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# **Oxytocin and Vasopressin Neuronal Activity In Response to Novel versus Familiar Object Exposure In Mice**

A thesis submitted in partial fulfilment  
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of

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# Abstract

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Oxytocin (OT) and vasopressin (AVP), peptides synthesised in the hypothalamic supraoptic and paraventricular nuclei, are released in response to social stimuli, especially those related to familiar conspecifics. Very little is known, however, about a link between OT/AVP and exposure to familiar versus novel non-social stimulation. Therefore, the current project examined whether the activity of neurons synthesising OT and AVP differs when animals are exposed to a novel versus familiar object introduced to the animal's environment. Mice were subjected to a short-term presence of a novel or familiar, distinctively shaped plastic object in a previously encountered arena and activation of OT and AVP neurons was subsequently assessed by the immunohistochemical analysis of co-localization between an immediate-early gene product, c-Fos, and OT/AVP. Exposure to a familiar object caused a significantly greater activation of OT and AVP neurons in the paraventricular nucleus of the hypothalamus, and an increasing trend approaching significance in the number of activated OT and AVP neurons in the supraoptic nucleus. In a follow-up in vivo pharmacology experiment, mice that received an intraperitoneal injection of a blood-brain barrier-penetrant OT receptor antagonist, L-368,899, prior to being exposed simultaneously to a familiar versus a novel object showed a significant increase in the amount of time spent with a familiar object and a decrease in time spent with a novel object. I conclude that introduction to novelty results in a decrease in OT neuronal activity as well as changes in AVP neuronal activity in the paraventricular nucleus of the hypothalamus.

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# List of Abbreviations

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- ARC – arcuate nucleus
- ASD – Autism Spectrum Disorder
- AVP – vasopressin
- AVPR1a – arginine vasopressin receptor 1a
- AVPR1b – arginine vasopressin receptor 1b
- AVPR2 – arginine vasopressin receptor 2
- BBB – blood brain barrier
- BNST – bed nucleus of the stria terminalis
- CeA – central amygdala
- CNS – central nervous system
- CSF – cerebral spinal fluid
- eGFP – enhanced green fluorescent protein
- FXS – Fragile X Syndrome
- HAB – high anxiety-related behaviour rats
- icv – intracerebroventricular
- ip – intraperitoneal
- LAB – low anxiety-related behaviour rats
- MPOA – medial preoptic area
- mRFP1 – monomeric red fluorescent protein 1
- NAcc – nucleus accumbens

NTS – nucleus of the solitary tract

OT – oxytocin

OTR – oxytocin receptor

PWS – Prader-Willi Syndrome

PVN – paraventricular nucleus of the hypothalamus

SON – supraoptic nucleus of the hypothalamus

WS – Williams Syndrome

# 1 Chapter 1

## Introduction

---

Oxytocin (OT) and arginine vasopressin (AVP), neuropeptides of the arginine vasotocin family, are evolutionarily conserved peptides that have important physiological and behavioural functions across the periphery and central nervous system (CNS) in mammals (Acher, Chauvet, & Chauvet, 1995; Lukas & Neumann, 2013). These behavioural functions include social interactions such as maternal care, aggression, pair bonding and sexual behaviour (Inga D Neumann, Burg, Neumann, & Burg., 2013). OT and AVP are also important mediators of the stress response, whereby expression of OT leads to the down regulation of the stress response while expression of AVP promotes angiogenesis (Robinson et al., 2002).

The focus of this thesis is on novelty versus familiarity in relation to inanimate objects. As the vast majority of current data comes from studies focusing on social interactions in the context of novel versus familiar conspecifics, the introduction to this thesis initially focuses on the social behavioural aspects of OT and AVP function but finally discusses the relatively scarce evidence that investigates OT and AVP's function in response to object novelty versus familiarity.

## 1.1 Oxytocin

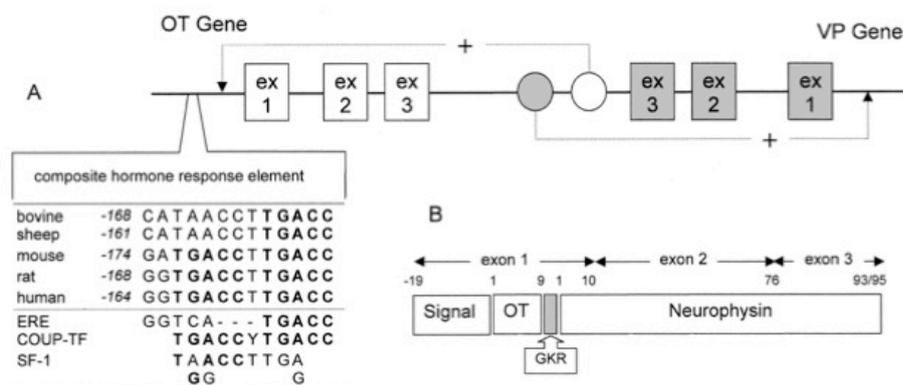
OT has been most extensively described in its involvement in parturition and lactation. Extensive research in these areas and the resulting significance of OT lead to the exploration of OT's roles in other physiological and behavioural functions.

The uterus of a pregnant female is one of the primary targets of OT. OT acts on the pregnant uterus by increasing the rate of contraction, leading to parturition (Fuchs, Fields, Freidman, Shemesh, & Ivell, 1995). OT antagonists are used to prevent preterm labour. Synthetic OT can be used to induce labour (P. D. Williams, Bock, Evans, Freidinger, & Pettibone, 1998). Uterine sensitivity to OT increases significantly around the time of labour. There is an upregulation of oxytocin receptors (OTR) which reaches its peak at the stage of early labour. The number of OTRs in a pregnant rat and a pregnant human are up to 200x that of a non-pregnant rat or human (Fuchs et al., 1995).

The milk ejection reflex is another physiological function related to reproduction in which OT is an essential component. The milk ejection reflex is the process in which milk is moved from the ducts to the nipple to allow food consumption by the infant. The suckling by the infant stimulates the PVN and SON to release OT into the bloodstream via the pituitary gland. OT then binds to the OTR located on the myoepithelial cells of the alveoli. The alveoli contract, this increased pressure pushes the milk into the ducts and is finally released through the nipple for feeding (Ramsay, Kent, Hartmann, & Hartmann, 2005).

