Wilmington, MA, USA), 8-10 weeks old, weighing between 110-130g were used for all experiments. Hamsters were individually housed in polycarbonate cages (23x43x20 cm) upon arrival and were kept in a 14:10 light/dark cycle with food and water available *ad libitum*. All experimental procedures were in accordance with the National Institutes of Health Guidelines for the Use of Animals and were approved by the Georgia State University Animal Care and Use Committee.

2.3.2 *Surgery, microinjections, and histology*

Hamsters were deeply anesthetized with 5% isoflurane in an induction chamber and maintained under anesthesia with 3.75% isoflurane throughout all surgical procedures. All animals were implanted with a 4mm, 26-gauge cannula guide aimed at the lateral ventricle. The stereotaxic coordinates were +0.8mm anterior to bregma, -1.1mm from the midline, and -2.2mm below dura. Hamsters were allowed one week to recover from surgery before any behavioral testing.

Microinjections were administered over the course of 1 minute into the lateral ventricle using an infusion pump (Harvard Apparatus), a 5μl Hamilton syringe, and a 14mm, 32-gauge microinjection needle. The volume of all microinjections was 1μl. The needle was left in place in the cannula guide for an additional minute following the injection to allow drug diffusion into the ventricle. Hamsters were sacrificed by lethal injection of sodium pentobarbital after testing and were injected with ink to verify the injection sites.

2.3.3 *Drugs*

The following drugs were used in ICV injections: OT (Bachem, CA, USA) and AVP (Fisher scientific, TX, USA) both at concentrations of 0.09μM, 0.9μM, 9μM, 90μM, and 900μM; [Thr^4,Gly^7]OT (TGOT, a highly selective OT receptor agonist, a gift of Dr. Maurice Manning) at concentrations of 270μM and 2700μM; [Phe^2]OVT (a highly selective V1a receptor agonist, a gift of Dr. Maurice Manning) at concentrations of 0.23μM, 2.3μM, and 23μM; desGly-NH$_2$-d(CH$_2$)$_5$[D-Tyr^2,Thr^4]OVT (a selective OTR antagonist, OTA, a gift of Dr. Maurice Manning) and d(CH$_2$)$_5$[Tyr(Me)^2]AVP (a selective V1aR antagonist known as Manning Compound, a gift of Dr. Maurice Manning) both at

concentrations of 18μM, 45μM, or 90μM in cocktail solutions mixed with 90μM OT or at 90μM when used alone; α-MSH at 360μM (Tocris, UK). The concentrations of OT and AVP administered were based on the concentrations used in previous studies that were found effective in modulating flank marking, aggression, and sexual receptivity in hamsters (Albers *et al.*, 1986; Ferris *et al.*, 1988; Whitman & Albers, 1995). The concentrations of the OT and AVP agonists were based on their relative efficacies compared to those of OT and AVP in binding to OTR and V1aR in rats, respectively (Manning *et al.*, 2012). The concentration of α-MSH used in these studies was based on those used to stimulate OT release in rats (Sabatier *et al.*, 2003). All control animals were given a 1μl injection of saline.

2.3.4 Behavioral Testing

Hamsters were handled daily for four days before any behavioral testing. In Experiment 1, flank marking behavior was recorded in each hamster's home cage for 5 minutes immediately following ICV injection of OT or AVP (0.09μM, 0.9μM, 9μM, 90μM, and 900μM). Each hamster was injected with OT and AVP at one of the above five concentrations in a counterbalanced order at 48 hour intervals (n=5 for doses 0.09μM, 0.9μM, and 9μM; n=4 for doses 90μM and 900μM). Flank marking was scored when a hamster pressed its flank gland region against a cage wall and moved forward (Ferris *et al.*, 1984).

In Experiments 2 & 3, flank marking was recorded in each hamster's home cage for five minutes immediately after ICV injection. In Experiment 2, animals received either 90μM OT (n=5), low or high doses of selective OTR agonist (n=9 and n=5, respectively), low, intermediate, or high doses of selective V1aR agonist (n=6, n=7, and

n=7, respectively). In Experiment 3, animals were given 90μM OT (n=5), mixed solutions of 90μM OT with OTA (n=5, n=9, n=7 for OT mixed with 90μM OTA, OT mixed with 45μM OTA, and OT with 18μM OTA, respectively), 90μM OT with V1aA (n=6, n=6, n=8 for OT mixed with 90μM V1aA, OT with 45μM V1aA, and OT with 18μM V1aA, respectively), OTA alone (n=6), and V1aA alone (n=7).

Experiment 4 was designed to examine whether α-MSH, which has been shown to be a potent releaser of endogenous OT in rats (Sabatier *et al.*, 2003), would stimulate flank marking. Because ICV injections of α-MSH did not stimulate flank marking in hamsters tested in their home cages, the following experiments examined whether α-MSH would influence flank marking in hamsters tested in cages containing the flank gland scent of other hamsters (i.e., odor-stimulated flank marking). Immediately following ICV injections, hamsters were placed in a testing arena (23x43x20 cm) that contained flank odors from another male hamster, and flank marks were recorded for 10 minutes (n=9 for both α-MSH and saline). The flank odors were freshly deposited in the arena by a donor that had been injected ICV with AVP and allowed to mark the arena 50 times (Gutzler *et al.*, 2011). In Experiment 4B, the V1aR (90μM) antagonist was injected ICV 1 hour before an injection of α-MSH or saline. Within subject design was used in the experiment and the order of α-MSH or saline injections was counter-balanced (n=5).

All the experiments took place during the first three hours of the dark phase of the daily light/dark cycle. All behavioral testing was videotaped and scored by an individual blind to the experimental conditions.

2.3.5 Data Analysis and Statistics

SPSS v21 was used to analyze all the data. The data are presented as mean ± standard error of the mean. Independent samples or paired t-tests were used for two-group comparisons and Analysis of Variance (ANOVA) was performed for comparisons among more than two groups. All comparisons were determined *a priori*; planned contrasts were performed following significant differences found in ANOVA tests. All tests were two tailed and differences were considered significant at $p \leq 0.05$.

2.4 Results

2.4.1 Dose-dependent effects of OT and AVP on Flank Marking

In order to examine the efficacies of OT and AVP in inducing flank marking, we microinjected OT and AVP ICV in a wide range of concentrations (0.09μM, 0.9μM, 9μM, 90μM, and 900μM). There was a significant drug by dose interaction $(F(1,4)=7.848, p<0.05)$. Therefore, analyses were conducted to examine the effects of dose on the OT and AVP groups separately. There was a significant main effect of dose on the number of flank marks recorded following microinjections of OT and AVP (OT groups: $F(4,18)=30.210, p<0.0001$; AVP groups: $F(4,18)=8.109, p<0.001$; Fig.1). Hamsters injected with 0.09μM AVP displayed significantly fewer flank marks than those injected with concentrations of 0.9μM, 9μM, 90μM, or 900μM AVP ($p<0.005$ for all comparisons). Hamsters injected with 0.09μM, 0.9μM, or 9μM OT, flank marked less than those injected with either 90μM or 900μM OT ($p<0.0001$ for all comparisons). Concentrations of 0.9μM and 9μM OT induced fewer flank marks compared to the same

concentrations of AVP when injected into the same animals (paired t-tests: $t(4)=4.691$, $p<0.01$; $t(4)=10.977$, $p<0.001$; respectively).

2.4.2 Effects of OTR and V1aR Agonists on Flank Marking

This experiment employed highly selective OTR and V1aR agonists to investigate whether flank marking was induced by activation of OT or V1a receptors. There was a significant main effect of drug treatment on flank marking ($F(5,33)=15.010$, $p<0.0001$, Fig.2). Hamsters injected with low or high concentrations of the selective OTR agonist displayed significantly fewer flank marks than those injected with $90\mu M$ OT ($p<0.005$ and $p<0.01$, respectively). In fact, 10 of the 14 animals injected with either low or high concentrations of the OTR agonist marked less than three times during the five minute test. The numbers of flank marks induced by the low and intermediate concentrations of the selective V1aR agonist did not significantly differ from that induced by OT ($p=0.616$ and $p=0.07$ for low and intermediate, respectively). Hamsters injected with the high concentration of V1aR agonist flank marked significantly more than did those animals injected with OT ($p<0.005$).

2.4.3 Effects of OTR and V1aR Antagonists on OT-induced Flank Marking

Experiment 3 examined whether selective OTR and V1aR antagonists (OTA and V1aA, respectively) block OT-induced flank marking. There was a significant main effect of drug treatment on the number of flank marks produced ($F(3, 22)=49.0$, $p< 0.0001$; Fig.3). Hamsters injected with OT and any of the three concentrations of V1aA flank marked significantly less than those injected with OT alone ($P<0.0001$ for all comparisons). In fact, all three concentrations of the V1aR antagonist almost completely blocked OT-induced flank marking. In contrast, the same concentrations of

the OTA did not significantly alter the amount of OT-induced flank marking (Fig.3).

There were no significant differences in the number of flank marks among hamsters

injected with OT and any of the three concentrations of OTA and those injected with OT

alone ($F_{(3, 22)}=0.674$, $p=0.577$). Hamsters in drug control groups injected with only

V1aA (90μM) or OTA (90μM) did not differ from saline control animals in flank

marking (flank marks: 90μM OTA: 0.0±0.0, n=7; 90μM V1aA: 0.9±1.3, n=6; saline:

0.1±0.2, n=7; $F_{(2,17)}=0.760$, $p=0.483$).

2.4.4 *Effects of Alpha-MSH on Odor-stimulated Flank Marking*

This experiment examined whether α-MSH, a peptide that stimulates

endogenous OT release in rats, would enhance odor-induced flank marking. Hamsters

injected with α-MSH displayed significantly more odor-induced flank marks than did

hamsters injected with saline (independent samples t-test: $t_{(16)}=2.338$, $p<0.05$;

Fig.4A). This enhancement in flank marking by α-MSH was blocked by an injection of

V1aR antagonist prior to testing. After administration of the V1aA, no differences in the

number of flank marks were observed between groups of hamsters injected with α-MSH

or saline (paired t-test: $t_{(4)}=0.611$, $p=0.574$; Fig.4B).

2.5 Discussion

The results of the present study support the hypothesis that OT can induce flank

marking by activating V1aRs and not OTRs. The data reveal that selective V1aR but not

OTR agonists mimic the effects of OT in inducing flank marking, and that selective V1aR

but not OTR antagonists block OT-induced flank marking. In addition, we

demonstrated that α-MSH, a peptide that stimulates endogenous release of OT (Sabatier

et al., 2003), enhances odor stimulated flank marking and that this enhancement by α-MSH can be blocked by applying a V1aR antagonist prior to testing. Together, these findings suggest that the endogenous release of OT might contribute to the regulation of flank marking as well as other social behavior by acting on V1aRs and that the V1aR should be considered to be an OTR as well as an AVP receptor.

Comparison of OT and AVP induced flank marking reveals that OT stimulates flank marking at concentrations between 9 and 90 μM, while AVP induces flank marking at concentrations between 0.09 and 0.9 μM, suggesting that OT is approximately 100-fold less potent in activating V1aRs than is AVP. Because the levels of OT and AVP released within the hamster brain are not known, it is not clear whether OT released endogenously might contribute to the regulation of the expression of flank marking. In an attempt to investigate whether endogenously released OT might contribute to the induction of flank marking normally stimulated by exposure to flank gland secretions of conspecifics (i.e., odor-stimulated flank marking), we injected α-MSH, which has been shown in rats to induce endogenous OT, but not AVP release, from hypothalamic neurons (Sabatier *et al.*, 2003). Although α-MSH was not sufficient to induce flank marking in the absence of conspecific odors, α-MSH did significantly enhance odor-stimulated flank marking, and this enhancement was blocked by a V1aR antagonist. The inability of α-MSH to induce flank marking in the absence of conspecific odors might be the result of an insufficient amount of endogenously released OT or the release of OT in a brain site outside the optimal region for induction of flank marking. It is also possible that α-MSH induced "priming" in the OT neurons so that more OT was released when the hamsters were subsequently exposed to conspecific

odors (Stoop, 2012). Even though α-MSH, by itself, did not induce flank marking, it did significantly enhance its expression when it was stimulated by relevant environmental cues.

In the present study, the effects of OTR/V1aR agonists and antagonists were investigated following their administration by ICV injection. Previous studies of the effects of AVP on flank marking examined its role in specific brain regions including the medial preoptic–anterior hypothalamus (MPOA-AH), periaqueductal gray, and lateral septum (Albers *et al.*, 1986; Irvin *et al.*, 1990; Hennessey *et al.*, 1992; Albers & Cooper, 1995). The effects of AVP injections within these regions on flank marking are similar to the effects of ICV administration. In both cases, AVP-stimulated flank marking has an ED50 (the dose that elicits a half-maximal behavioral response) of 0.9μM, and the effect lasts for 5-10 minutes (Albers *et al.*, 1986; Ferris *et al.*, 1988; Caldwell & Albers, 2003; Ferris *et al.*, 2013). Although several studies have examined the ability of OT to induce flank marking following its injection into the MPOA-AH, complete dose-response relationships are not available in most cases (Albers *et al.*, 1986; Harmon *et al.*, 2002). In general, the effects of different concentrations of OT into the MPOA-AH in inducing flank marking are consistent with the effects of OT given ICV in the present study (cf. Ferris *et al.,* 1984).

Recent evidence from other studies also supports the hypothesis that OT can act on V1aRs. Central administration of OT appears to produce analgesic effects (Ge *et al.*, 2002; Gao & Yu, 2004) by activating V1aRs (Schorscher-Petcu *et al.*, 2010). OT does not induce analgesia in V1aR knock-out mice and OT-induced analgesia in wild-type mice can be blocked by pretreatment of V1aR but not OTR antagonists (Schorscher-Petcu *et al.*, 2010). Another study also suggests that OT can produce behavioral effects

by acting on V1aRs; OT rescues autistic-like deficits of OTR null mice in sociability, aggression, as well as cognitive flexibility by acting on V1aRs (Sala *et al.*, 2011).

In summary, the present study demonstrated that OT induces flank marking by activating V1aRs. Our results raise the important possibility that endogenously released OT might influence other social behaviors by acting on V1aRs. These studies emphasize the importance of pharmacological profiling when studying the behavioral responses to OT and AVP.

2.6 Acknowledgements

2.7   Chapter 2 Figures
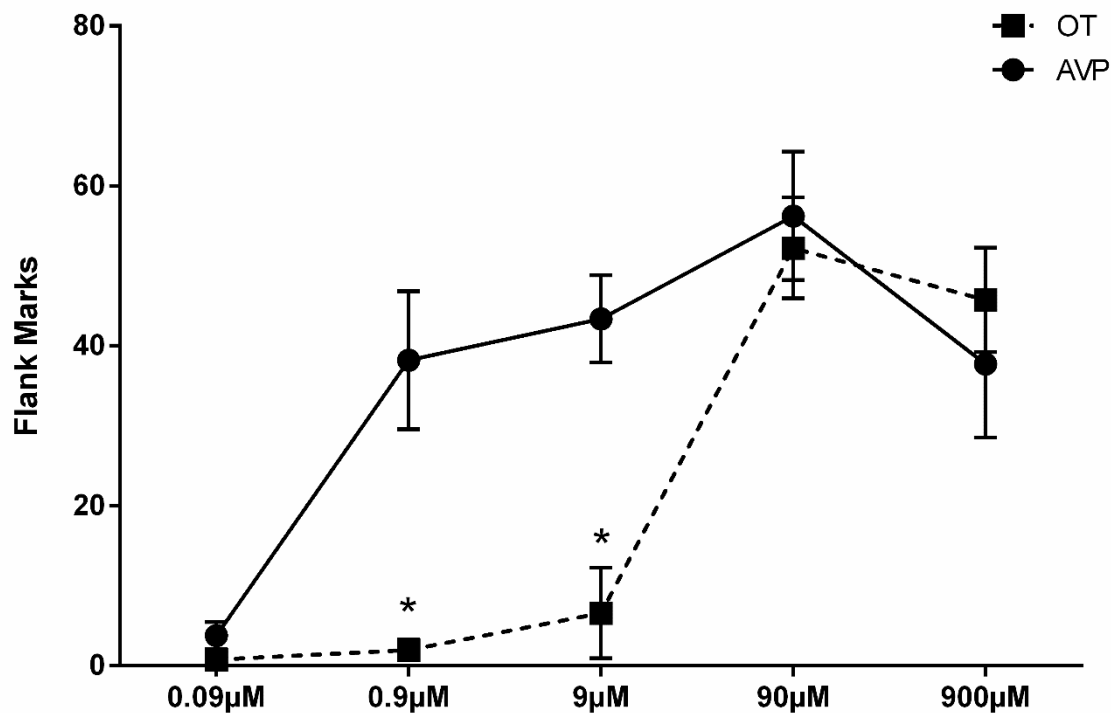
Figure 2.1 Effects of OTR and V1aR agonists on flank marking.