### 1.1.1 Neurobiology of oxytocin

The OT gene is located on chromosome 2 in mice and on chromosome 20 in humans. The gene contains three exons and two introns, with intergenic regions that vary between species. The intergenic regions of the genes contain regulatory DNA sequences that control expression of OT (Gainer, Fields, & House, 2001). OT is a neurohypophysial nonapeptide hormone with a disulphide bridge linking Cys 1 and 6. The peptide consists of a six amino acid cyclic COOH-terminal alpha-amidated three-residue tail, resulting in the neutral OT amino acid isoleucine (Gimpl & Fahrenholz, 2001; Lee, Macbeth, Pagani, & Young, 2009). This neutrality of the amino acid isoleucine is essential in the recognition and binding of OT receptors (Gimpl & Fahrenholz, 2001). Figure 1.1.1 depicts the arrangement of the OT gene and its enhancers (Gimpl & Fahrenholz, 2001).



**Figure 1.1.1. The structural organisation of the oxytocin (OT) and vasopressin (AVP) genes.** *These genes are transcribed (as shown by arrows) in opposite directions perhaps supporting the theory that these genes may have arisen from duplication of an ancestral gene. Enhancers of OT and AVP genes are shown as well as the composite hormone response element that is located upstream to the OT gene promoter which is conserved across species (Gimpl & Fahrenholz, 2001).*

Primary synthesis of OT occurs in the magnocellular neurons of the paraventricular nucleus (PVN) and the supraoptic nuclei (SON) of the hypothalamus (I. Neumann, Koehler, Landgraf, & Summy-Long, 1994). The peptide is then transported from these regions to the posterior pituitary where it is stored and released into the periphery (Inga D Neumann et al., 2013). This pathway is known as the hypothalamic-hypophysial axis. OT is also synthesized in parvocellular neurons whose target terminals are within the CNS. (Caldwell, Lee, Macbeth, & Young, 2008). OT can be individually released into the periphery and CNS but can also be released simultaneously with AVP (I. Neumann et al., 1994).

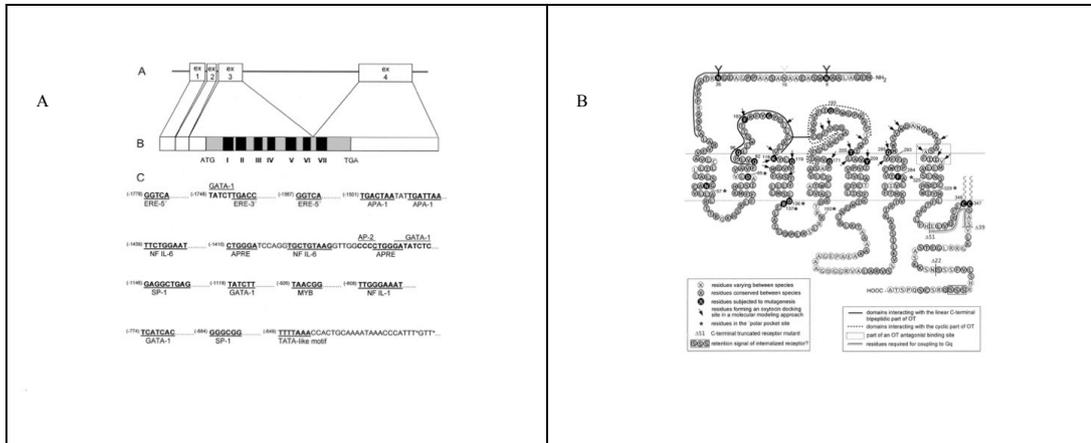
Transgenic rodent models are a beneficial tool for visualising OT originating cells, pathways, and their terminal targets. Fluorescent visualisation in transgenic rats has been used to examine OT expression in the PVN, SON and dorsal horn of the spinal cord following adjuvant arthritis chronic inflammation. The transgenic rats were used to express the OT and the monomeric red fluorescent protein 1 (mRFP1) fusion gene. The magnocellular and parvocellular OT pathways were visualized and it was concluded that OT-mRFP1 was more highly expressed in OT cells with OTR targets within the brain and periphery following periods of chronic

inflammation. Using fluorescence, this study showed that chronic inflammation activated OT neurons within the magnocellular and parvocellular divisions of the PVN which suggest that OT expression may also have a role in sensory modulation within the body (Matsuura et al., 2015).

OT is currently known to have one receptor. The OTR is a member of the G protein-coupled receptor family (Barberis, Mouillac, & Durroux, 1998),(Caldwell et al., 2008). G protein-coupled receptors are receptors that pass through the cell membrane and bind to receptor agonists which activate a transduction signalling pathway and results in targeted cellular responses that are a direct effect of the binding of the ligand, i.e. OT or AVP. (Trzaskowski et al., 2012),(Lee et al., 2009).

OTRs are widely distributed throughout the CNS. In most species OTRs within the CNS are commonly found in the olfactory bulb, hippocampal formation, central amygdala (CeA), lateral amygdala, nucleus accumbens (NAcc), neocortex and the hypothalamus (Insel, Gelhard, & Shapiro, 1991). In humans, OTRs within the CNS are found within the nucleus of the vertical limb of the diagonal band of Broca, the substantia nigra pars compacta, the ventral part of the lateral septal nucleus, the preoptic/anterior hypothalamic area, the posterior hypothalamic area, and the substantia gelatinosa of the caudal spinal trigeminal nucleus and of the dorsal horn of the upper spinal cord, the basal nucleus of Meynert, as well as in the medio-dorsal region of the nucleus of the solitary tract (Loup, Tribollet, Dubois-Dauphin, & Dreifuss, 1991). These differences in the distribution of OTRs within the CNS, is a possible explanation of the differences in social behaviours observed between species (Lee et al., 2009). Figure 1.1.2,

taken from Gimpl et al. (Gimpl & Fahrenholz, 2001), show the receptor gene and a schematic of the OT receptor with specific amino acid residues involved in ligand binding.



**Figure 1.1.2. The human oxytocin receptor gene.** (A) The organization of the human oxytocin (OT) receptor gene. (B) A schematic representation of the human OT receptor. This image shows the amino acid residues involved in ligand binding and the intracellular transduction events that happen after OT binding.