There was a significant main effect of dose on the number of flank marks that OT or

AVP induced.   For the doses of 0.9μM and 9μM, OT induced fewer flank marks

compared to AVP when injected into the same animals. Note: * indicates a significant

difference between OT and AVP.
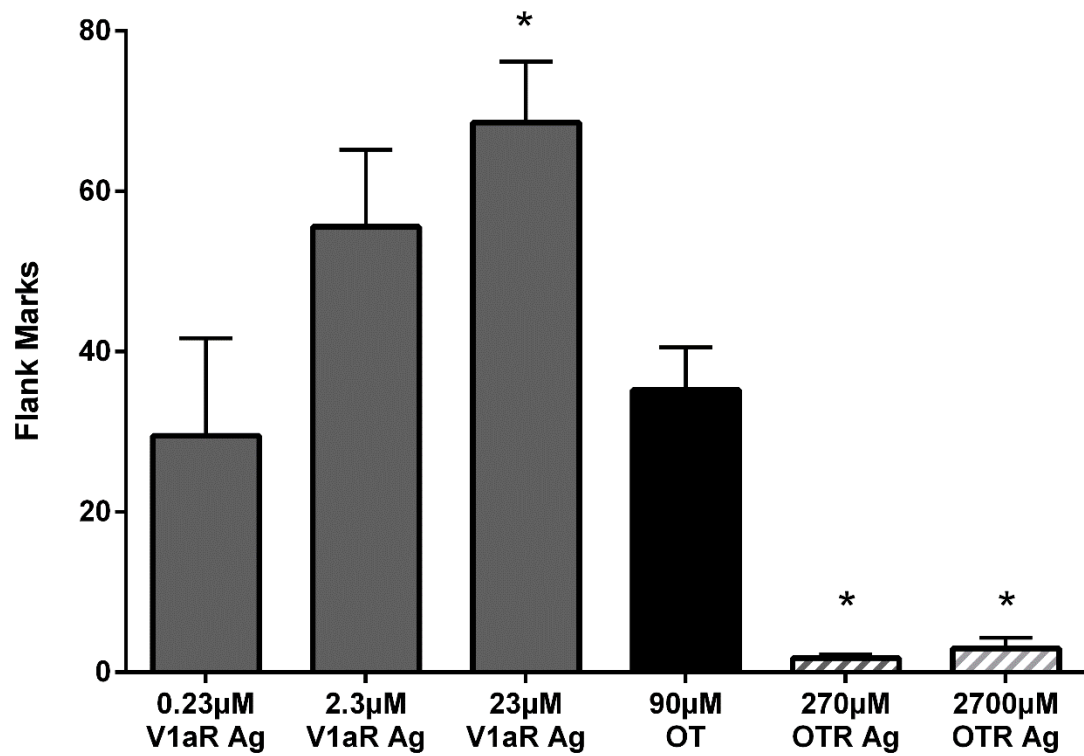
Figure 2.2 Effects of OTR and V1aR agonists on flank marking.

There was a significant main effect of drug treatment among the groups. V1aR Agonist

(Ag); OTR Agonist (Ag). Note: * indicates a significant difference compared to OT.
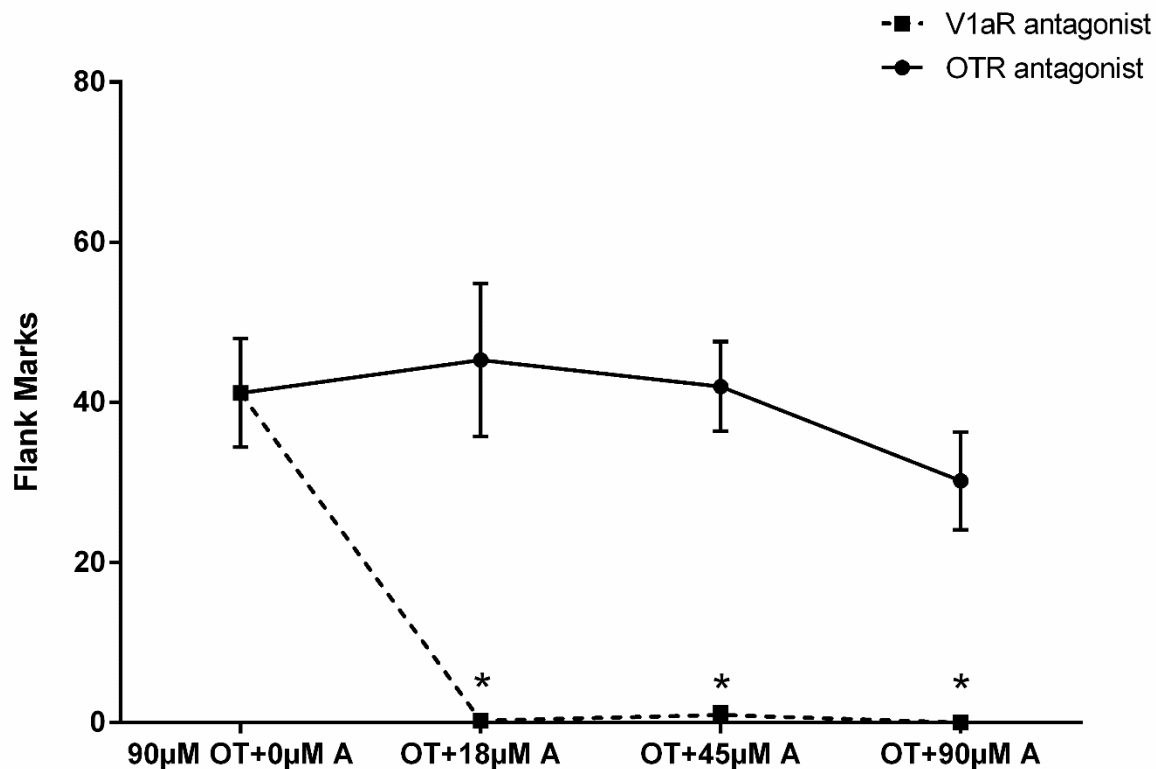
Figure 2.3 Effects of OTR and V1aR antagonists on OT-induced flank marking.

All three concentrations of the V1aR antagonist completely blocked OT induced flank marking. The same concentrations of the OTR antagonist did not significantly affect flank marking. Note: * indicates a significant difference compared to the OT group. A: antagonist.
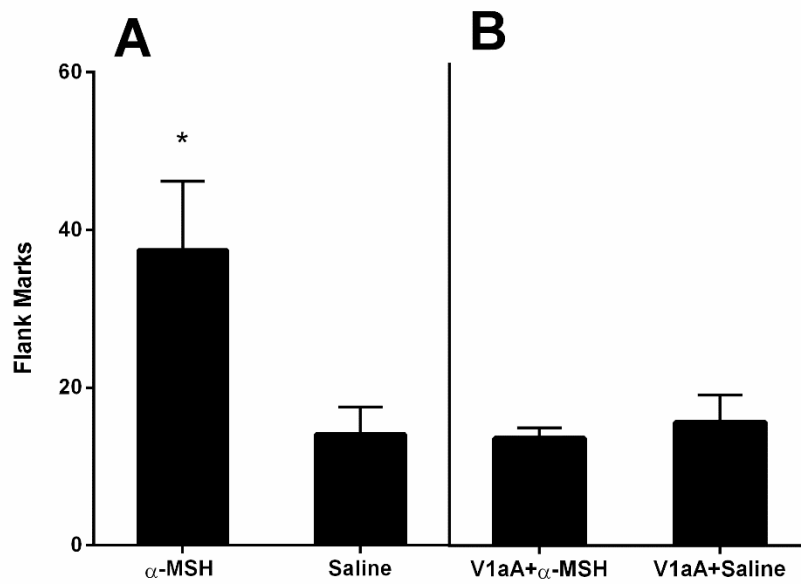
Figure 2.4 Effects of alpha-MSH on odor-stimulated flank marking and involvement of V1aR.

(A) Hamsters injected with α-MSH flank marked more than those injected with saline. (B) V1aA blocked this α-MSH-induced enhancement in flank marking. Note: * indicates a significant difference compared to the saline group.

CHAPTER 3: OXYTOCIN (OT) AND ARGININE-VASOPRESSIN (AVP) ACT ON OT RECEPTORS AND NOT AVP V1A RECEPTORS TO ENHANCE SOCIAL RECOGNITION IN ADULT SYRIAN HAMSTERS (MESOCRICETUS AURATUS)

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3.1 Abstract

Social recognition is a fundamental requirement for all forms of social relationships. A majority of studies investigating the neural mechanisms underlying social recognition in rodents have investigated relatively neutral social stimuli such as juveniles or ovariectomized females over short time intervals (e.g., 2 hrs). The present study developed a new testing model to study social recognition among adult males using a potent social stimulus. Flank gland odors are used extensively in social communication in Syrian hamsters and convey important information such as dominance status. We found that the recognition of flank gland odors after a brief exposure lasted for at least 24 hrs, substantially longer than the recognition of other social cues in rats and mice. Intracerebroventricular injections of OT and AVP prolonged the recognition of flank gland odor for up to 48 hrs. Selective OTR but not

V1aR agonists, mimicked these enhancing effects of OT and AVP. Similarly, selective OTR but not V1aR antagonists blocked recognition of the odors. In contrast, the recognition of non-social stimuli was not blocked by either the OTR or the V1aR antagonists. Our findings suggest both OT and AVP enhance social recognition via acting on OTRs and not V1aRs and that the recognition enhancing effects of OT and AVP are limited to social stimuli.

Keywords: social behavior; nonapeptides; neuropeptides; neurohypophyseal hormones; social communication; flank marking; chemosensory, olfaction;; odor recognition; memory

3.2 Introduction

The ability to recognize one individual from another of the same species is a fundamental requirement for nearly all forms of social relationships including pair bonding, maternal behavior and dominance (Petrulis, 2009; Gabor *et al.*, 2012). Social recognition is expressed in many different levels of complexity from the relatively simple ability to discriminate between familiar and unfamiliar social cues to the true recognition of specific individuals as well as recognition of kin (Johnston & Bullock, 2001; Choleris *et al.*, 2009). There is a wealth of data demonstrating that species such as mice, rats, hamsters, and gerbils can display basic levels of social discrimination in the laboratory (Halpin, 1976; Johnston, 1993; Choleris *et al.*, 2009; Gabor *et al.*, 2012).

To date, a majority of social recognition studies have focused on the ability of an adult rodent to recognize a juvenile or ovariectomized female of the same species (Le Moal *et al.*, 1987; Ferguson *et al.*, 2000; Ferguson *et al.*, 2001; Winslow & Insel, 2004; Veenema *et al.*, 2012). This approach was taken to investigate the social recognition of a relatively "neutral" social stimulus. Rats and mice fail to recognize a juvenile or an ovariectomized female within 2~3 hrs after an initial encounter in most behavioral tests (Ferguson *et al.*, 2001; Winslow & Insel, 2004; Veenema *et al.*, 2012), perhaps because of the neutral nature of the social stimulus. In the present study we investigated the duration of social recognition in adult male Syrian hamsters (*Mesocricetus auratus*) using odors obtained from flank glands as the social cue. Flank gland odor is a powerful social cue that serves to communicate a variety of different types of social information in hamsters including dominance status (Johnston & Lee, 1976; Ferris *et al.*, 1984; Ferris *et al.*, 1987). As such, investigating the recognition of flank gland odors in adult males provides a social recognition test with a high degree of relevance and validity for understanding of social memory.

The nonapeptides oxytocin (OT) and arginine vasopressin (AVP) are both involved in the regulation of social recognition in many species including mice and rats (Albers, 2012; Gabor *et al.*, 2012). For instance, mice lacking the OT gene have severe impairments in their ability to recognize other individuals (Ferguson *et al.*, 2000; Winslow & Insel, 2004) and centrally or peripherally injected AVP facilitates social memory in rats (Le Moal *et al.*, 1987; Veenema *et al.*, 2012). OT and AVP have a high degree of similarity in their structure and in the structure of their canonical receptors (Gimpl & Fahrenholz, 2001; Maybauer *et al.*, 2008; Manning *et al.*, 2012; Song *et al.*, 2014). Recently it has been demonstrated that both OT and AVP can produce

functionally significant responses by activating AVP V1a receptors (V1aRs) (Schorscher-Petcu *et al.,* 2010; Sala *et al.,* 2011; Ramos *et al.,* 2013; Qiu *et al.,* 2014; Albers, 2015). For example, both OT and AVP can induce communicative behavior in hamsters when injected into the lateral ventricle and do so by activating V1a receptors and not OT receptors (OTRs) (Song *et al.,* 2014).

In the present study, we demonstrate that adult male hamsters can recognize social odors from other adult male hamsters for at least 24 hrs and that centrally administered OT and AVP significantly enhance the duration of social recognition in this species. Further, we demonstrate for the first time that OT and AVP can produce behavioral effects by acting on OTRs and not V1aRs using highly selective OT and AVP1a receptor agonists and antagonists.

3.3 Materials and Methods

3.3.1 Animals

Adult male Syrian hamsters (Charles River Laboratories Inc., Wilmington, MA, USA), 2-5 months old, weighing between 110-150g were used in all experiments. All experimental hamsters were individually housed in polycarbonate cages (23x43x20 cm) for 14 days prior to experimental use. Scent donor hamsters had been housed in groups (4 per cage) when used as non-aggressive intruders in a previous experiment. These hamsters were then housed singly for at least 7 days prior to scent donation for the current experiment. Hamsters were kept on a 14:10 light/dark cycle with food and water *ad libitum*. All experimental procedures were in accordance with the National

Institutes of Health Guidelines for the Use of Animals and were approved by the Georgia State University Animal Care and Use Committee.

3.3.2 *Surgery, microinjections, and histology*

Hamsters were deeply anesthetized via 5% isoflurane in an induction chamber and maintained with 3.75% isoflurane throughout all surgical procedures. Each subject was implanted with a 4mm, 26-gauge cannula guide aimed at the left lateral ventricle. The skull was leveled and the guide cannula was implanted using the stereotaxic coordinates: +0.8mm anterior to bregma, -1.1mm from the midline, and -2.2mm below dura. Hamsters were allowed one week to recover from surgery before behavioral testing.

Introcerebroventricular (ICV) injections were administered over the course of 1min into the lateral ventricle using an infusion pump (Harvard Apparatus), a 5μl Hamilton syringe, and a 14mm, 32-gauge needle. The volume of all microinjections was 1μl. Post-injection, the needle was left in the cannula guide for an additional minute to allow drug diffusion into the ventricle. Hamsters were sacrificed by lethal injection of sodium pentobarbital after testing and were injected with ink to verify the injection sites.

3.3.3 *Drugs*

The following drugs were injected: 9μM OT (Bachem, CA, USA) and 9μM AVP (Fisher scientific, TX, USA); 27μM [Thr4,Gly7]OT (TGOT, a highly selective OT receptor agonist, a gift of Dr. Maurice Manning); 0.23μM [Phe2]OVT (a highly selective V1a receptor agonist, a gift of Dr. Maurice Manning); 90μM desGly-NH2-d(CH2)5[D-Tyr2,Thr4]OVT (a selective OTR antagonist, OTA, a gift of Dr. Maurice Manning) and

90μM d(CH2)5[Tyr(Me)2]AVP (a selective V1aR antagonist known as Manning

Compound, a gift of Dr. Maurice Manning). The concentration of OT and AVP

administered was based on the concentrations used in previous studies that were found

effective in altering other social behavior in hamsters and other studies on social

recognition in rats (Dantzer *et al.*, 1988; Engelmann & Landgraf, 1994; Song *et al.*,

2014). The concentrations of the OT and AVP agonists were based on their relative

efficacies compared to those of OT and AVP in binding to OTR and V1aR in rats,

respectively (Manning *et al.*, 2012). The concentration of both OTR and V1aR

antagonists were based on the concentration used in previous studies that was found to

block social behavior in hamsters and rats (Ferris *et al.*, 1988; Nephew & Bridges,

2008). All control animals were given a 1μl injection of saline.