The OTR gene is expressed differently in various tissues. In the uterus and the hypothalamus, the amount of OTR gene expression is heavily dependent on sex steroids, especially estradiol. One study, using estrogen receptor knock out mice, showed that while the basal level of OTR expression was not dependent on the amount of estrogen present in the brain, it was necessary for the binding of the OT receptor within the CNS (L. J. Young, Wang, Donaldson, & Rissman, 1998). Although estrogen is thought to be important in the gene expression of the OTR, it is unclear whether OTR expression is predominantly regulated by the presence of estrogen (Gimpl & Fahrenholz, 2001). OT and AVP receptors show a high degree of sequence identity. Therefore, OT agonists/antagonists have been shown to have some affinity for AVP receptors (Barberis et al., 1998).

### **1.1.2 Oxytocin and the regulation of social behaviour in rodents**

The ability to recognise a conspecific is important for survival of self and species (Francis et al., 2014). Upon recognition of a conspecific, the ability to elicit an appropriate response and form a social memory of an individual is also important in order to survive within a social group. OT is a neuropeptide that has regulatory roles in a variety of these social processes including social behaviour, social memory and social recognition. Using OTR knock out mice, OT and its receptors has been shown to be involved in reacting to stressful stimuli and regulating social behaviour (Ferguson et al., 2000; Francis et al., 2014). OT influences the formation of social memory by affecting the processing of olfactory cues (Sanchez-Andrade & Kendrick, 2009). The formation of social memories is imperative for behaviour that fulfils an animal's need to live in a social group to be displayed (Sanchez-Andrade & Kendrick, 2009).

#### **1.1.2.1 Oxytocin and aggression**

Aggressive behaviour is usually a response to novel aspects of the animal's environment and is a means of survival. Aggression can be seen between same sex individuals, individuals of the opposite sex, as a defensive act of territory, or following capture of prey. Aggression can loosely be defined as inflicting harm or injury on another who is motivated to avoid this harm or injury (Takahashi & Miczek, 2014).

Maternal aggression is an important protective mechanism to ensure that offspring are not under threat from a novel social stimulus (Bosch & Neumann, 2012). These threats (in the laboratory) usually include conspecifics, these conspecifics can include adult male intruders or novel, female individuals (Bosch & Neumann, 2012). An initial study used to determine any link between OT and aggression showed that OT knock out mice are less aggressive than their wild type conspecifics (W. S. Young, 3rd et al., 1998). These initial findings suggest that in wild type mice, OT plays a role in aggressive behaviour. Maternal aggression has been shown to be linked with the emotionality of the species being studied. This emotionality correlates with the amount of OT released within the brain at the time of stress and perceived aggression (Bosch, Meddle, Beiderbeck, Douglas, & Neumann, 2005). High anxiety-related behaviour (HAB) rats have been bred in the laboratory to be model organisms for high anxiety behaviour. In contrast, low anxiety-related behaviour rats (LAB) have been bred in the laboratory to exhibit low levels of anxiety. HAB lactating rats were more aggressive than LAB lactating rats when exposed to the maternal defense test. The HAB rats displayed more raised back defensive postures and an increased anxiety level upon intruders. OT release in the PVN and CeA also increased in the HAB rats and only minimally increased or did not increase at all in the LAB rats (Bosch et al., 2005). LAB rats that were infused with OT into the PVN or CeA also showed an increased level of aggressive behaviour when faced with an intruder. These findings suggest that OT release in the PVN and CeA were critical for the regulation of aggression in maternal care of offspring (Bosch et al., 2005). Rodent models with lesions of the PVN also show a decrease in aggression and maternal like behaviours. Lactating rats with PVN lesion showed a decrease in the frequency of attack and the duration of attack on an imposing male compared to

the control with an intact PVN (Consiglio & Lucion, 1996). This finding indicates that the aggressive behaviours seen in nursing rats towards intruders may be linked to the PVN, the main source of OT. OT injected into the BNST was also shown to reduce maternal aggression by reducing the frequency of biting by the Wistar rat dam (Bosch, 2011).

#### **1.1.2.2 Oxytocin and anxiety**

OT also has regulatory roles in anxiety and stress-coping. OT has been shown to be an anxiolytic neuropeptide (Lukas & Neumann, 2013). Following the introduction to a stressful stimulus, the OT system is activated. This has been shown through increased OT gene expression within the SON, increased electrophysiological activity of OT neurons, and peripheral and intracerebral OT release (Gibbs, 1984; Lang et al., 1983; I. D. Neumann & Slattery, 2016; Onaka & Yagi, 1993). In male rats exposed to novelty or social defeat, there is a rapid increase in OT release into the periphery as well as the SON (I. D. Neumann, 2007). An increased release of OT into the periphery and PVN has also been seen in female rats who are defeated by an aggressive lactating dam (I. D. Neumann, Toschi, Ohl, Torner, & Kromer, 2001). It is reasonable to conclude that anxiety inducing stimuli activate the OT system, release peripheral and central OT and provide an analgesic effect. This analgesia reduces stress levels in the affected individual and allows an appropriate behavioural response to the novel or stress inducing stimulus (I. D. Neumann, Torner, & Wigger, 2000). Following the release of OT in the brain, OT acts as a modulator of behavioural activities related to anxiety. These anxiolytic effects are particularly increased during sexual

activity and lactation (I. D. Neumann et al., 2000). Female OT knock out mice show greater levels of anxiety behaviour than wild type mice during maze tests (Amico, Mantella, Vollmer, & Li, 2004). OT and its anxiolytic effects are important in an individual's response to social environments. In stress free environments, the OT system is at a low level of activity and has not been shown to have an effect on anxiety maintenance. This suggests that the OT system has a specific role in response to novel, stressful environments (I. D. Neumann & Slattery, 2016). An anxiolytic effect has been seen in female HAB rats after chronic intracerebroventricular (icv) injection of OT. When LAB female rats were given OTR antagonists they displayed an increased level of anxiety behaviours compared to LAB rats who were not treated with OTR antagonists (Slattery & Neumann, 2010).

### **1.1.2.3 Oxytocin's role in social cognition: social approach, social recognition, and social memory**

In order for social interactions to occur, an individual must be willing to approach another and/or be approached by another while displaying a minimal agonistic behavioral response. This desire for social interaction is involved in the ability to live in social groups and to aide in social memory (Lukas, Toth, Veenema, & Neumann, 2013). In order for any type of social interaction to occur, the individual must overcome social avoidance behaviours and initiate social approach (I. D. Neumann & Landgraf, 2012). The social preference test paradigm measures an individual's time spent with a social stimulus such as a conspecific compared with the time spent with a non-social stimulus, such as an inanimate

object. This choice is defined as a social preference (I. D. Neumann & Landgraf, 2012). OT has been shown to facilitate social approach and to prevent social avoidance in both rats and mice (Anacker & Beery, 2013; Ben-Ami Bartal, Decety, & Mason, 2011). OT neurons are active in the CNS during social preference behaviours. When this activity was simulated using administration of an OTR antagonist, the antagonist impaired social approach and increased social avoidance. This increase in avoidance was reversed when mice and rats were given synthetic OT following a ten minute episode of social defeat (Lukas et al., 2011). Another social paradigm used for establishing and measuring social approach has also been used to show OT's importance in social approach. This paradigm uses electric foot shocks during the investigation of a conspecific. This paradigm results in social fear conditioning which reduces social investigation and social preference of the conspecific for a short and prolonged period of time (Toth, Neumann, & Slattery, 2012). This avoidance of social approach due to the social fear conditioning has been shown to be reversed by the central administration of OT (Lukas & Neumann, 2013).

As well as social approach, the recognition of individuals and the discrimination between individuals is also an important part of the social process. In laboratory animals, social memory can be measured using social discrimination tests. These tests expose an experimental rodent to a conspecific for a period of time, the conspecific is then removed for a period of time, from 30 minutes up to days (I. D. Neumann & Landgraf, 2012). The familiar conspecific is then replaced alongside a novel conspecific. Social memory results in the experimental rodent spending less time investigating the familiar rodent, and more time investigating the novel conspecific (Engelmann, Landgraf, & Wotjak, 2004). This social investigation

includes using olfactory systems to investigate the anogenital region of conspecifics (I. D. Neumann & Landgraf, 2012). Studies investigating both OT and OT receptor knock out mice have shown the importance of the neuropeptide OT in the regulation of social recognition and memory (Lukas et al., 2013). Rats examined for their abilities to discriminate between known and novel conspecifics have shown that exposure to a previously introduced conspecific within 180 minutes of the pre-exposure are able to discriminate between a pre-exposed conspecific and a novel conspecific. These rats were then given icv administration of OT. This administration showed an effect on conspecific recognition. OT receptor antagonists have been shown to have an effect on social memory. When administered immediately after the introduction of the experimental rodent to a conspecific, the OT receptor antagonist interfered with the rat's ability to establish a memory of, and therefore recognize, a conspecific (Engelmann, Ebner, Wotjak, & Landgraf, 1998). This suggests that OT and/or its receptors are involved in the process of forming short and long term memory of conspecifics (Engelmann et al., 1998).

#### **1.1.2.4 Affiliative behaviours: maternal behaviours and pair bonding**

OT and AVP play roles at various stages. These roles include inducing delivery as well as working after the delivery to initiate and maintain nursing and maternal behaviours. This physiological adaptation to reproduction is seen as an increased amount of the neuropeptides OT and AVP being produced as well as an increase in the number of receptors produced at this time (Bosch & Neumann, 2012).

Maternal care and maternal aggression, which characterise maternal behaviour, allows a female animal to ensure her offspring's survival. Maternal care involves providing the infant with food, warmth, and adequate social stimuli. Maternal aggression constitutes the protection of the infant upon a potential threat (Bosch & Neumann, 2012). Arched back nursing is a posture of feeding that has a direct correlation with maternal care. Rats who spent more time in the arched back feeding position showed a higher level of maternal care including licking, grooming, and feeding. When OT receptors were blocked, the time mothers spent arched back nursing decreased along with other forms of maternal care given to the infants (Pedersen & Boccia, 2003). Lesion of the PVN as well as the use of OT antisense techniques result in increased maternal aggression in rats allowing us to conclude that projections of OT neurons from the PVN have a role in modulating maternal aggression (Giovenardi, Padoin, Cadore, & Lucion, 1998). Studies done on maternal aggression have shown that changes in maternal OT levels in the CNS increase in the days before parturition and increases to a maximum level in the early lactation phases. OT levels then drop off at the time of weaning (Caughey et al., 2011). These neuropeptide fluctuations are likely the cause of behavioural changes seen in mother rats when presented with an intruder. When lactating females were presented with male intruders, 84.5 percent reacted with fighting behaviours in order to protect their young (Erskine, Denenberg, & Goldman, 1978). Studies in rats have shown that the blockade of the OT receptor with an OT antagonist following parturition leads to a reduction in the onset of maternal behaviour. This reduction of maternal behaviour consisted of time spent grouping pups, time spent on the nest with pups, time grooming pups and time feeding pups (van Leengoed, Kerker, & Swanson, 1987). OT knock out mice

showed a reduction in the maternal care provided to foster pups compared to wild type mice. OT knock out mice that were placed with foster pups showed significantly less maternal behaviour such as retrieving pups, pup-licking frequency, and time spent crouching over pups on the nest. This suggests that OT may play a role in the motivation to retrieve pups and to take them to a safe location (Pedersen, Vadlamudi, Boccia, & Amico, 2006).

Pair bonding is a reproductive social behaviour that is important in reproduction and care of offspring. Pair bonding is facilitated by OT and has been extensively studied in the prairie vole which exhibits life-long monogamous relationships (Lukas & Neumann, 2013). Pair bonding is defined and assessed by the partner preference paradigm in which an increased amount of time is spent in close proximity to the pair bonded partner than in close proximity to a novel conspecific (L. J. Young & Wang, 2004). Pair bonding in females is facilitated by the release of OT. In female prairie voles, icv injection of synthetic OT induces pair – bonding between a female and male prairie vole without previous mating. Pair – bonding prior to mating has also shown to be prevented when pre-treatment of icv OTR antagonist is used (L. J. Young & Wang, 2004). OTR binding in the NAcc has also been shown to be increased in a prairie vole in comparison to a non-monogamous female montane vole. This difference in OTR binding further supports the specific role of OT and pair-bonding in prairie voles (Insel & Shapiro, 1992).