3.3.4 Behavioral Testing

3.3.4.1 Recognition of social odors

The goal of Experiment 1 was to determine how long hamsters can recognize a

conspecific odor. Hamsters were placed in a cage (23x43x20 cm) with a glass

microscope slide (25mm x 75mm x 1mm) taped to a cage wall approximately 2cm from

the floor for 3min (Trial 1); the slide was scented with a fresh flank gland odor, collected

from another adult male conspecific. Odors were deposited on the slides by gently

holding hamsters and rubbing the slides against the flank gland regions 20 times. After

this initial exposure to the odor stimulus hamsters were given a second odor exposure

20 minutes (min) (n = 9), 24 hours (hr) (n = 9), 48 hr (n = 7), or 7 days (n = 7) later.

This odor test consisted of placing the hamster in a cage with one slide taped to one cage

wall and another slide taped to the opposite cage wall for 3min (Trial 2). One slide was scented with fresh flank gland odor of the same conspecific from Trial 1 (familiar odor) and the other slide with fresh flank gland odor of a novel male conspecific (novel odor). The familiar and novel stimulus hamsters were from two groups of animals that were different in age by 1-3 months; half of each of the two groups formed familiar hamsters and the other half formed novel hamsters. Each of the experimental hamsters had one stimulus hamster from each group as familiar and novel hamsters. Thus, the familiar and novel odors were not from the same litters for each subject and the intrinsic properties of each group that might affect investigation time were counterbalanced. Testing took place in a room with red dim lights and within the first 3 hr of the dark phase of the light-dark cycle. Time spent sniffing the odor was recorded and scored later by a trained experimenter blind to experimental conditions.

The data collected in Experiment 1 showed that hamsters can recognize a previously encountered flank gland odor from a novel flank gland odor for 20 min and 24 hr but not for 48 hr or 7 days after the initial encounter. The goal of Experiments 2&3 was to test whether OT, AVP and selective OTR and V1aR agonists were able to increase the duration of social recognition from 24 hr to 48 hr. The protocol for Experiments 2&3 was the same as in Experiment 1 except hamsters were injected with either OT, AVP, a selective OTR agonist, a selective V1aR agonist, or saline into the ventricle immediately following Trial 1. Briefly, hamsters were placed in a cage to allow for investigation of a conspecific odor for 3h (Trial 1). In Experiment 2, hamsters were injected with OT (n = 8), AVP (n = 7), or saline (n = 6) immediately following the initial odor exposure. In Experiment 3, hamsters were injected with the OTR agonist (n = 9), the V1aR agonist (n = 9), or saline (n = 9) immediately following the initial odor

exposure. Forty-eight hours later, they were again placed in a cage to allow for investigation of an odor from the previously encountered conspecific (familiar odor) and an odor from a novel conspecific (novel odor) for 3min (Trial 2).

Experiment 4 determined whether OTR and V1aR antagonists injected into the ventricle could block social recognition 20min after an initial odor exposure. The protocol for Experiment 4 was the same as in Experiments 2&3 except the time between the first odor exposure and the odor test was 20 min. The OTR (n = 11), V1aR (n = 8) antagonists or saline (n = 10) were injected before the initial odor exposure in order to allow time for the antagonists to act before the first odor exposure.

3.3.4.2 Recognition for non-social stimulus

The goal of Experiments 5&6 was to examine whether the effects of OT and AVP were specific to social odors or whether these neuropeptides influenced the recognition of non-social odors as well. Lemon extract and a cocktail of lemon extract and vanilla extract were used as non-social odors in these experiments. The lemon and vanilla extracts were purchased at a grocery store and the cocktail was made of 90% of lemon and 10% of vanilla. Hamsters' initial preference for the lemon scent was determined after the lemon and the cocktail of lemon and vanilla were simultaneously presented to hamsters in a pilot experiment. In the experiments thereafter, the lemon scent was presented to hamsters for 3 min as the first odor. In the second odor exposure, both the lemon and the cocktail were simultaneously presented to hamsters for 3min in Trial. The odors were presented to hamsters in a similar manner as in Experiments 1-4. Each odor (10ul) was pipetted onto the center of a microscope slide and then the slide was taped to a wall of the cage, approximately 2cm above the floor. The slides of lemon and

the cocktail were placed on opposite sides of the cage and their locations were counterbalanced. Experiment 5 examined how long hamsters could recognize a non-social odor after an initial exposure. The odor test occurred 20 min (n = 6), 60 min (n = 6), and 24 hr (n = 6) after the initial odor exposure. Because this initial experiment found that the lemon scent could be recognized for 20 min and 60 min, but not 24 hr, the ability of the OTR and V1aR antagonists to block recognition was tested after 20 min. In Experiment 6, hamsters were randomly divided into 3 groups and were injected with 90uM OTR antagonist (n =12), 90uM V1aR antagonist (n=6), or saline (n = 9) immediately before the initial odor exposure. The odor test took place 20 min after the initial odor exposure.

3.3.5 *Data analyses and statistics*

SPSS V21 was used to analyze all the data. All data are presented as mean ± standard error of the mean. In all experiments, percentage of time spent sniffing a specific odor over the total sniffing time was presented in order to reduce the variation among the hamsters in their overall sniffing levels. Paired-sample Student's *t*-test was used to detect differences in the percentages of time spent with a previously exposed odor/scent versus the percentage of time spent with a novel odor/scent. One-way ANOVA test was used to detect differences in the percentage of time spent with the novel odor versus the initial odor among animals injected with saline and drugs of interest (Difference Percentage = (Time spent with the Novel – Time spent with the Familiar) / (Time spent with the Novel + Time spent with the Familiar)). All *post hoc* comparisons were determined *a priori*; planned contrasts were performed following

significant differences found in ANOVA tests. All tests were two tailed and differences were considered significant at $p \leq 0.05$.

3.4 Results

3.4.1 Recognition of social odors

Experiment 1: How long do hamsters recognize a previously encountered social odor?

Hamsters were first exposed to a flank gland odor for 3 min and then tested 20 min, 24 hr, 48 hr, or 7 days later, in a social discrimination test with the same flank gland odor and a novel flank gland odor. Hamster that were tested 20 min and 24 hr after the initial odor exposure spent more time sniffing the novel odors compared to the familiar odors (20 min, $t(8) = 5.070$, $p < 0.05$; 24 hr, $t(8) = 2.561$, $p < 0.05$, Fig. 1), whereas those tested 48 hr or 7 days later spent similar amounts of time sniffing both odors (48 hr, $t(7) = 1.214$, $p = 0.270$; 7 days, $t(6) = -0.293$, $p = 0.780$, Fig. 1). These data indicate hamsters can recognize a conspecific odor 20 min and 24 hr, but not 48 hr or 7 days after an initial 3 min exposure.

Experiment 2: Do OT and AVP increase the duration of recognition of social odors?

Hamsters were first exposed to flank gland odor for 3 min and then given ICV injections of OT, AVP, or saline. Forty-eight hr later hamsters were then exposed to flank gland odor from the same individual used in the initial odor exposure and a flank gland odor from a novel hamster. Hamsters injected with saline spent similar amounts

of time sniffing the novel odor and the odor presented initially (t(5) = 0.278, p = 0.792); this is consistent with the outcomes from Experiment 1 (Fig. 2A). In contrast, hamsters that were injected with OT or AVP spent more time investigating the novel odor compared to the odor presented initially (OT: t(7) = 4.202, p<0.05; AVP: t(6) = 2.643, p<0.05, Fig. 2A). Thus, OT or AVP administration after exposure to the initial odor increased the time hamsters were able to discriminate between familiar and novel odors from 24 to 48 hr. ANOVA indicated that there was a significant difference in the Difference Percentages among the three groups (F(2,18) = 4.152, p < 0.05, Fig. 2B). Planned *post hoc* contrasts indicated that the Difference Percentages in OT and AVP injected hamsters were higher than that in saline injected hamsters (p < 0.05) and there was no difference of the Difference Percentages between the OT and AVP injected hamsters (p = 0.524).

Experiment 3: Do selective OTR and V1aR agonists increase the duration of recognition of social odors?

The experimental protocol in the following experiment was the same as that in Experiment 2 except the drugs injected were selective OTR, V1aR agonists or saline (control). Because there was no difference between saline injected hamsters in Experiment 2 and this experiment, data from the two groups were pooled together. Again, time spent sniffing the novel odor and the odor presented initially was not different in these hamsters injected with saline (t(8) = -0.386, p = 7.10, Fig. 3A). In hamsters injected with the selective V1aR agonist the time spent sniffing the novel odor and the odor presented initially did not was not significantly different (t(8) = -0.136, p = 0.895, Fig. 3A). In contrast, hamsters injected with the OTR agonist, spent more time

sniffing the novel odor than the odor presented initially (t(8) = 3.452, p < 0.05, Fig. 3A).

There was a difference in the Difference Percentages among the three groups (F(2,24) =

3.921, p < 0.05, Fig. 3B). *Post hoc* tests indicated the hamsters injected with the OTR

agonist had a significantly higher Difference Percentage than the hamsters injected with

V1aR agonist (p < 0.05) or the hamsters injected with (p < 0.05). No significant

differences were observed in Difference Percentages between the groups injected with

the V1aR agonist and the group injected with saline (p = 0.760).

Experiment 4: Do OTR and V1aR antagonists reduce the duration of recognition

of social odors?

Hamsters were initially exposed to a flank gland odor immediately following ICV

injections of OTA, V1aA, or saline. Twenty min later hamsters were then exposed to

flank gland odor from the same individual used in the initial odor exposure and a flank

gland odor from a novel hamster. Hamsters injected with saline spent significantly more

time sniffing the novel odor than the familiar odor (t(9) = 3.547, p < 0.01, Fig. 4A),

consistent with data obtained in Experiment 1. Hamsters injected with the selective

V1aR antagonist also spent significantly the time spent sniffing the novel odor than the

odor presented initially did not was not significantly (t(7) = 3.189, p < 0.05, Fig. 4A). In

contrast, hamsters injected with the OT antagonist spent similar amounts of time

investigating the odor presented initially and the novel odor (t(9) = 0.485, p = 0.638,

Fig. 4A). There was a significant difference in the Difference Percentages among the

three groups (F (2,23) = 4.754, p < 0.05, Fig. 4B). *Post hoc* tests revealed that the

Difference Percentages in saline and V1a antagonist injected hamsters were higher than

that in OT antagonist injected hamsters (p < 0.05 for both saline and V1aA). No

significant differences were observed in Difference Percentages between the groups injected with the V1aR antagonist and the group injected with saline (p = 0.760).

3.4.2 Recognition of non-social odors

Experiment 5: How long do hamsters recognize a previously encountered non-social scent?

Hamsters were first exposed to a lemon odor for 3 min and then were tested 20 min, 60 min or 24 hr later in an odor discrimination test with the lemon odor and an odor composed of a cocktail of lemon and vanilla together. As a control for an initial preference for the scents, another group of hamsters was not pre-exposed to the lemon odor but was directly exposed to the lemon odor and the cocktail odor. Control hamsters preferred the lemon odor over the cocktail (initial preference). To determine the retention of the recognition of the lemon odor during the pre-exposure, the percentage of time on the cocktail odor versus on the lemon odor was compared between hamsters that were pre-exposed to the lemon and hamsters that were not pre-exposed. There was a significant difference in the percentages of time spent sniffing the cocktail odor versus the lemon among the 4 hamster groups ($F(3,20) = 3.879$, $p < 0.05$, Fig. 5). *Post hoc* tests showed hamsters that were first exposed to lemon and then tested for the two scents with ITIs of 20min and 60min had greater Difference Percentages than that of hamsters that were not pre-exposed to lemon (20min, $p < 0.05$; 60min, $p < 0.01$). Difference Percentages did not differ between hamsters that were tested with an ITI of 24h and hamsters that were not pre-exposed (p = 0.327). These data indicate

hamsters can recognize non-social scents 20min and 60min, but not 24h after an initial 3min encounter.

Experiment 6: Do OTR and V1aR antagonists reduce the duration of recognition of non-social odors?

Hamsters were initially exposed to lemon odor immediately following ICV injections of OTA, V1aA, or saline. Twenty minutes later hamsters were then exposed to lemon odor and the cocktail odor. Another group of hamsters was not initially exposed to lemon odor but was directly exposed to the lemon odor and the cocktail odor as an initial preference control (there was no difference in the duration of sniffing between these hamsters and those used for the same purpose in Experiment 5, so they were pooled together for data analysis). Again, to detect the recognition of the lemon odor during the pre-exposure, the percentage of time on the cocktail odor versus on the lemon odor was compared between hamsters that were pre-exposed to the lemon and those that were not pre-exposed. There was a significant difference in the percentages of time spend sniffing the novel cocktail odor compared to the lemon odor among the 4 hamster groups ($F(3,34) = 14.930$, $p < 0.001$, Fig. 6). All the hamsters that were initially exposed to lemon, regardless of drug administration, had greater Difference Percentages than hamsters that were not initially exposed to the lemon odor ($p < 0.001$ for all comparisons). Difference Percentages did not differ among the hamsters that were injected with OTA, V1aA, or saline ($F(2,24) = 0.767$, $p = 0.475$).

3.5   Discussion

In the present study adult male hamsters were found to recognize the social odors of other adult males for 20 min and 24 hr, but not 48 hr or 7 days. The duration of this social recognition is substantially longer than that seen in many previous tests of social recognition that employed more neutral social stimuli, e.g., juvenile rats. An advantage of using a social odor as opposed to another animal as the social stimulus is that it eliminates the possibility that the properties of the stimulus will change as the result of social interactions between the stimulus animal and the test animal. As such, this social recognition test provides a simple and robust approach with a great deal of relevance and validity that can be used to investigate neurobiological mechanisms of social recognition.

The injection of either AVP or OT in the lateral ventricle prolonged recognition of flank gland odors to at least 48 hr. The OTR agonist but not the V1aR agonist mimicked these recognition enhancing effects of OT and AVP. Similarly, the OTR but not the V1aR antagonist blocked recognition of the odor. These findings indicate that both OT and AVP can significantly increase the duration of social recognition and do so by acting on OTRs and not V1aRs. Importantly, our studies also demonstrated that the effects of AVP and OT do not extend to the recognition of non-social odors. Hamsters recognized lemon scent for up to 60 min but not 24 hr. Unlike in the social recognition experiments, neither the OTR nor V1aR antagonists reduced the duration of the recognition of lemon scent. These data support the hypothesis that OT and AVP enhance the recognition of social but not non-social stimuli.

The duration of recognition of the social stimulus in the present study (at least 24 hrs) is considerably longer than the duration of recognition seen in most previous

studies in rats and mice (2~3 hrs) (Dantzer *et al.*, 1987; Winslow & Insel, 2004; Choleris *et al.*, 2009; Engelmann *et al.*, 2011; Gabor *et al.*, 2012). The recognition of social odors may be particularly important for species like Syrian hamsters because individuals do not encounter other individuals frequently in the wild (Gattermann *et al.*, 2008). The longer duration of social recognition may also result from using flank gland odors as the social cue because these odors represent a powerful social stimulus that serves to communicate important social information such as dominance status (Ferris *et al.*, 1987). The recognition and memory of social stimuli can be quite sophisticated in rodents. Social recognition can be influenced by social and environmental context including the sex of the stimulus animal, the place where the initial exposure occurs (home cage, clean cage, or cage of another individual) (Zheng *et al.*, 2013), and whether the stimulus animal was infected with parasites (Kavaliers *et al.*, 2005).   Rodents are also capable of kin recognition and the true recognition of specific individuals (e.g. Mateo & Johnston, 2003; Johnston & Peng, 2008; Petrulis, 2009). It will be important to determine if OT and AVP may be involved in mediating some of the more complex forms of social recognition.

A considerable body of evidence has shown that OT and AVP can act either peripherally or centrally to influence social recognition in rats and mice (Dantzer *et al.*, 1987; Engelmann & Landgraf, 1994; Ferguson *et al.*, 2001; Winslow & Insel, 2004; Larrazolo-Lopez *et al.*, 2008; Lukas *et al.*, 2011; Albers, 2012; Gabor *et al.*, 2012). Peripheral AVP administration increases social recognition of juveniles in mice as well as in rats (Dantzer *et al.*, 1987; Bluthe *et al.*, 1993) and peripheral administration of low doses of OT facilitates social recognition of juveniles in a dose-dependent manner (Popik *et al.*, 1992). In the CNS, OT and AVP can act in multiple brain sites to influence

social recognition. Medial amygdala (MeA) has been reported as an essential site for OT's effects on social recognition. Antagonism of OTRs in the MeA impairs social recognition of juveniles and OT injection into the MeA rescues the deficits in social recognition seen in OT knock-out mice (Ferguson *et al.*, 2000; Ferguson *et al.*, 2001). Endogenous OT release in the main olfactory bulb (MOB) can enhance the retention of social memory and this enhancement can be blocked by an OTR antagonist (Larrazolo-Lopez *et al.*, 2008). AVP appears to influence social recognition by acting on the lateral septum (LS) and the MOB. Administration of AVP in the LS extends the recognition of juveniles and antagonism of V1aRs in this area impairs the recognition (Dantzer *et al.*, 1988; Veenema *et al.*, 2012). AVP injected into the LS restores social recognition in AVP-deficit of Brattleboro rats (Engelmann & Landgraf, 1994). AVP injection in the MOB also prolongs the recognition of juvenile rats (Dluzen *et al.*, 1998) and V1aR antagonism in the MOB impairs recognition (Tobin *et al.*, 2010). Taken together, these studies show there are multiple regions in the CNS where OT and AVP can influence social recognition.

There are two studies that suggest that V1a receptors are important for social recognition in rats. Infusions of antisense V1a oligonucleotides into the LS impair social recognition (Landgraf *et al.*, 1995) and over-expression of V1aRs in the LS by viral vectors enhances recognition in rats (Landgraf *et al.*, 2003). Other studies have investigated the role of OTRs and V1aRs in social recognition using knock-out mice (Bielsky *et al.*, 2004; Choleris *et al.*, 2007). Severe impairments in social recognition are found in OTR knock-out and knock-down mice (Choleris *et al.*, 2007; Macbeth *et al.*, 2009). Conflicting results, however, have been reported in studies employing the same strains of V1aR knock-out mice. One group found that V1aR knock-out mice did not

habituate to the presence of ovariectomized females, suggesting an impairment in social

recognition (Bielsky *et al.*, 2004; Bielsky *et al.*, 2005). In contrast, another study using

V1aR knock-out mice from the same source in a similar habituation and dishabituation

paradigm found a subtle olfactory deficit but normal social recognition (Wersinger *et*

al., 2007). Our data are consistent with the later study suggesting that OTRs and not

V1aRs mediate social recognition. The reasons for the discrepancy in studies regarding

V1aRs are unknown. It is possible that the V1aR is involved in other behaviors such as

anxiety-related behavior that may indirectly affect social recognition in a less profound

way. It could be that different testing models such as using a juvenile animal versus

social odors may underlie these different results. It is also possible, of course, that the

role of V1aRs in social recognition may vary across species.

The present study provides the first evidence that OT and AVP act on OTRs and

not V1aRs to produce behavioral effects. Recently, another study from our lab found

that both OT and AVP induce social communication via acting on V1aRs and not OTRs

in hamsters (Song *et al.*, 2014). Other studies have also found that both OT and AVP

can act on V1a receptors to influence behavioral and physiological responses

(Schorscher-Petcu *et al.*, 2010; Sala *et al.*, 2011; Ramos *et al.*, 2013; Qiu *et al.*, 2014;

Albers, 2015). Taken together, these data suggest that while OT and AVP can activate

both OTRs and V1aRs to influence social behavior OTRs regulate some social behaviors

and V1aRs regulate other social behaviors. At present there is no evidence that a

combination of OTR and V1aR activation is involved in regulating the same behavior, in

contrast, the existing data suggest that each behavior is regulated by either OTRs or

V1aRs. It is interesting to note that while both OTRs and V1aRs are found in limbic

structures such as the extended amygdala they rarely overlap (Veinante & Freund-

Mercier, 1997). As such, the activation of small anatomically separable populations of OTRs or V1aRs by the local synaptic release of neuropeptide may influence a single social behavior while the release of large amounts of AVP and/or OT by volume transmission could potentially activate large numbers of both OTRs and V1aRs and thereby influence the expression of multiple behaviors.

3.6 Conclusion

The present study employed a new test of social recognition of adult conspecifics wherein flank gland odors of adult male hamsters were used as the social cue to investigate neurobiological mechanisms underlying social recognition. Centrally administered OT and AVP significantly prolong the recognition of social cues from other adult individuals but these enhancing effects of OT and AVP do not extend to recognition of non-social cues. Further, both OT and AVP were found to act on OTRs but not V1aRs to influence social recognition, thus providing the first evidence that AVP and OT can act on OTRs to regulate social behavior. These findings emphasize the potential significance of the interactions between OT, AVP and their receptors in regulating complex social behavior in the central nervous system.

3.7 Acknowledgements

3.8   Chapter 3 Figures
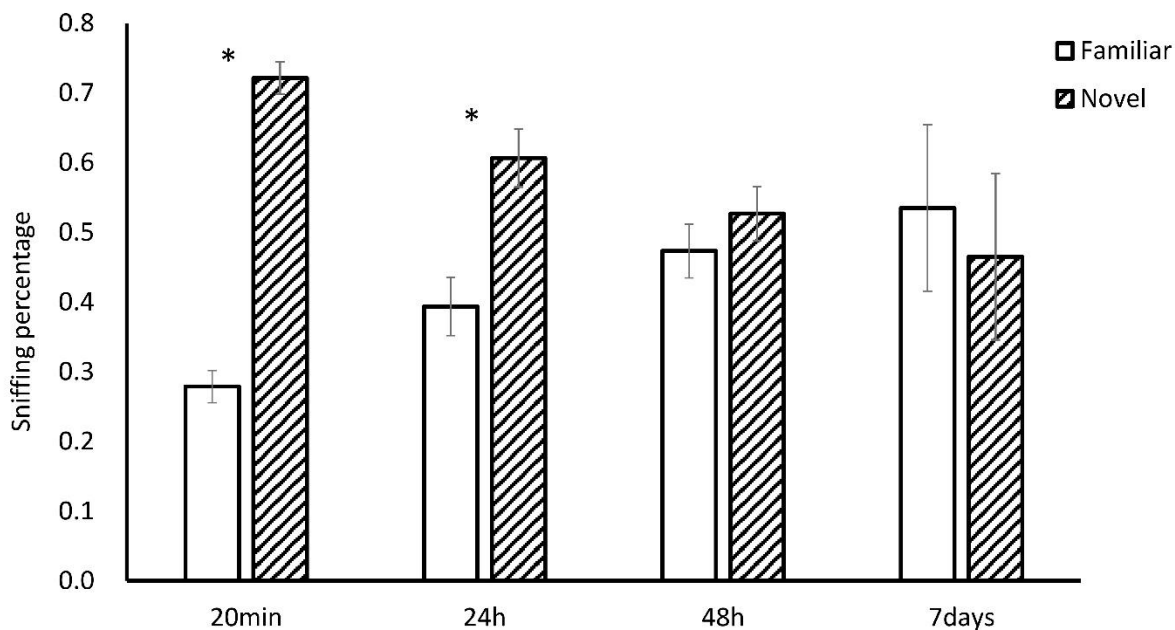
Figure 3.1 Duration of social recognition in hamsters

Hamsters spent more time on a novel odor versus a familiar odor that they encountered 20min and 24hr earlier. But they spent similar amount of time on both odors when they encountered the 'familiar odor' 48hr or 7 days earlier. * indicates a significant difference between Familiar and Novel.
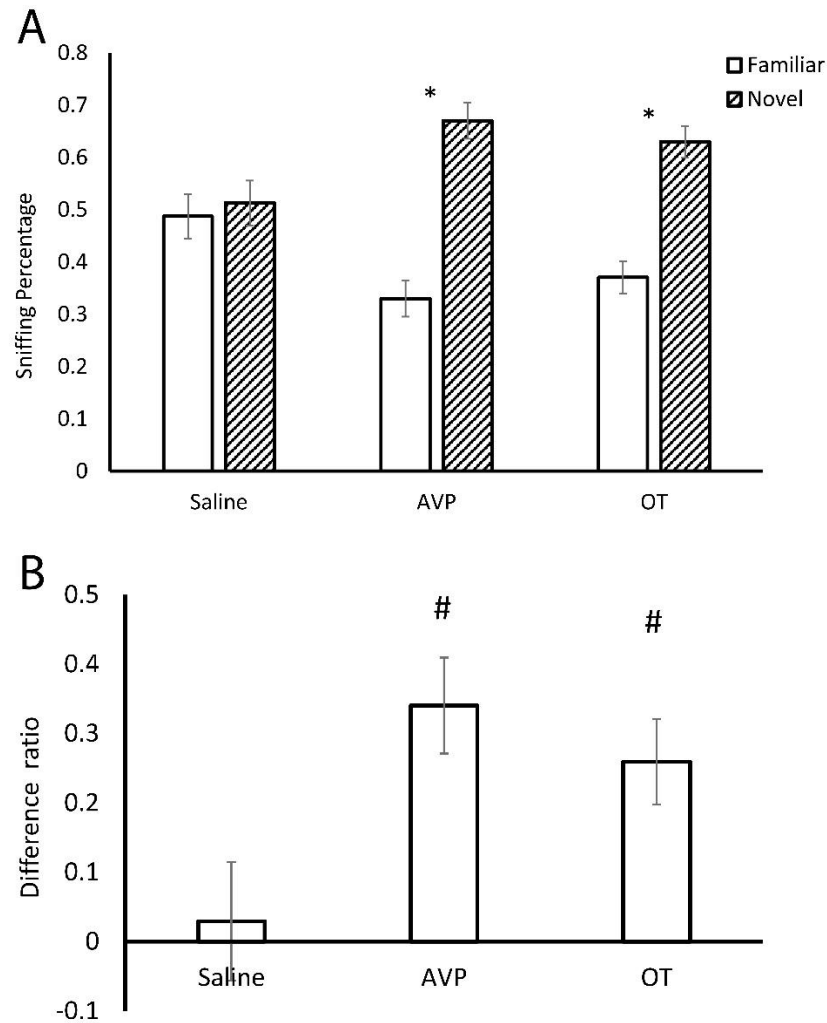
Figure3.2 OT and AVP enhance social recognition

Hamsters injected with OT or AVP spent less time in the familiar odor 48hr after their initial exposure, whereas control hamsters spent the same amount of time on the familiar and novel odors. B. Hamsters injected with OT or AVP had greater percentages of extra time over the novel odor than saline control hamsters. * indicates a significant difference between Familiar and Novel. # indicates a significant difference compared with saline.
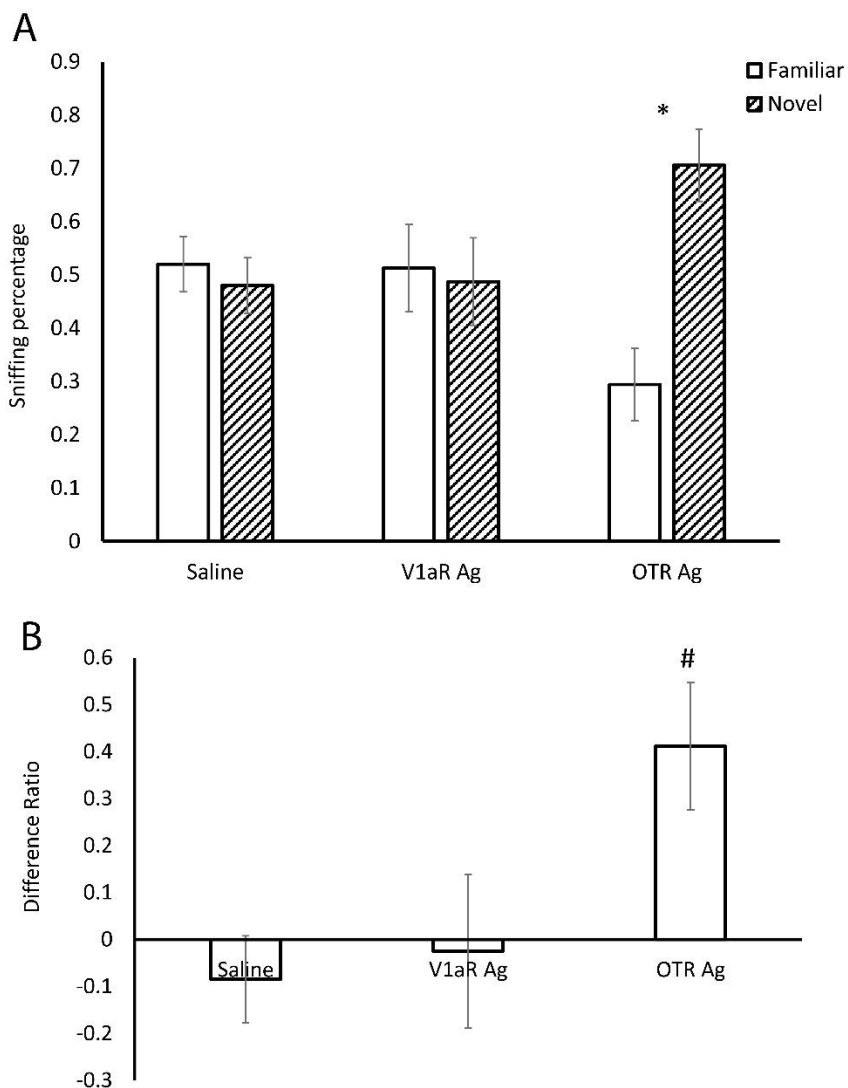
Figure 3.3 Effects of V1aR and OTR agonists on social recognition

Hamsters injected with OTR Ag spent less time with the familiar odor 48hr after their initial exposure, whereas control hamsters as well as hamsters injected with V1aR Ag spent the same amount of time with both the familiar and novel odors. B. OTR Ag hamsters had a greater percentage of extra time over the novel odor than saline and V1aR Ag hamsters. * indicates a significant difference between Familiar and Novel. # indicates a significant difference compared with saline. Ag: agonist
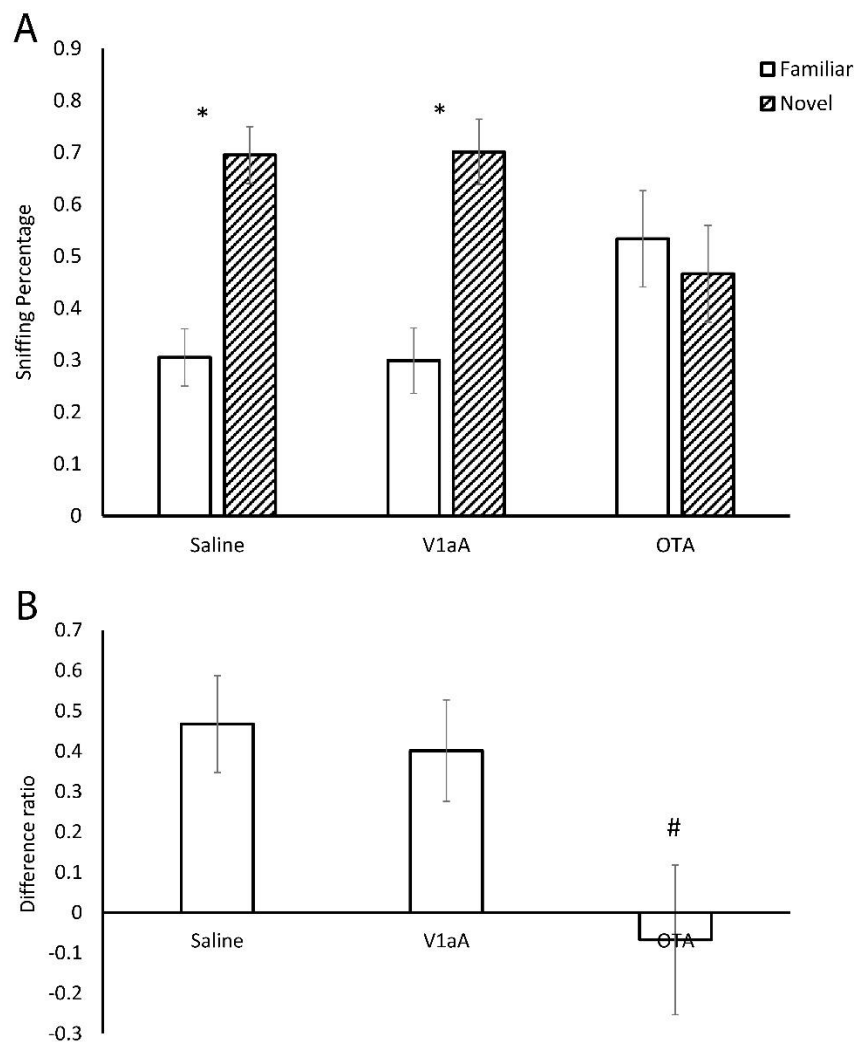
Figure 3.4 Effects of V1aR and OTR antagonists on social recognition