When OT's neuronal contributions in pair bonding were studied, it was found that no matter the species studied or its monogamous or non-monogamous lifestyle, OT's immunoreactivity of neurons was consistent in a number of locations across

numerous brain regions including the PVN, SON, and the BNST (K. A. Young, Gobrogge, Liu, & Wang, 2011). Further studies found that while OT neurons were not significantly different in monogamous and non-monogamous species, the number and patterns of OT receptors did differ between monogamous and non-monogamous species (Hammock & Young, 2002). Therefore, OT receptors may be responsible for these differences in reproductive strategies between monogamous and non-monogamous species of rodents.

### **1.1.3 OT has been linked to social abnormality**

The OT receptor gene has been shown to have a haploinsufficiency effect (Sala et al., 2013) on the phenotype of an individual. SIM1 haploinsufficiency results in the reduction of the bHLH-PAS transcript. The SIM1 transcript is needed for the correct formation and development of the neurons within the PVN and SON. Because the neurons that extend from the PVN and SON play a critical role in homeostatic processes and the secretion of OT and AVP, the SIM1 gene is an effective method for analysing the effects of gene deletion or haploinsufficiency (Swanson & Sawchenko, 1983). Mice born with the full deletion of the SIM1 gene die shortly after birth while mice born with SIM1 haploinsufficiency display a phenotype with a reduction in the number of OT and AVP neurons (Duplan, Boucher, Alexandrov, & Michaud, 2009). Mice who are heterozygous for the OTR gene (*OTR*<sup>+/-</sup>) are shown to have a gene dosage effect on behavioural phenotype (Sala et al., 2013). Sala et al found that (*OTR*<sup>+/-</sup>) mice had 50% less OT receptors in the brain than homozygous (*OTR*<sup>+/+</sup>) mice. OTR null mice (*OTR*<sup>-/-</sup>) have increased aggression, impaired cognition, impaired sociability and a preference for social novelty while (*OTR*<sup>+/-</sup>) have normal social functioning in

aggression and cognition (Sala et al., 2013). This suggest that the OTR has a haplosufficiency effect with gene dosage affecting the severity of social abnormality depending on the transcription of the gene.

OT dysregulation has been investigated in Autism Spectrum Disorder (ASD), Prader-Willi Syndrome (PWS), and Williams Syndrome (WS). These neurodevelopmental disorders are linked with behavioural and social deficits in individuals in which they effect (Francis et al., 2014). ASD arises from interactions between genetic predisposition and environmental factors (Bailey et al., 1995). Currently there is no treatment for ASD but there are several current studies using intravenous OT or intranasal OT to treat the core symptoms of ASD. Andari et al. found that ASD individuals treated with IN-OT interacted in a social partner game more than the control and had enhanced feelings of trust with that person. These same persons were then studied for gazing time at a face following IN-OT administration and the gazing time was increased. This study found that when patients were administered OT, they had more appropriate social behaviours and responded more strongly to others (Andari et al., 2010). PWS is characterised by behavioural difficulties, stubbornness, difficulty reading facial expressions, compulsive behaviours, difficulties in language development and eating abnormalities (Francis et al., 2014). People with PWS have a decreased number of neurons that produce OT in the PVN along with a decrease in the amount of OT found in the CSF (Martin et al., 1998; Swaab, Purba, & Hofman, 1995). Dombret et al. showed that mice with a loss of the Maged1 gene, used as a model for PWS, treated with OT were shown to have increased social interactions and an increased level of OT in the hypothalamus (Dombret et al., 2012). Human individuals affected by PWS who were treated with IN-OT showed a decrease in

disruptive behaviours and an increase of trust in others (Tauber et al., 2011). WS has a behavioural phenotype of being socially fearless with poor judgement and a high drive to have social interactions. These individuals also have high anxiety levels (Francis et al., 2014). WS seems to be the reciprocal of ASD as people with WS have an increase in OT levels compared to controls and display an increase in approach to strangers and a decrease in the ability to recognise and adapt to social behaviours (Dai et al., 2012). These findings suggest that OT may have a dosage effect with high doses of OT possibly leading to WS and low doses possibly leading to ASD. As there seems to be disruptions in the OT system, and resulting behavioural disorders, OT is a good candidate for treatment research in these genetic diseases.

## **1.2 Vasopressin**

The main focus of studies on physiological roles of AVP has revolved around osmolality regulation (including downstream consequences for cardiovascular parameters) as well as circadian rhythmicity. Circulating levels of AVP control the regulation of water re-absorption. As blood osmolality increases, hypothalamic neurons release AVP mostly targeting AVPR2s in the kidney where permeability of water is increased. This causes an increase in water conservation and a decrease in blood osmolality (Caldwell et al., 2008). AVP and AVPR1a, but not OT, have been found in the suprachiasmatic nucleus (SCN) where they are involved in regulating circadian rhythmicity (Li, Burton, Zhang, Hu, & Zhou, 2009; Moore, 1983). Each animal's behaviour and physiology is influenced by

this circadian rhythm or clock within the brain. AVP is found to be approximately five times higher in the cerebral spinal fluid (CSF) during the morning hours than during the hours of the night (Reppert, Artman, Swaminathan, & Fisher, 1981). An AVPR1a reporter has been used to show that a deficiency in this reporter can lead to a decrease in the circadian rhythmicity of *V1aR* deficient (*V1a<sup>-/-</sup>*) mice. This finding links VP to important social behaviour in rodents such as sleep – wake cycles (Li et al., 2009).

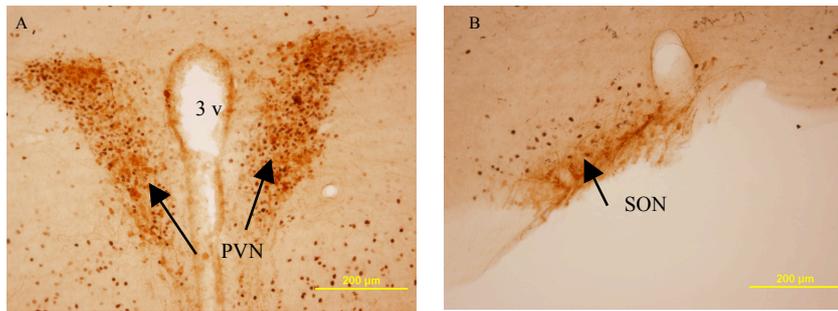
Fewer studies have investigated AVP and its importance in social behaviours compared to the vast amount of studies that have been performed on the importance of OT in social and physiological functions (Insel & Shapiro, 1992; Lukas et al., 2011; Sala et al., 2013). Since OT and AVP are so genetically and structurally similar, even exhibiting affinity for the other's receptor (Gimpl & Fahrenholz, 2001), it is important to understand AVP's roles and effects within the periphery and CNS in relation to behaviour and novelty. Specifically, any effects AVP may have on an individual's preference for a familiar versus a novel conspecific or object.