Hamsters injected with saline and V1aA spent more time with a novel odor versus a familiar odor they encountered 20min earlier; hamsters injected with OTA spent similar amount of time on both odors. B. Hamsters injected with OTA had a smaller percentage of extra time than hamsters injected with saline or V1aA. OTA: OTR antagonist; V1Aa: V1aR antagonist. * indicates a significant difference between Familiar and Novel. # indicates a significant difference compared with saline.
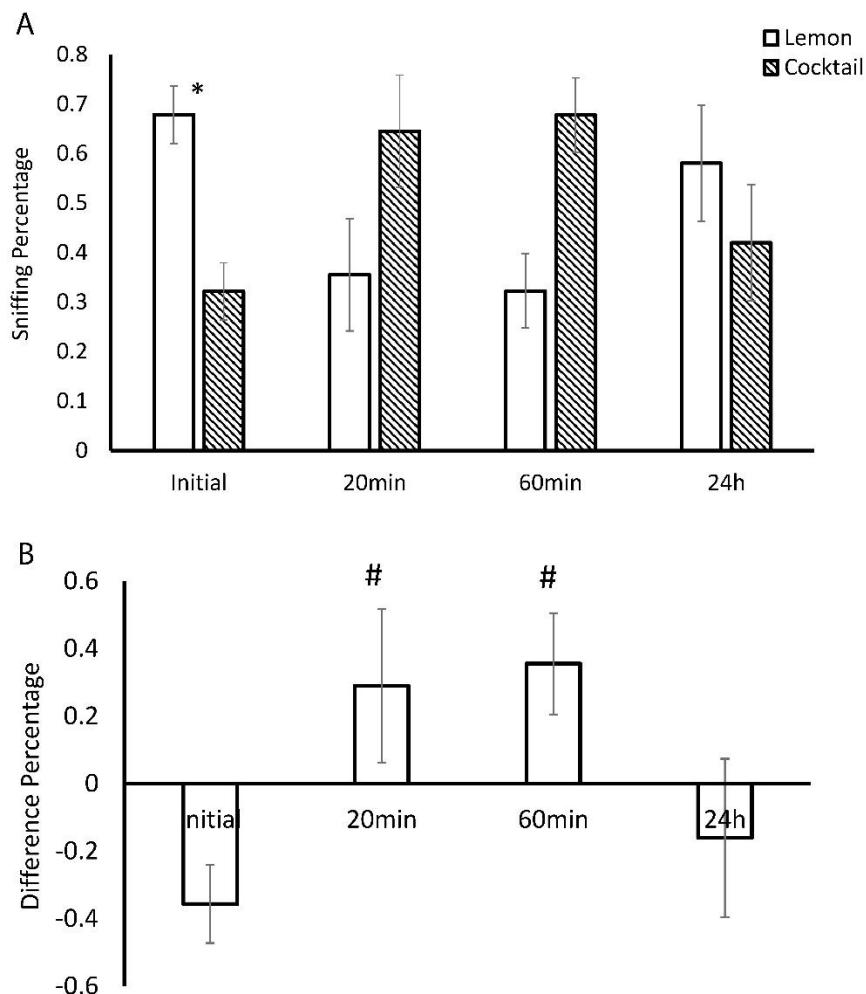
Figure 3.5 Duration of recognition for non-social cues

Hamsters that were not pre-exposed to lemon (the Initial group) spent more time with lemon when presented with a lemon and vanilla cocktail, whereas hamsters that were pre-exposed to lemon did not. B. Hamsters that were pre-exposed 20min and 60min earlier had significantly greater Difference Percentages than hamsters in the Initial group. Hamsters that were pre-exposed 24h earlier had similar Difference Percentages than the Initial group. * indicates a significant difference between Lemon and Cocktail. # indicates a significant difference compared with Initial.
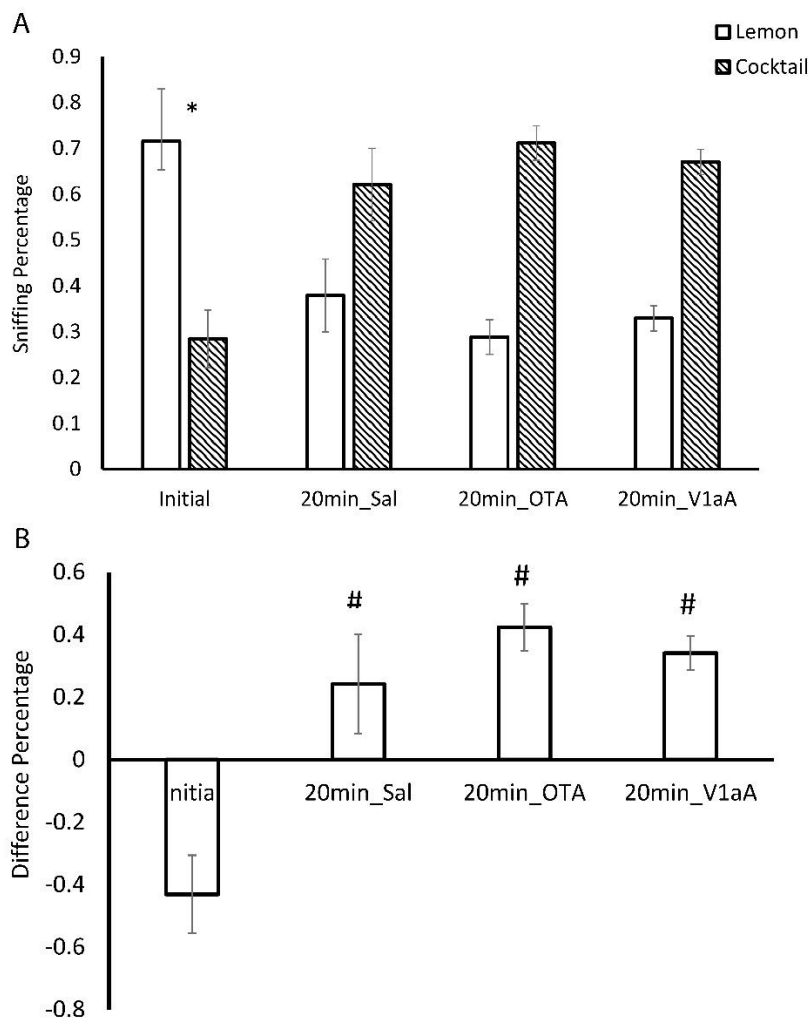
Figure 3.6 Neither V1aR nor OTR antagonist blocked non-social recognition

Hamsters that were not pre-exposed (Initial group) spent more time with lemon; whereas hamsters that were injected Sal, OTA, or V1aA did not. B. Hamsters that were injected with Saline, OTA, or V1aA had greater Difference Percentages than hamsters in the Initial group. OTA: OTR antagonist; V1Aa: V1aR antagonist. * indicates a significant difference between Lemon and Cocktail. # indicates a significant difference compared with Initial.

**CHAPTER 4 ACTIVATION OF OXYTOCIN RECEPTORS IN THE VENTRAL TEGMENTAL AREA IS ESSENTIAL FOR THE REWARDING PROPERTIES OF SOCIAL INTERACTIONS**

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4.1 Abstract

Social reward plays a fundamental role in shaping human and animal behavior. The rewarding nature of many forms of social interaction including sex behavior, parental behavior, and social play has been revealed using well-established paradigms such as the conditioned place preference test. Many motivated social behaviors are regulated by the nonapeptides oxytocin (OT) and arginine vasopressin (AVP) through their actions in multiple brain structures. Interestingly, there is little data on whether OT or AVP might contribute to the rewarding properties of social interaction by their actions within brain structures that play a key role in reward mechanisms, particularly in the ventral tegmental area (VTA). The goal of the present study was to investigate the role of OT and AVP in the VTA in regulating social reward by injecting OT and AVP active drugs into the VTA of male hamsters and examining whether it would alter the rewarding properties of social interactions. Our data showed social interaction between two male hamsters induced conditioned place preference in saline control animals.

Interestingly, OT and AVP injected into the VTA induced a two-fold increase in the place preference when compared to control injections of saline. Finally, because OT and AVP can act on each other's receptors to influence social behavior, we also determined which receptor is responsible for mediating these effects by applying highly selective OTR and V1aR agonists and antagonists. Our results not only showed that OT and AVP activate OTRs and not V1aRs to enhance social reward, they also showed the OTR activation in the VTA is essential for the expression of social reward.

Keywords: social interaction, social behavior, social salience, conditioned place preference, neuropeptides

4.2 Introduction

The fact that many forms of social interaction have rewarding properties plays fundamental role in human and animal behavior. Indeed, the frequency with which groups of humans gather together for entertainment illustrates the powerful rewarding properties of social interactions. In animals there is substantial evidence that many forms of social interaction have significant rewarding properties and thereby play a critical role in social motivation (Trezza *et al.*, 2011). Using the conditioned place preference as well as operant lever-pressing and T-maze tests, the rewarding nature of social behaviors including sex behavior, parental behavior, and social play have been revealed (Meisel *et al.*, 1996; Mattson & Morrell, 2005; Trezza *et al.*, 2011; Peartree *et al.*, 2012). Interestingly, even losing a social encounter can be more rewarding than

being alone as long as the loss is not too severe (Gil *et al.*, 2013b). The mechanisms underlying social reward are critical for the expression of adaptive social behavior and dysfunctions in the regulation of social reward can result in the development of devastating psychiatric disorders (Bora *et al.*, 2009).

Oxytocin (OT) and arginine-vasopressin (AVP) are evolutionarily conserved mammalian neuropeptides that play essential roles in regulating a variety of motivated social behaviors including aggression, social recognition, parental behavior and social communication (Ishak *et al.*, 2011; Albers, 2012; 2015). The effects of OT and AVP in the brain are mediated primarily through the activation of OT receptors or V1a AVP receptors. Many of the behavioral effects of OT and AVP are the result of their actions in structures that have been proposed to form a social behavior neural network (SBNN) (Newman, 1999). There is increasing evidence that the SBNN, composed of neural groups or "nodes" including, but not limited to, the extended amygdala, the bed nucleus of the stria terminalis (BNST), lateral septum (LS), periaqueductal gray (PAG), medial preoptic area (MPOA), ventromedial hypothalamus (VMH), and anterior hypothalamus (AH), controls the expression of social behavior (Crews, 1997; Albers, 2012; Goodson & Kingsbury, 2013; Albers, 2015).

Motivated social behaviors also require the activity of the mesolimbic dopamine (DA) system, a network of reciprocally connected brain regions that determine the salience of stimuli, assign motivational value, and initiate appropriate action (Love, 2014; Caldwell & Albers, 2015). The ventral tegmental area (VTA) is a key region in this motivational network providing many of the dopamine containing projections that innervate a variety of cortical and limbic structures. While the SBNN and the mesolimbic DA system are distinct from one another, they interact supporting decision

making about the expression of motivated social behaviors (O'Connell & Hofmann, 2011a; b).

While OT and AVP can act within the mesolimbic DA system to influence the expression of various forms of social behavior there is very little data on whether OT or AVP might contribute to the rewarding properties of social behavior by their action within these structures. The purpose of the present study was test the hypothesis that activation of OT and/or V1a receptors in the VTA mediates the rewarding properties of social interaction. Because OT and AVP can influence social behavior by acting on each other's receptors (Sala *et al.*, 2011; Song *et al.*, 2014) we also determined which receptors are responsible for inducing social reward by applying highly selective OT and AVP receptor agonists and antagonists.

4.3 Materials and Methods

4.3.1 Animals

Syrian hamsters (*Mesocricetus auratus*), purchased from Charles River Laboratories Inc., Wilmington, MA, USA, were used in all experiments. The experimental hamsters were 10-12 weeks old, weighed 110-130g, and were singly housed in polycarbonate cages (23x43x20 cm) upon arrival to our vivarium. The stimulus hamsters were 8-10 weeks old, weighed 90-100g, and were housed in a group of 4 per cage upon arrival. All hamsters were kept on a 14:10 light/dark cycle with food and water ad libitum. All experimental procedures were in accordance with the National

Institutes of Health Guidelines for the Use of Animals and were approved by the Georgia State University Animal Care and Use Committee.

4.3.2 Surgery, and microinjections

Surgery: Seven days after arrival, hamsters were anesthetized with 5% isoflurane in an induction chamber and maintained with 3.75% isoflurane throughout all surgical procedures. A 4mm, 26-gauge cannula guide was implanted in each experimental hamster aimed at the ventral tegmental area (VTA) (-2.9mm anterior to bregma, -0.6mm from the midline, and -2.9mm below dura). The guide was affixed to the skull using wound clips and Ortho-Jet dental acrylic (Lang Dental, Wheeling, IL, USA). Ketefen (1ml/kg) was injected I.P. for analgesia and hamsters were monitored daily for 3 days and given additional ketefen as required.

Microinjections: Hamsters were gently restrained and microinjections were given over the course of 1min using an infusion pump (Harvard Apparatus), a 1µl Hamilton syringe, and a 12mm, 32-gauge microinjection needle. The volume of all microinjections was 250nl. Following the injection, the needle was left in the cannula guide for an additional minute to allow drug diffusion into the VTA. At the end of the experiment experimental hamsters were sacrificed by lethal injection of sodium pentobarbital (2ml/kg) and were injected with ink to verify the injection sites (see representative cannula placement in Fig. 1).

4.3.3 Drugs

The following drugs were used: 9µM, 90µM OT (Bachem, CA, USA) and AVP (Fisher scientific, TX, USA); 27µM [Thr4,Gly7]OT (OTAG, a highly selective OT receptor agonist, a gift of Dr. Maurice Manning); 0.23µM [Phe2]OVT (V1aAG, a highly selective V1a receptor agonist, a gift of Dr. Maurice Manning); 90µM desGly-NH2-d(CH2)5[D-Tyr2,Thr4]OVT (OTA, a selective OTR antagonist, a gift of Dr. Maurice Manning) and 90µM d(CH2)5[Tyr(Me)2]AVP (V1aA, a selective V1aR antagonist known as Manning Compound, a gift of Dr. Maurice Manning). The concentrations of OT and AVP administered were based on the concentrations used in previous studies that were found effective in altering other social behavior in hamsters (Harmon *et al.*, 2002; Albers *et al.*, 2006; Song *et al.*, 2014). The concentrations of the OT and AVP agonists were based on their relative efficacies compared to 9µM OT and AVP in binding to OTR and V1aR in rats, respectively (Manning et al., 2012). All controls were given a 250nl injection of saline.

4.3.4 Behavioral testing

Male hamsters were tested using conditioned place preference (CPP) to examine whether social interaction with another male adult hamster could induce conditioned place preference, and if so, how much change in chamber preference would be induced by social interaction. Drugs of interest and saline were injected into the VTA and tested for their effects on the magnitude of change in chamber preference induced by social interaction, as a measure of the magnitude of social reward. The CPP apparatus consisted of a white chamber (61cm x 46cm x 38cm), a black chamber (61cm x 46cm x

38cm), and a transitional area (38cm x 25cm x 38cm). Hamsters were given two 15min pretests, five pairs of conditioning, and one 15min post-test. In the pretest, hamsters were tested for their initial preference of the white and black chambers by allowing them to freely explore the whole apparatus for 15min. The pretest was repeated again 24 hrs later and the average was used for data analysis. Immediately before each conditioning session, drugs of interest or saline were injected into the VTA; hamsters were then placed in the non-preferred chamber for social interactions and in the empty preferred chamber for social isolation each day for 5 days. Hamsters went through one trial of social interaction and one trial of social isolation with 1 hr apart each day in a counter-balanced manner. After conditioning, hamsters were tested again for their chamber preference in the post-test in the same way as in the pretests. Hamsters were not injected with drugs or saline during the pretest and the posttest.

The goal of Experiment 1 was to determine whether microinjections of AVP and OT into the VTA could alter the magnitude of social reward. Hamsters were assigned to five groups and were injected with 90μM AVP, 9μM AVP, 90μM OT, 9μM OT and saline. In this experiment, we also measured the effects of OT and AVP in the VTA on social behavior including aggression, sociability, grooming, and flank marking, during the social interaction conditioning. The frequency of crossing the gates to both chambers during posttests was also scored to evaluate differences in the locomotive behavior after conditioning among the animals. After we found the enhancing effects of both OT and AVP in social reward in Experiment 1, Experiment 2 was conducted to test whether selective OTR and V1aR agonists could mimic the effects of OT and AVP in order to determine which receptor is involved in the regulation of social reward. Because there were no differences in the behavioral effects in controls injected with saline in this

experiment and in Experiment 1, the data from saline controls were pooled for analyses. Experiment 3 determined whether antagonism of OTRs or V1aRs would reduce the rewarding aspects of social interaction, i.e., reduce the ability of social interaction to induce conditioned place preference. Because no difference was found in the saline control animals used in this experiment and in Experiments 1&2, the data were pooled together again for analyses.

4.3.5 Data analyses and statistics

All the data are presented as mean ± standard error of the mean. SPSS V21 was used to analyze the data. Paired student *t*-test was used to detect differences in time spent in the social-interaction chamber between pretests and post-tests in all animal groups. One-way ANOVA test was used to detect differences in the change of time spent in the social interaction chamber among animals injected with drugs of interest and saline control. All post-hoc comparisons were determined *a priori*; planned contrasts were performed following significant differences found in ANOVA tests. All tests were two tailed and differences were considered significant at $p \leq 0.05$.

4.4 Results

Experiment 1 tested whether OT or AVP injected into the VTA influences the magnitude of social reward. In the pretest all the hamsters spent similar amounts of time in their non-preferred chamber (ANOVA, $F_{(4,35)}=0.363$, $p>0.05$ Fig.2A) and in their preferred chamber (data not shown). After 5 conditioning trials in the non-preferred chamber socially interacting within another hamster and 5 conditioning trials

in the preferred chamber in social isolation, all hamsters had a significant increase in the time spent in the non-preferred chamber, i.e., the social-interaction chamber (paired t tests, saline: t(9)=5.222, $p<0.01$, 90μM AVP: t(7)=5.245, $p<0.01$, 9μM AVP: t(8)=6.432, $p<0.01$, 90μM OT: t(5)=5.311, $p<0.01$, and 9μM OT: t(6)=5.487, $p<0.01$, , Fig.2B). Although there was a difference in the magnitudes of increase in the time in the social-interaction chamber among all the hamsters (F(4,35)=2.933, $p<0.05$) the hamsters injected with OT and AVP (at both doses) had a significantly greater increase in the time spent in the social interaction chamber compared to hamsters injected with saline (planned contrasts $p<0.05$ for all 4 comparisons).

Because OT and AVP were injected into the VTA just before hamsters were placed in the chamber for each conditioning session, we also measured social behavior during the session to examine whether OT or AVP in the VTA affects the expression of social behaviors including aggression, sociability, grooming, and flank marking. All hamsters exhibited similar levels of aggression (F(4,35)=0.242, p=0.912, Fig. 3A) and did not differ in the duration of non-social behavior (F(4,35)=1.953, p=0.123). There were, however, significant differences in the duration of social behavior (F(4,35)=3.092, p<0.05), in the duration of grooming (F(4,35)=5.548, p<0.01), and in the frequency of flank marking (F(4,35)=3.531, p<0.05). Posthoc planned contrasts indicated that hamsters injected with 90μM AVP trended to spend less time engaged in social behavior than controls injected with saline (p=0.07). The same group of hamsters also spent more time grooming (p<0.05) and flank marked more than controls injected with saline (p<0.05, Fig.3B). However, there were no significant differences between any other drug injected groups (i.e. 9μM AVP, 90μM or 9μM OT) and controls injected with saline in the duration of social behavior, grooming, or the frequency of flank marking. There

was no significant difference among the 5 groups of hamsters in the frequencies of crossing the gates to both chambers ($F_{(4,35)}=2.505$, $p>0.05$, Fig.4).

Experiment 2 investigated whether highly selective OT and V1a receptor agonists could mimic the effects of OT and AVP on social reward. In the pretest there were no between group differences in the time spent in the non-preferred chamber ($F_{(2,27)}=0.269$, $p>0.05$, Fig.5A). After conditioning in the non-preferred chamber with social interaction and in the preferred chamber with social isolation, hamsters injected with saline and the OTR agonist spent significantly more time in the social-interaction chamber in the posttest versus in the pretest (saline: $t_{(14)}=5.116$, $p<0.001$; OTAG: $t_{(6)}=6.387$, $p<0.01$, Fig.5A). Hamsters injected with the V1aR agonist did not spend significantly more time in the social interaction chamber after conditioning although there was a trend in that direction ($t=1.941$, $p=0.09$, Fig.4A). There was a significant difference in the magnitude of increase in the time spent in the social-interaction chamber among groups ($F_{(2,25)}=4.168$, $p<0.05$, Fig.5B). Hamsters injected with the OTR agonist had a greater increase than those injected with the V1aR agonist or saline in the time spent in the social-interaction chamber ($p<0.05$ for both comparisons, Fig.5B).

The aim of Experiment 3 was to test the hypothesis that activation of either OT or V1a receptors is necessary for the rewarding properties of social interaction. All the hamsters spent similar amount of time in their non-preferred chamber in the pretest ($F_{(2,30)}=0.014$, $p>0.05$, Fig.6A). Hamsters injected with saline and the V1aR antagonist spent significantly more time in the social-interaction chamber after conditioning (saline: $t_{(17)}=7.104$, $p<0.001$; V1aA: $t_{(5)}=2.638$, $p<0.05$ Fig.6A). Hamsters injected with the OTR antagonist, however did not spend significantly more

time in the social interaction chamber after conditioning although there was a trend in that direction (t(8)=2.091, p=0.07, Fig.6A). After conditioning there were significant differences in the magnitude of increase in the time spent in the social-interaction chamber among the groups (F(2,30)=3.737, p<0.05, Fig.6B). Hamsters injected with the OTR antagonist spent significantly less time in the social-interaction chamber compared to hamsters injected with saline or the V1aR antagonist (p<0.05 for both comparisons, Fig.6B). There was no difference in the magnitude of the increase in the time spent in the social-interaction chamber between hamsters injected with the V1aR antagonist and those with saline (p>0.05 for both comparisons, Fig.6B).

Histological analysis of the sites of injection revealed most placements were found between Bregma -4.3mm and Bregma -4.9mm. The brain sites of the microinjection needle tips of representative animals in the study are shown in Fig. 1. Placements were considered accurate if they were located within the structure of interest. Animals were excluded if histological analysis revealed poor cannula placements.

4.5 Discussion

Our data support the hypothesis that activation of OT receptors in the VTA is necessary for the rewarding properties of social interactions. Although both OT and AVP can enhance social reward by their actions within the VTA, the results obtained with highly selective OT and V1a agonists and antagonists indicate that these neuropeptides influence social reward by acting on OT and not V1a receptors. More specifically, the highly selective OT agonist, but not the V1a agonist significantly

increased the time spent in the social-interaction associated chamber after conditioning.

Most importantly, the highly selective OT antagonist, but not the V1a antagonist

significantly reduced the time spent in the social-interaction associated chamber after

conditioning. These data indicate that activation of OT receptors in a key element of the

mesolimbic DA system, the VTA, plays an essential in mediating the rewarding

properties of social interactions.

There are an increasing number of examples of the ability of OT and AVP to

cross-activate each other's receptors in the brain. Previous studies have shown that OT

can modulate social behaviors such as social communication by acting on V1aRs and not

OT receptors (Sala *et al.*, 2011; Song *et al.*, 2014) and that AVP can enhance social

recognition of adult conspecifics by acting on OT receptors and not V1a receptors (Song

et al., 2015). The present data are yet another example that cross-activation between

OT and AVP that can occur in the regulation of social behavior. The absence of

selectivity between OT and AVP receptors in rodents suggest that a functional

distinction between OT and AVP may be overstated. Of course, it remains important to

demonstrate that endogenously released OT and AVP cross-activate each other's

receptors in functionally significant ways.

OT and AVP play important roles in motivated social behavior by acting within

multiple 'nodes' of the SBNN including hypothalamus, amygdala, BNST, and lateral

septum (Ferris *et al.*, 1984; Huhman & Albers, 1993; Bamshad & Albers, 1996; Martinez

et al., 2010; Gil *et al.*, 2011; Bredewold *et al.*, 2014). There is also growing evidence that

OT and AVP can act within multiple sites of the dopamine reward circuitry to influence

social behaviors (Lim & Young, 2004; Baskerville & Douglas, 2010; Numan & Young,

2015). Despite the key role of the VTA in the midbrain reward circuitry, the present

study provides the first evidence that activation of OT receptors in the VTA is essential for the rewarding properties of social interactions. A recent study in humans suggests the VTA may be a region for OT and AVP to affect the saliency of socially relevant cues (Groppe *et al.*, 2013). Their data from functional magnetic resonance imaging indicate that intranasal OT significantly enhances VTA activation in response to cues signaling social reward, i.e., friendly faces in humans. There is also recent evidence that OT receptors in other elements of the midbrain reward circuitry are involved in mediating the rewarding properties of social interactions. Inactivation of OTRs in the NAcc, particularly those located in the presynaptic serotonin neurons were found to reduce social reward resulting from social interactions among male mice(Dolen *et al.*, 2013). As a result, activation of OT receptors within multiple sites of the SBNN and the mesolimbic DA system may link the social and motivational elements underlying social reward.

4.6 Conclusion:

OT and AVP injected into the VTA enhance the rewarding properties of social interactions via acting on OTRs and not V1aRs. Importantly, the present study also showed that OTR activation in the VTA is essential for the rewarding properties of social interactions.

4.7   Chapter 4 Figures

Bregma -4.3mm   Bregma -4.6mm   Bregma -4.9mm
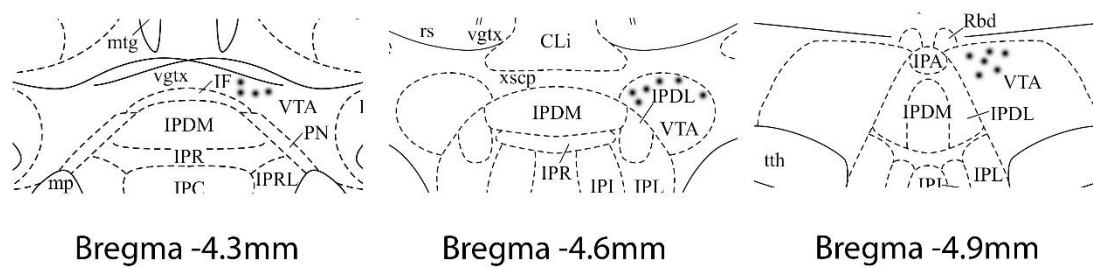
Figure 4.1 Representatives of VTA cannulation placement in all studies.
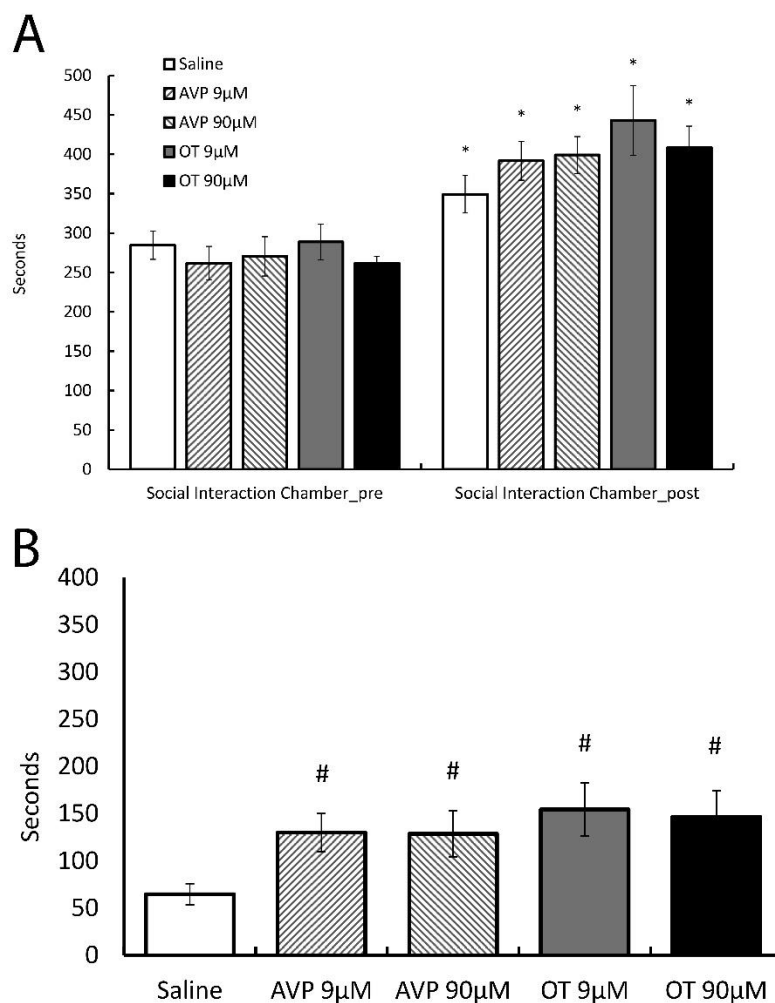
Figure 4.2 Effects of OT and AVP in the VTA on CPP.

A. Left. All hamsters spend similar amounts of time in the social interaction chamber in the pretest. A. Right. All groups of hamsters increased the time spent in the SIC in the posttest as compared to the pretest. B. Increase of the time spent in the social interaction chamber in the post-test as compared to the pretest. Hamsters injected with AVP and OT at both doses have a larger increase after conditioning compared with saline animals. Note: * indicates a difference in the post-test compared with the pretest. # indicates a difference compared with saline.
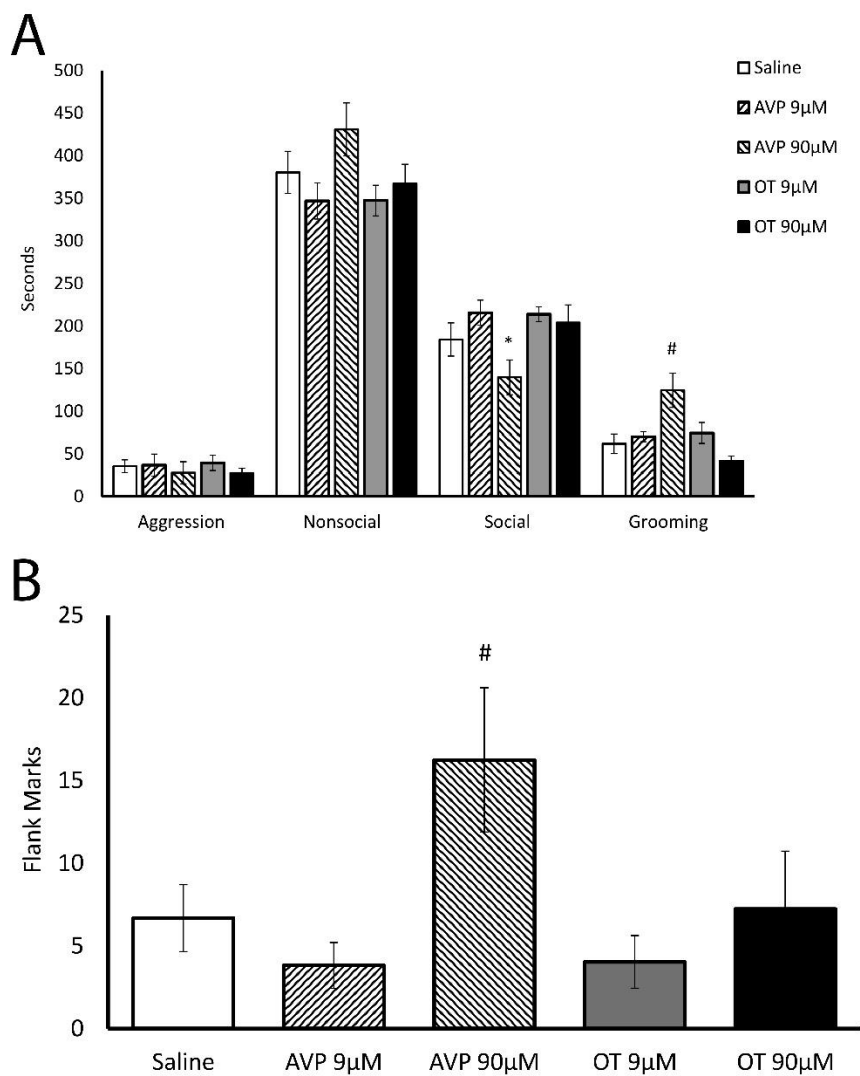
Figure 4.3 Effects of OT and AVP in the VTA on social behavior.