### **1.2.1 Neurobiology of vasopressin**

The AVP gene is located on chromosome 2 in mice and on chromosome 20 in humans. The gene contains three exons and two introns with intergenic regions that vary between the species. The intergenic regions of the genes contain regulatory DNA sequences that control expression of AVP (Gainer et al., 2001). AVP differs from OT by only two amino acids at positions 3 and 8 (Lee et al., 2009). While the AVP gene is on the same chromosome as the OT gene, AVP is

orientated in an opposite transcriptional direction, suggesting that it evolved from a duplication of the same gene (Caldwell et al., 2008) (see Figure 1.4). AVP is also a neurohypophyseal nonapeptide hormone but because of the change in amino acids, it results in a basic amino acid (lysine, arginine) instead of the neutral amino acid found in OT (Gimpl & Fahrenholz, 2001). This Lys, Arg change in the AVP compound is essential in the recognition of AVP by the AVP receptors (Gimpl & Fahrenholz, 2001). See Figure 1.1.1 for the structural organisation of the AVP gene and its transcriptional direction.

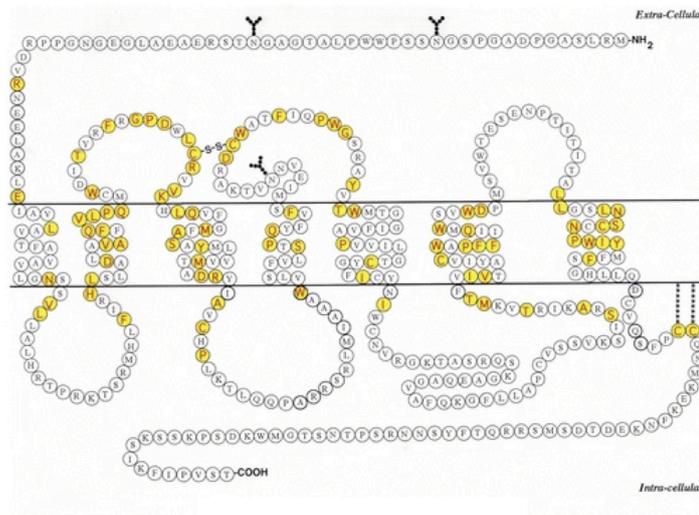
Alongside OT, the primary synthesis of AVP occurs in the magnocellular neurons of the PVN and the SON of the hypothalamus (I. Neumann et al., 1994). The peptide is then transported from these regions to the posterior pituitary where it is stored and released into the periphery (Inga D Neumann et al., 2013). This pathway is known as the hypothalamic-hypophyseal axis. As well as in magnocellular neurons, AVP is synthesized in parvocellular neurons whose axons remain within the CNS. The release of AVP from these projections allows the peptide to act within the CNS at its target receptors (Caldwell et al., 2008). AVP can be released individually into the periphery and CNS but can also be released simultaneously with OT (I. Neumann et al., 1994).



**Figure 1.2.2. Localization of vasopressin (AVP) cells within the paraventricular nucleus of the hypothalamus (PVN) and the supraoptic nucleus (SON) in a cross section of the mouse brain stained for AVP. (A) depicts a bilateral image of AVP neurons stained within the PVN. (B) depicts AVP neurons stained within the SON. 3v: third ventricle.**

AVP receptors are members of the G protein-coupled receptor family (Barberis et al., 1998). AVP has three receptors, AVPR1a, AVPR1b, and AVPR2 (Caldwell et al., 2008). AVPR1a and AVPR1b bind to GTP binding proteins and act through the phospholipase C activity while AVPR2 couple to G's and act through the cyclic AMP system (Bankir, 2001). AVP receptor binding in the brain is found in the bed nucleus of the stria terminalis, the amygdala, the NAcc, the diagonal band of Broca, the dorsal hippocampus, and the caudate nucleus (Dumais & Veenema, 2016). The AVPR1a is found in the liver, kidney and vasculature (Caldwell et al., 2008). The AVPR1b is found in the anterior pituitary but is detected in many other tissues throughout the body (Caldwell et al., 2008). AVPR2 is also found in the kidney (Caldwell et al., 2008). Again, AVP receptors show a high degree of sequence identity with OT receptors, therefore AVP agonists/antagonists have been shown to have affinity for OT receptors (Barberis et al., 1998). This alternate binding may have interaction consequences or physiological effects on the organism (Francis et al., 2014). The following image

depicts the AVPR1a and its ligands for binding (Thibonnier, Coles, Thibonnier, & Shoham, 2001).



**Figure 1.2.3. Vasopressin 1a receptor and its ligands for binding.** This image shows the amino acid residues that are involved in ligand binding and the intracellular transduction events that happen after vasopressin binding.

Enhanced green fluorescent protein (eGFP) transgenic rats are used to track AVP expression and its targets. The VP-eGFP fusion gene has been used to study AVP release in response to nociceptive stimulation (Suzuki et al., 2009). Suzuki et al. measured AVP-eGFP fusion gene expression following subcutaneous injection of saline or formalin into the paws of rats. This study, using the visualisation of the eGFP, showed that AVP levels in plasma were increased 15 min after formalin injection. AVP levels in the PVN were also increased, especially in the parvocellular division of formalin injected rats (Suzuki et al., 2009).

## **1.2.2 Vasopressin and the regulation of social behaviour in rodents**

Although fewer studies have focused on the importance of AVP in response to social stimuli, AVP has regulatory roles in a variety of social processes including social behaviour, social memory and social recognition. AVP has receptors in the PVN and SON that are important for regulating social behaviour and reacting to stimuli which may be stressful (Francis et al., 2014).

### **1.2.2.1 Vasopressin and anxiety**

AVP also has regulatory roles in anxiety and stress-coping. While OT has been shown to be an anxiolytic neuropeptide (Lukas & Neumann, 2013), AVP has been shown to have anxiogenic effects (Heinrichs, von Dawans, & Domes, 2009). Studies exploring AVP's link with anxiety have shown that anxiety in rats is increased by AVPR1a agonists while AVPR1a antagonists reduce anxiety (Mak, Broussard, Vacy, & Broadbear, 2012), (Appenrodt, Schnabel, & Schwarzberg, 1998). Transgenic mice that lack the AVPR1a and genetically mutated rats that do not produce AVP show lower levels of anxiety than wild type rats (Zelena et al., 2008). As well as anxiogenic effects, the release of AVP from the PVN and SON following stress and anxiety triggers the release of adrenocorticotropin from the anterior pituitary and glucocorticoids from the adrenal glands. The release of these glucocorticoids produces an array of effects in response to the stressful stimulus. These responses include mobilising energy, increasing cardiovascular tone and delaying processes in the body that are not critical and controlling osmolality (Fodor et al., 2016).

### **1.2.2.2 Vasopressin and aggression**

AVP is co-released with OT within the brain and has been shown to increase aggression in lactating rats protecting their offspring. Following the infusion of AVPR1a antagonist, the time of initiation of first attack in Wistar rats was reduced. There was also a reduction in the overall aggressive behaviour of female, lactating Wistar rats (Bosch, 2011). AVP has been used in HAB and LAB dams to show changes in levels of maternal aggression as a result of AVP release in the brain. As discussed previously, HAB rats show more maternal care and aggression than LAB female rats. In more aggressive HAB female rats, a single injection of an icv AVPR1a antagonist decreases the frequency of aggressive behaviours while an icv infusion of synthetic AVP in LAB female rats increases the frequency of aggressive behaviours following intrusion by a conspecific (Bosch, 2011). The release of AVP has been shown to rise in the CeA of HAB rats, but not LAB rats, when rats are exposed to the maternal defense test. This rise in AVP within the CeA correlated with the observation of increased maternal aggression in the HAB rats, and not the LAB rats, when the rats were exposed to novel conspecifics (Bosch & Neumann, 2010).

### **1.2.2.3 Vasopressin and affiliative behaviours: maternal behaviours and pair bonding**

AVP levels in the CNS increase in the days before parturition, finally increasing to a maximum level in the early lactation phases. AVP levels then drop at the time of weaning (Caughey et al., 2011). When lactating females were presented

with male intruders, over 80 % initiated aggressive behaviours in order to protect their young (Erskine et al., 1978).

A role of male prairie voles, facilitated by pair bonding, is to guard nests from intruders (K. A. Young et al., 2011). There is a difference in importance of OT and AVP between the sexes in relation to pair bonding. In male prairie-voles, AVP is the neuropeptide associated with pair-bonding. Centrally administered AVP in male prairie-voles increases partner preference without prior mating. During mating, male prairie-voles have an increased release of AVP in the ventral pallidum. This region also has an increased number of AVPR1a receptors in comparison to non-monogamous montane voles (Insel & Shapiro, 1992).

#### **1.2.2.4 Vasopressin's role in social cognition: social approach, social recognition, and social memory**

The importance of AVP in social approach has not been studied in as much detail as OT. One investigation in the role of AVP in social approach showed that an AVPR1a antagonist had no effect on the social approach behaviour of male Wistar rats (Lukas et al., 2011). Another study showed that the use of an AVPR1b antagonist reduced social avoidance as a result of social defeat in mice (Litvin, Murakami, & Pfaff, 2011). Icv injection of AVP into male rats has been shown to result in prolonged social memory formation. Rats exposed to an icv AVP injection following exposure to a conspecific were able to increase their conspecific social recognition time by 120 minutes. These studies have shown that AVP injected into the central nervous system can increase the rat's ability to form and consolidate social memories (Bielsky & Young, 2004).

### **1.3 Oxytocin and vasopressin: empathy and pro-social behaviour**

There is strong support that empathy has significant evolutionary underpinnings and is thought to have evolved alongside parental care as it is important for a mother care such as nurturing and feeding. This initial maternal empathy is thought to have evolved over time into the ability to recognise and respond to the behaviours of others (Gonzalez-Liencre, Shamay-Tsoory, & Brune, 2013). The ability to recognize another individual's situation was of an evolutionary advantage to a species (McEwen & Akil, 2011). The experience of empathy allows individuals to live in social groups and allows bonding between individuals such as pair bonding and parent-offspring bonding. The definition of empathy is broad, and ranges from having feelings of concern for others to having feelings and emotions that mimic another individual's feelings and emotions (McEwen & Akil, 2011). The experience of empathy and empathetic motivation leads into pro-social behaviour. Pro-social behaviour is defined as an action or actions that are intended to benefit another individual (Ben-Ami Bartal et al., 2011). Rats have been shown to be capable of empathetic and pro-social behaviour. Rats that were previously housed together showed empathy and pro-social behaviour when their familiar conspecific was placed in a restrainer. The free rat would, quickly and intentionally, open the restrainer and free the familiar conspecific even when other rewards were offered such as chocolate. These rats showed that an individual acts pro-socially, via helping behaviours, when the individual detects and responds to a familiar conspecific's distress. The rats did not open the door to the restrainer when the restrainer was empty or when it housed a toy (Ben-Ami Bartal et al., 2011). A second example of rats showing empathy and pro-social behaviour can

be seen when known conspecifics were soaked with water. A rat that witnessed a soaked, familiar conspecific inside a water area rapidly showed door opening behaviour. When the same rats were put in the arena and a conspecific was not inside the water area, the rats showed no interest in opening the water area door. Rats that had previously experienced the soaking water area opened the door for a familiar conspecific more quickly than rats who had not previously experienced soaking. This study also showed that rats would help a familiar conspecific escape the soaking water area before they would obtain a food reward. The results show, especially following the increased release time by rats who had previously experienced the water area, that rats can behave pro-socially and may be experiencing feelings of empathy towards their familiar conspecifics (Sato, Tan, Tate, & Okada, 2015).