All hamsters have similar levels of aggression and nonsocial behavior. AVP (90μM) injected animals have a trending difference in the duration of social behavior compared to controls. The same group has higher levels of grooming and flank marking behavior. Note: * indicates a trending difference (p=0.07) compared to saline controls; # indicates a significant difference compared to controls.
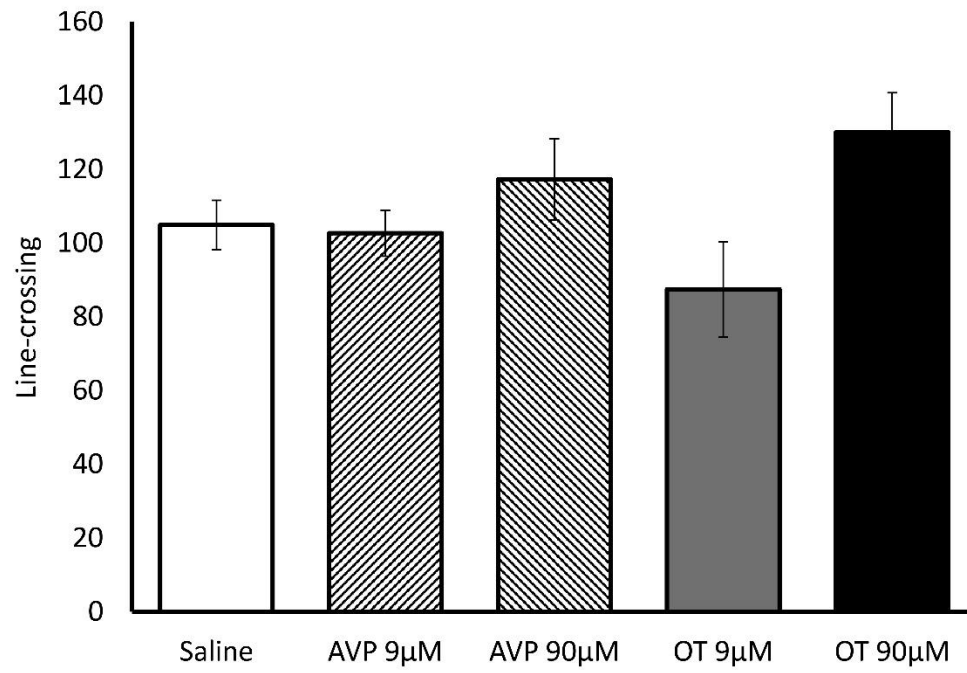
Figure 4.4 Line-crossing of all hamsters in the post-test.

There was no significant difference in line-crossing among all the hamsters.
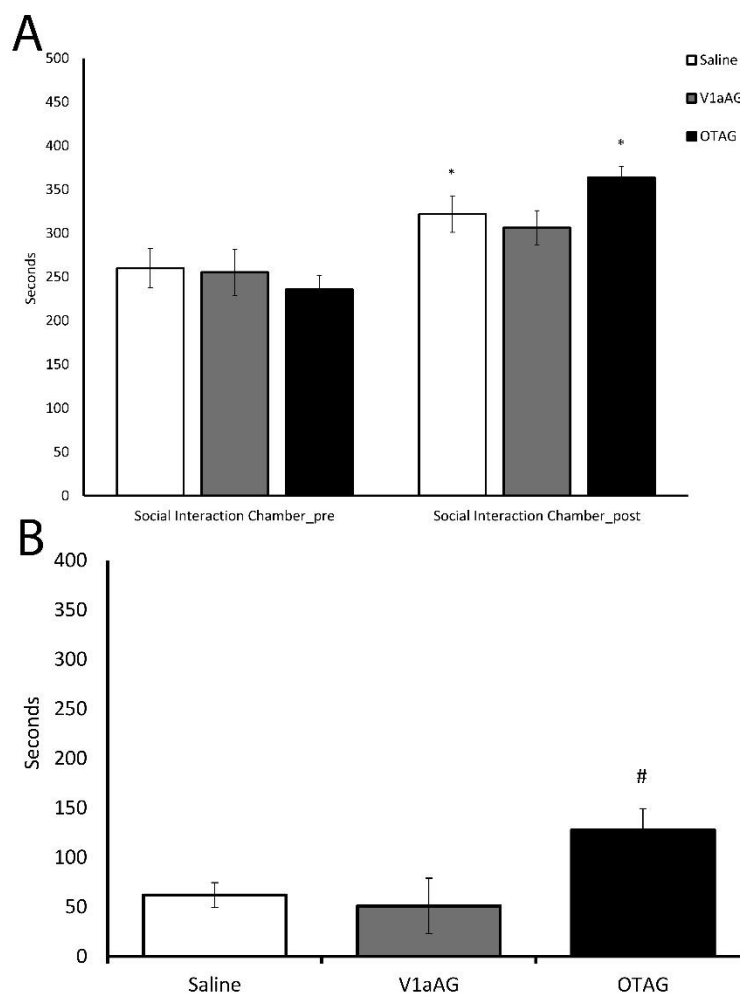
Figure 4.5 Effects of OTR and V1aR agonists on CPP

A. Time spent in the social interaction chamber in the pretest and the post-test.

Hamsters injected with saline and OTAG increase time spent in the social interaction

chamber in the posttest vs in the pretest. B. Increase of time spent in the social

interaction chamber in the post-test vs in the pretest. Hamsters injected with OTAG

have a larger increase after conditioning compared with saline animals. Note: *

indicates a difference in the post-test compared with the pretest. # indicates a

difference compared with saline. V1aAG: V1aR agonist; OTAG: OTR agonist.
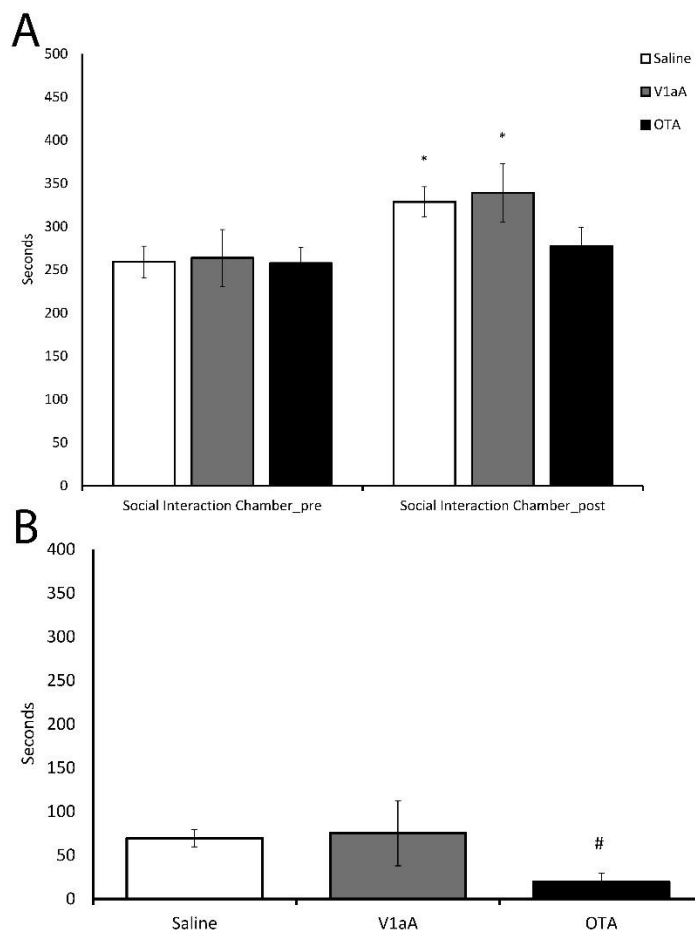
Figure 4.6 Effects of OTR and V1aR antagonists on CPP

A. Time spent in the social interaction chamber in the pretest and post-test. Hamsters injected with saline and V1aA increase time spent in the social interaction chamber in the posttest vs in the pretest. B. Increase of time spent in the social interaction chamber in the post-test vs in the pretest. Hamsters injected with OTA have a smaller increase after conditioning compared with saline animals. Note: * indicates a difference in the post-test compared with the pretest. # indicates a difference compared with saline. V1aA: V1aR antagonist; OTA: OTR antagonist.

CHAPTER 5 GENERAL DISCUSSION AND CONCLUSION

5.1 Summary

The goal of this dissertation was to examine the roles of cross-activation in the OT and AVP systems and its role in the regulation of social behavior. In Chapter 2, we found OT induces social communication (i.e. flank marking behavior) via activating V1aRs, though it is less potent than AVP. The V1aR but not OTR agonist mimics the OT effects on flank marking and the V1aR but not OTR antagonist blocks OT-induced flank marking. Furthermore, Chapter 2 also showed endogenous released OT stimulated by the central injection of alpha-MSH enhances odor-induced flank marking, indicating endogenous OT may be involved in the regulation of flank marking behavior. Follow-up experiments showed inhibition of endogenous OT by an antagonist for MC4 receptor (the receptor that alpha-MSH binds) reduces odor-induced flank marking, also supporting this notion. Taken together, these data indicate endogenous OT may activate V1aRs in the regulation of flank marking in Syrian hamsters. I next explored the possibility that cross-talk between OT and AVP is involved in the regulation of other social behaviors. In Chapter 3, I tested the effects of OT and AVP on social recognition by using a new testing model where flank gland odors were used as social cues. I first demonstrated a substantially longer memory for social recognition (24hr) compared to what has been shown in most previous studies (i.e., 2hr) that used 'relatively neutral social stimuli' such as juveniles and ovariectomized females. I then showed both OT and AVP injected into the intracerebroventricle (ICV) enhance social recognition for up to 48hr. I also found that the OTR and not the V1aR agonist lengthens the social memory,

suggesting that AVP may regulate social recognition by acting on OTRs. Furthermore, our data also indicate that these recognition-enhancing effects of OT and AVP do not extend to non-social cues. In Chapter 4, I found both AVP and OT injected into the VTA enhance social reward in male Syrian hamsters tested in a CPP paradigm. In these studies, we injected AVP, OT or saline in the VTA of male hamsters and conditioned them to social interaction with a non-aggressive male conspecific. Hamsters injected with saline spent more time in the social-interaction associated chamber after conditioning, suggesting social interaction is rewarding for hamsters. In comparison, hamsters injected with AVP or OT had a larger increase in the time spent in the social-interaction associated chamber after conditioning compared to those injected with saline. Follow-up experiments showed that the OTR but not V1aR agonist mimics these reward-enhancing effects of OT and AVP, suggesting that OTRs but not V1aRs mediate social reward. Importantly, our data also showed OTR antagonism in the VTA blocks social reward that is seen in saline controls, indicating activation of OTRs in the VTA is essential for the expression of social reward. Taken together, all these data suggest both OT and AVP in the central nervous system, presumably acting on multiple brain sites, facilitate social behaviors in that they induce the performance of social behavior, prolong the recognition of social interaction, and enhance the rewarding nature of social interaction.

5.2   Implications of this work

Our studies showed strong evidence that OT and AVP can cross-activate each other's receptors in the regulation of a variety of social behaviors. OT and AVP can be

released via classic synaptic transmission in a highly localized manner from neuronal terminals. They can also be released via volume transmission from large dense-core vesicles that can be found in all areas of neurons including axons, soma, and dendrites. While synaptic transmission may result in activation of their correspondent receptors by the two neuropeptides, volume transmission has the potential to result in cross-activation of OTRs and V1aRs by AVP and OT via diffusion of the peptides into brain regions that express these receptors. Our findings indicate OT and AVP activate both V1aRs and OTRs but that V1aRs mediate some behaviors and OTRs mediate other behaviors. It is not clear how synaptic transmission and volume transmission interact in the signaling of OT and AVP in the brain, but we speculate that different patterns of synaptic and non-synaptic activations may be responsible for the expression of different social behavior (See Figure 5.1).

We also speculate that volume transmission might not be the only reason that cross-talk between OT and AVP can occur. Because there is no efficient OTR and V1aR antibodies available, there are few data on direct innervations of OT and AVP on post-synaptic neurons that express either OTRs or V1aRs. It might seem unlikely, but it is really unknown whether OT axons actually make synapses on V1aRs expressing neurons or AVP axons make synapses on OTRs expressing neurons. It is also not known where OT/AVP release from neuron terminals through synaptic transmission will activate both OTRs and V1aRs that are in close vicinity or even co-expressed in the same neurons.

Our findings also indicate that due to the non-selectivity of OTRs and V1aRs in rodents the distinct functions of OT and AVP may be overstated. OT and AVP are certainly released under different mechanisms (Ebner *et al.*, 2000), though there is some overlap when they are both being released in the brain (Wotjak *et al.*, 2001). But

in terms of their effects, what ultimately matters is the specific receptor activation or the activation of a specific receptor combination and their downstream pathways. Unfortunately, besides the one study that shows specific inhibition of OTRs expressing neurons on V1aRs in the CeA where they antagonistically regulate fear response (Huber *et al.*, 2005), there are few data on the relationship between OTRs and V1aRs in the regulation of social behavior. The existence of hetero-dimers between OTR and V1aRs has been reported in vitro cellular systems, it remains a challenge to investigate whether OTRs and V1aRs dimerize in the central nervous system or in the peripheral system (Albizu *et al.*, 2006; Chini & Manning, 2007). Therefore, although our data show OTRs mediate some social behaviors and V1aRs mediate others, it remains to be determined whether OTRs and V1aRs can act synergically to regulate the same social behavior.

Lastly, from the original discovery of the OT and AVP systems, OTRs appear to be associated with giving birth and maternal bonding, and V1aRs appear to be associated with homeostasis. Perhaps OTRs mediates 'altruism-related' behaviors and V1aRs mediates 'egocentrism-related' behaviors. Thus, such a hypothesis is consistent with our data showing OTRs are involved in recognition and reward, and V1aRs are involved in active social communication to defend territory and guard mates. Future experiments are needed to test this hypothesis.

5.3 Cross-talk of OT and AVP in other social behaviors

5.3.1 *Aggressive behavior*

Aggression is a complex behavior that can be divided into many different categories including offensive aggression, defensive aggression, predatory aggression,

and maternal aggression. There are a number of studies that have reported the involvement of OT and AVP in the regulation of offensive aggression and maternal aggression. AVP microinjections in the anterior hypothalamus increase offensive aggression (Ferris *et al.*, 1997; Caldwell & Albers, 2004; Albers *et al.*, 2006; Gobrogge *et al.*, 2007) and V1aR antagonists inhibit aggression in male Syrian hamsters (Potegal & Ferris, 1989). Sex differences may exist in the effects of AVP on offensive aggression and there are data that show AVP in the AH of female hamsters inhibits aggression (Gutzler *et al.*, 2010). Oxytocin in the AH is also found to inhibit aggression in female hamsters (Harmon *et al.*, 2002). Both AVP and OT are involved in maternal aggression; AVP deficiency suppresses aggression in lactating female rats (Fodor *et al.*, 2014) and OT knock-out mice show less maternal aggression compared with wild-type mice (Young *et al.*, 1998). Considered together, both AVP and OT appear to inhibit aggression in females and it is unclear whether the same receptor is involved in this.

5.3.2 *Pair bonding*

A pair bond is described as an enduring preferential association formed between two sexually mature adults, and is characterized by selective contact, affiliation, and copulation with the partner over a stranger (Young *et al.*, 2011). Many studies on the effects of OT and AVP on pair bonding in mammalian species were conducted in prairie voles (*Microtus ochrogaster*). OT in the NAcc is necessary for pair bond formation in female prairie voles as OT facilitates and blockage of OTRs impairs pair bonding (Williams *et al.*, 1994; Insel & Hulihan, 1995). AVP in the lateral septum (Liu *et al.*, 2001) and ventral pallidum (Pitkow *et al.*, 2001; Lim & Young, 2004) is necessary for pair bonding in male prairie voles and V1aR antagonism in these two regions disrupts

this behavior. Effects of OT, AVP, and their homologs on pair bonding have also been reported in other socially monogamous species such as coppery titi monkeys (Freeman *et al.*, 2014), zebra finches (Kelly & Goodson, 2014), and cichlids (O'Connor *et al.*, 2015).

5.3.3 *Sexual behavior*

A wealth of data shows OT/AVP family of peptides plays an important role in sexual behavior. OT in the MPOA facilitates male reproductive behavior such as anogenital investigation and ejaculation (Gil *et al.*, 2011). Sex experience enhances OTR gene and protein expressions in the MPOA (Gil *et al.*, 2013a). Arginine vasotocin promotes sexual behavior in male Xenopus tropicalis (Miranda *et al.*, 2015). AVP in the MPOA-AH facilitates lordosis in female Syrian hamsters (Huhman & Albers, 1993). Copulation cues increase Fos expression in AVP neurons in the amygdala of male rats (Hari Dass & Vyas, 2014). Interestingly, an OT/AVP-like signaling system in Caenorhabditis elegans is also found to regulate reproductive behavior including mate search, mate recognition, and mating, suggesting an ancient role of OT and AVP in sexual behavior (Garrison *et al.*, 2012). Taken together, all these data support the involvement of both OT and AVP in the regulation of sexual behavior in a wild range of animal species.

5.3.4 *Parental behavior*

One of the first functions that was reported on central OT administration was its role in maternal behavior. ICV administration of OT was found to induce maternal behavior in virgin rats (Pedersen & Prange, 1979); the same research group also found AVP in the ICV could also induce maternal behavior only with a later onset (Pedersen *et al.*, 1982). Later studies suggested multiple brain sites that may be important for OT

and AVP to regulate maternal behavior including auditory cortex (Marlin *et al.*, 2015), prefrontal cortex (Sabihi *et al.*, 2014), medial preoptic area (Caughey *et al.*, 2011), and the VTA (Baskerville & Douglas, 2010). See reviews (Bosch & Neumann, 2012; Bridges, 2015) for detailed information on their sites of action and the interaction of OT and AVP with other neurotransmitters in the regulation on maternal behavior.

5.4   Future directions

5.4.1 Binding affinities of OT and AVP to V1aRs and OTRs in hamsters

The present work has shown both OT and AVP can activate OTRs and V1aRs to influence social behavior in hamsters. Although there are some data showing differential efficacies of OT and AVP in binding their receptors in species including rats, mice, and humans (Manning *et al.*, 2012), the efficacy data are lacking in Syrian hamsters. Our behavioral data from Chapter 2 showed the AVP is about 100 times more potent than OT in binding to V1aRs. Our data from Chapters 3 & 4 were not able to detect affinity differences of AVP and OT in binding to OTRs, possibly due to the much smaller differences in affinity as seen in other species. Species differences, however, have been shown in the selectivity of AVP and OT binding to their receptors. For instance, OT is selective in binding to OTRs in humans whereas it is not selective to OTRs in rats and mice (see Table 1 in Chapter 1). The functional significance of these differences is not known. Therefore, it is important to determine the binding affinities of OT and AVP to V1aRs and OTRs in hamsters. One way to accomplish this is to utilize *in vitro* over-expression of V1aRs and OTRs and conduct binding assays.

5.4.2 Functional significance of cross-talk between OT and AVP systems

OT and AVP can activate V1aRs to regulate some social behaviors such as social communication and activate OTRs to regulate other social behaviors such as social recognition and social reward. So far there are few data showing the functional significance of this cross-talk on a physiological level. In Chapter 2, we demonstrated endogenously released OT acts on V1aRs to induce social communication by injecting α-MSH in the lateral ventricle. Injecting α-MSH was the best approach that was practically available for me; but α-MSH also interacts with other neurotransmitters including DA (Leonard *et al.*, 1976; Lindblom *et al.*, 2001), which certainly complicates the interpretations of our findings. Although if volume transmission can be demonstrated to be important in neuropeptide signaling then OT and AVP cross-talk may be a fairly common phenomenon. In future experiments, it is important to examine the mechanisms under which volume transmission is regulated, to test more rigorously to what extend this cross-talk occurs physiologically, and to determine how volume transmission interacts with synaptic activation which is presumably non-cross-activation to regulate social behaviors.

5.5 Conclusion

This dissertation work demonstrates OT and AVP can activate both V1aRs and OTRs in the regulation of social behavior but that V1aRs mediate some social behaviors (e.g. social communication) and OTRs mediate other social behaviors (e.g. social recognition and social reward). This work also shows the social behavior facilitating effects of OT and AVP in that they induce social communicative behavior, prolong

recognition of social cues used in social interaction, and enhance the magnitude of social reward. This work also discusses the functional significance of this cross-talk between OT and AVP systems. These findings provide vital information to further our current understanding on how the signaling of OT and AVP may work in the central nervous system.

5.6   Figures for Chapter 5
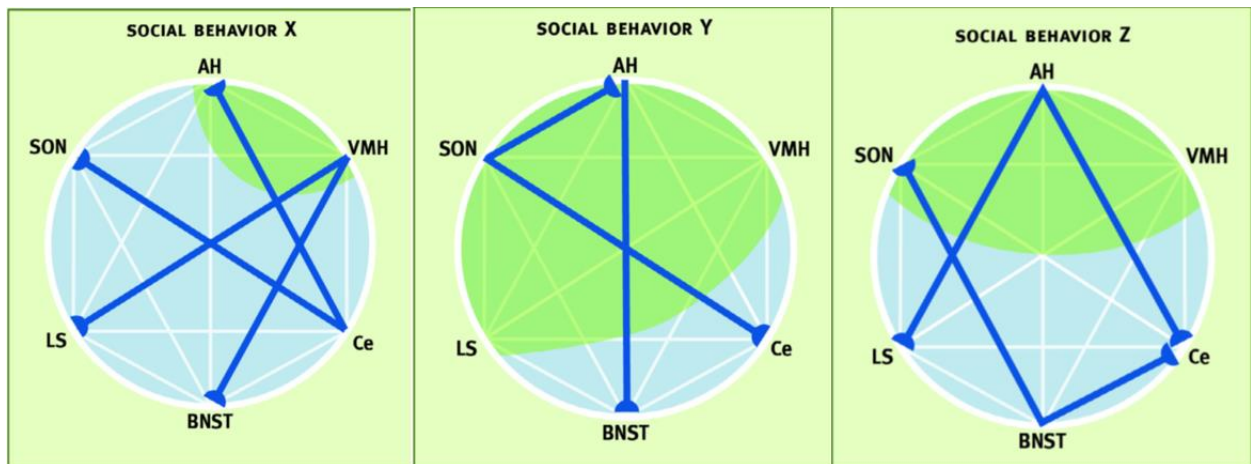
Figure 5.1 Schematic graphs on the hypothetical interaction of synaptic versus volume transmission in the regulation of different social behaviors. Blue lines indicates synaptic connections between brain sites. Green areas indicate volume transmission where all the receptors (OTRs and V1aRs) within the range are activated. Adapted from (Albers, 2015).

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Wersinger, S.R., Caldwell, H.K., Martinez, L., Gold, P., Hu, S.B. & Young, W.S., 3rd (2007) Vasopressin 1a receptor knockout mice have a subtle olfactory deficit but normal aggression. *Genes, brain, and behavior*, 6, 540-551.

Whitman, D.C. & Albers, H.E. (1995) Role of oxytocin in the hypothalamic regulation of sexual receptivity in hamsters. *Brain Research*, 680, 73-79.

Williams, J.R., Insel, T.R., Harbaugh, C.R. & Carter, C.S. (1994) Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster). *J Neuroendocrinol*, 6, 247-250.

Winslow, J.T. & Insel, T.R. (2004) Neuroendocrine basis of social recognition. *Curr Opin Neurobiol*, 14, 248-253.

Wotjak, C.T., Naruo, T., Muraoka, S., Simchen, R., Landgraf, R. & Engelmann, M. (2001) Forced swimming stimulates the expression of vasopressin and oxytocin in magnocellular neurons of the rat hypothalamic paraventricular nucleus. *Eur J Neurosci*, 13, 2273-2281.

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

Young, W.S., III, Shepard, E., DeVries, A.C., Zimmer, A., LaMarca, M., Ginns, E., Amico, J., Nelson, R., Hennighausen, L. & Wagner, K.-U. (1998) Targeted Reduction of Oxytocin Expression Provides Insights into its Physiological Roles. In Zingg, H., Bourque, C., Bichet, D. (eds) *Vasopressin and Oxytocin*. Springer US, pp. 231-240.

Zheng, D.J., Foley, L., Rehman, A. & Ophir, A.G. (2013) Social recognition is context dependent in single male prairie voles. *Anim Behav*, 86.

Curriculum Vitae

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EDUCATION:

DEGREE: Bachelor of Arts

MAJOR and ADVISORS: Biology

INSTITUTION: Northeastern Forestry University. Harbin, China (1996-2000)

DEGREE: Master of Science

MAJOR and ADVISORS: Behavioral Ecology, Dingzhen Liu

INSTITUTION: Beijing Normal University, Beijing, China (2000-2003)

DEGREE: Master of Arts

MAJOR and ADVISORS: Psychology, Robert Johnston (deceased)

INSTITUTION: Cornell University, Ithaca, NY (2003-2006)

GPA: 3.90/4

DEGREE: Doctor of Philosophy (in progress)

MAJOR and ADVISORS: Neuroscience, Elliott Albers

INSTITUTION: Georgia State University, Atlanta, GA (2010- in progress)

GPA: 3.85/4

RESEARCH INTERESTS

Functions of neuropeptides and other neurochemical signals in controlling social behaviors such as aggression, communicative behavior, reproductive behavior, social recognition, and social reward

PROFESSIONAL ORGANIZATIONS

Animal Behavior Society (2005)

Society for Behavioral Neuroendocrinology (2013, 2015)

Society for Social Neuroscience (2013-present)

International Society for Research on Aggression (2014)

Society for Neuroscience (2012-present)

PUBLICATIONS

1. Gattermann, R, Johnston, R. E., Yigit, N., Fritzsche, P., Larimer, S. C., Ozkurt, S., Neumann, K., **Song Z.,** Colak, E., Johnston, J. and McPhee, M. E. Golden hamsters are nocturnal in captivity but diurnal in nature. Biol Lett. 2008 Jun 23; 4(3):253-5.

2. Larimer, S. C., Fritzsche, P., **Song, Z.,** Johnston, J., Neumann, K., Gattermann, R., McPhee, M. E. and Johnston, R. E. Foraging behavior of golden hamsters (Mesocricetus auratus) in the wild. Journal of Ethology Volume 29, Issue 2 (2011), 275-283, DOI: 10.1007/s10164-010-0255-8

3. **Song, Z.,** McCann, K.E., McNeill, J.K.t., Larkin, T.E., 2nd, Huhman, K.L. & Albers,

H.E. (2014) Oxytocin induces social communication by activating arginine-vasopressin V1a receptors and not oxytocin receptors. Psychoneuroendocrinology, 50c, 14-19.

4. Wang, M., Li, M., Geng, X., **Song, Z.,** Albers, H.E., Maoquan, Y., Zhang, X., Xie, J., Qu, Q., He, T., (2015) Altered neuronal activity in primary motor cortex and globus pallidus after dopamine depletion in rat. Journal of the Neurological Sciences, 348, 231-240.

5. **Song, Z.,** Larkin, II, T.E, O'Malley, M, and Albers, H.E. Both arginine-vasopressin and oxytocin enhance social recognition via acting on oxytocin receptors. *Submitted to Hormones and Behavior on 9/20/2015.*

6. **Song, Z.,** Larkin, II, T.E, Borland, J.M, and Albers, H.E. Microinjection of arginine-vasopressin in the ventral tegmental area enhances conditioned place preference for social interaction via acting on oxytocin receptors. *In prep.*

INVITED TALKS

1. **Song, Z.,** McCann, K.E., McNeill, J.K., Larkin, T.E., 2nd, Huhman, K.L. & Albers, H.E. Mar., 2015. Oxytocin induces social communication by activating arginine-vasopressin V1a receptors and not oxytocin receptors. Animal Behavior Conference held at Indiana University, Bloomington, IN.

2. **Song, Z.** Dec., 2015. Cross-talk between oxytocin and vasopressin systems in the brain: roles in the regulation of social behavior. Becker's lab. Molecular and Behavior Neuroscience Institute, University of Michigan, Ann Arbor, MI.

POSTER PRESENTATIONS

1. **Song, Z**, Johnston, R, Larimer, S, Johnston, J. Animal Behavior Society, Salt Lake, Utah, 2005. Poster: Foraging Behavior in Female Golden Hamsters in the Wild.

2. **Song, Z,** McCann, K, McNeill, J, Huhman, K, Albers, E. Society for Neuroscience, New Orleans, 2102. Poster: Oxytocin: Role in reward and "prosocial" behavior.

3. **Song, Z,** McCann, K, McNeill, J, Huhman, K, Albers, E. Society for Neuroscience, San Diego, CA, 2013. Poster: Oxytocin induces social communication in male Syrian hamsters by activating V1a and not OT Receptors.

4. **Song, Z,** Larkin, T, Albers, E. International Society for Research on Aggression, Atlanta, GA, 2014. Poster: Alpha-Melanocyte-stimulating Hormone Enhances Flank Marking Behavior in Male Syrian Hamsters by Activating Arginine Vasopressin (AVP) V1a Receptors.

5. **Song, Z,** Larkin, T, Albers, E. Society for Neuroscience, D.C., 2014. Poster: Microinjection of arginine-vasopressin (AVP) in the ventral tegmental area (VTA) enhances conditioned place preference for social interaction.

6. Ross, A, **Song, Z,** Albers, E. Society for Neuroscience, D.C., 2014. Poster: Sex differences in the regulation of arginine-vasopressin (AVP) V1a receptors by social experience: Role in the expression of aggression.

7. Terranova, J, **Song, Z,** Larkin, T, Norvelle, A, Albers, E. Society for Neuroscience, D.C., 2014. Poster: Serotonin 1a receptor activity in the anterior hypothalamus contributes to sex differences in aggression in Syrian hamsters.

8. Walton, J.C., Perry, D.M., **Song, Z.,** Larkin, T., Albers, H.E. 2015. Brain and behavior program annual retreat at Georgia State University. A novel role for non-classical peptide release in the social behavior of Syrian hamsters.

9. **Song, Z,** Larkin, T, O'Malley, M, Albers, E. Society for Behavioral Neuroendocrinology, Pacific Grove, CA. 2015. Poster: Both oxytocin and arginine vasopressin enhance social recognition by acting on oxytocin receptors

10. Terranova, J, **Song, Z,** Larkin, T, Hardcastle, N, Norvelle, A, Albers, E. Society for Behavioral Neuroendocrinology, Pacific Grove, CA. 2015. Poster: Sex differences in the effects of serotonin (5-ht) and arginine vasopressin (AVP) on the neural control of aggression

11. Ross, A, **Song, Z,** Larkin, T, Albers, E. Society for Behavioral Neuroendocrinology, Pacific Grove, CA. 2015. Poster: Sex differences and social experience in the regulation of arginine-vasopressin (AVP) V1a and serotonin (5HT) 1a receptors and aggression in Syrian hamsters

12. **Song, Z,** Larkin, T, O'Malley, M, Albers, E. Society for Neuroscience, Chicago, IL. 2015. Poster: Oxytocin (OT) and arginine-vasopressin (AVP) act on OT receptors (OTRs) to influence social but not non-social recognition in Syrian hamsters (*mesocricetus auratus*)

13. Terranova, J, **Song, Z,** Larkin, T, Hardcastle, N, Norvelle, A, Albers, E. Society for Neuroscience, Chicago, IL. 2015. Poster: Sex differences in the effects of dominance status on serotonin (5-HT) and vasopressin (AVP) cell activity in Syrian hamsters (*Mesocricetus auratus*)

14. Ross, A, Larkin, T, **Song, Z,** Albers, E. Society for Neuroscience, Chicago, IL. 2015. Poster: The role of sex differences and social experience in the regulation of arginine-vasopressin (AVP) V1a receptors and aggressive behavior in Syrian hamsters (*Mesocricetus auratus*)

15. Partrick, K, Thompson, B, Larkin, T, **Song, Z,** Huhman, K. Society for Neuroscience,

Chicago, IL. 2015. Poster: Brain derived neurotrophic factor impairs consolidation of conditioned defeat learning but does not impair learning of social cues

TEACHING EXPERINCE:

1. Teaching assistant in *Introduction to Biological psychology* (Cornell University, Fall 2004)

2. Teaching assistant in *Biological Psychology Laboratory* (Cornell University, Spring&Fall 2005)

3. Lab lecturer in BIOL 1104 *Introduction to Biology* (Georgia State University, Spring&Fall 2012, Spring&Fall 2013)

4. Supervisor in *research practicum program* (Psyc 4760) in the psychology department, GSU, Spring&Fall, 2014.

STUDENT MEOTORSHIP

1. Shavindya Kaluarachchige 2011

2. Jonnsung Kim Summer 2011 (University of Notre Dame)

3. Ngoc-Thao Nguyen 2011-2012

4. Virginia Walker 2012

5. Ansa Riaz 2012-2013

6. Alex Ausborn 2014

7. Maureen O'Malley 2014-2015

8. Abiel Berhance 2015-present

9. Taylor Kahl 2015-present

EXTRACURRICULAR ACTIVITIES

1. Served as a judge for Psychology Undergraduate Research Conference (PURC) at GSU in 2011.

2. Volunteered in the organizing committee in Society for Behavioral Neuroscience Conference in 2013.

3. Volunteered in Georgia Science Olympiad in 2013.