As OT and AVP have been affiliated with a wide range of social behaviours, OT and AVP may also be involved in the regulation of empathy and pro-social behaviours that are shown above. One experiment analysed a single polymorphic region (SNP) within the OTR and a polymorphic repeat allele within the AVPR1a. The experiment selected regions that have been previously shown to contribute to empathetic and prosocial behaviour (Uzefovsky et al., 2015). The OTR SNP rs53576 has been shown to increase behaviours of empathy in both clinical and non-clinical test subjects (Bryan et al., 2009) and the AVPR1a-327 repeat allele has been associated with lower amounts of altruistic giving and autism (Avinun et al., 2011), (Kim et al., 2002). The presence of these two polymorphisms, the OTR SNP rs53576 or the AVPR1a-327 repeat allele, in individuals resulted in a lower level of empathy when the individuals were assessed using questionnaires

that reported levels of empathy (Uzefovsky et al., 2015). This experiment showed a link between OT, AVP, and empathy.

#### **1.4 Oxytocin, vasopressin and Novelty: focus on responsiveness to objects**

An individual's preference for familiar versus novel conspecifics has been shown to be effected by OT (Madularu, Athanassiou, Yee, Kenkel, et al., 2014). Social discrimination tests, as described previously, showed that without an experimental variant, an individual would spend more time exploring a novel conspecific than a familiar conspecific, these individuals are able to discriminate between a previously explored conspecific and a new one (Engelmann et al., 2004). OT has effects preference for familiar versus novel conspecifics in prairie voles. OT administered to female prairie voles increased time spent with a familiar conspecific versus a novel conspecific. These familiar conspecific preferences are diminished using OTR antagonists in the same animals (Madularu, Athanassiou, Yee, & Mumby, 2014).

Familiarity and novelty are characteristics that apply not only to individuals, but also to inanimate to objects. Novelty is a change from the expected event or stimulus based on previous experience and internal estimates of outcome of the new stimulus (Antunes & Biala, 2012). The drive to explore novel objects is thought to simultaneously create conflict within rodents by evoking avoidance and approach behaviours. Approach behaviours reflect an animal's willingness to explore a novel object while avoidance behaviours reflect the animal's fear of novelty (Dulawa, Grandy, Low, Paulus, & Geyer, 1999). Animals tend to explore novelty because of their natural drive to do so, this allows us to assume it is

beneficial to the individual. Although, this natural drive can be observed, the exact physiological changes leading to this drive are still unknown (Baxter, 2010). Two papers, one using rats and one using non-human primates, suggest that preference for novelty may stem from incest avoidance and preference for unfamiliar individuals (Carter, DeVries, & Getz, 1995; Parr, Heintz, Lonsdorf, & Wroblewski, 2010). In contrast to exploring novelty, when individuals are in safe, familiar environments, they have lower anxiety levels (Anacker & Beery, 2013). The exploration of a novel environment or stimulus can be a risky behaviour as it may increase the level of exposure one experiences in relation to predators or sources of infection. Therefore, in order to approach a novel stimulus, one not only needs to not only have the curiosity to explore but also needs to suppress risk avoidance (Smith, Wilkins, Mogavero, & Veenema, 2015). While individuals explore novelty, there are also avoidance behaviours because there is safety in familiarity. Although it is clear that OT facilitates preference for familiar conspecifics, it is unclear if OT, or its closely related neuropeptide AVP, facilitates preference for inanimate, or novel, non-social objects.

One study using calves to investigate the serum levels of OT and the resulting investigative nature of the calf showed that an increased level of serum OT concentration resulted in an increased amount of time investigating novel objects (Chen, Tanaka, Ogura, Roh, & Sato, 2015). A 1993 experiment showed that rats put in a novel environment of a painted black box coupled with an auditory stimulus had decreased plasma levels of AVP compared to a control and had no change in OT expression (Onaka & Yagi, 1993). Another study using prairie voles to assess object preferences found that saline treated controls displayed preference for novel objects opposed to familiar objects, OT treated voles did not

demonstrate an object preference, and OTR antagonist treated voles showed preference for the novel object (Madularu, Athanassiou, Yee, Kenkel, et al., 2014). A similar study using rats to assess object preference injected OT into the left ventricle. After 30 minutes, injected rats showed a preference for the familiar object while saline injected rats showed preference for novel objects. Four hours after injection, both the OT injected rats and the saline injected rats showed a preference for novel objects (Madularu, Athanassiou, Yee, & Mumby, 2014). Together, these studies suggest that OT either has no effect or increases an individual's exploration of a familiar object while AVP levels decrease in response to novelty. While these studies have interesting conclusions about the importance of OT and preference for novelty, they have not analysed the neuronal activity of OT in response to novel versus familiar exposure to objects.

Effects of a novel environment or stimulus on the expression of OT and AVP may be helpful in determining whether these neuropeptides are important in the brain's regulation of the response to novel stimuli. As discussed previously, OT and AVP have been implicated in the stress response. Central and peripheral levels of OT increase in response to stressful stimuli including novel environments, conditioned fear and noxious stimuli (I. Neumann et al., 1994; Onaka, 2004). A novel object compared to a familiar one may be stressful to an individual. OT and AVP's roles in stressful responses as well as their roles in familiar versus novel preference suggest that they may be good candidates for studying neuronal changes within the brain as a response to novelty. As we are aware that OT and AVP are highly expressed in the PVN and SON (W. S. Young, 3rd & Gainer, 2003), it is reasonable to explore these areas in relation to neuronal activity in mice exposed to a familiar versus a novel stimulus.

## 1.5 Specific Aims

While current literature is successful in identifying many of the physiological and behavioural responses associated with OT, AVP and their receptors, there is still little evidence to confirm if there are any changes of OT and/or AVP neuronal activity within the paraventricular nucleus and supraoptic nucleus of the hypothalamus in response to a novel versus a familiar stimulus. It is expected that activity of OT and AVP neurons will be suppressed in animals exposed to a novel (thus, anxiety inducing) object stimulus. Therefore, the current set of studies will determine (a) OT-R blockade's effects on the amount of time spent with a novel object (b) the PVN/SON OT neuronal activity changes in response to exposure to a novel object (c) the PVN/SON AVP neuronal activity changes in response to exposure to a novel object. These findings will lead to a better understanding of neuroendocrine responses to novelty, underpinning further research on health pathologies that are associated with abnormal behaviours in novel social and inanimate environments, e.g., as seen in individuals with social abnormalities.

## **2 Chapter 2**

### **Methods**

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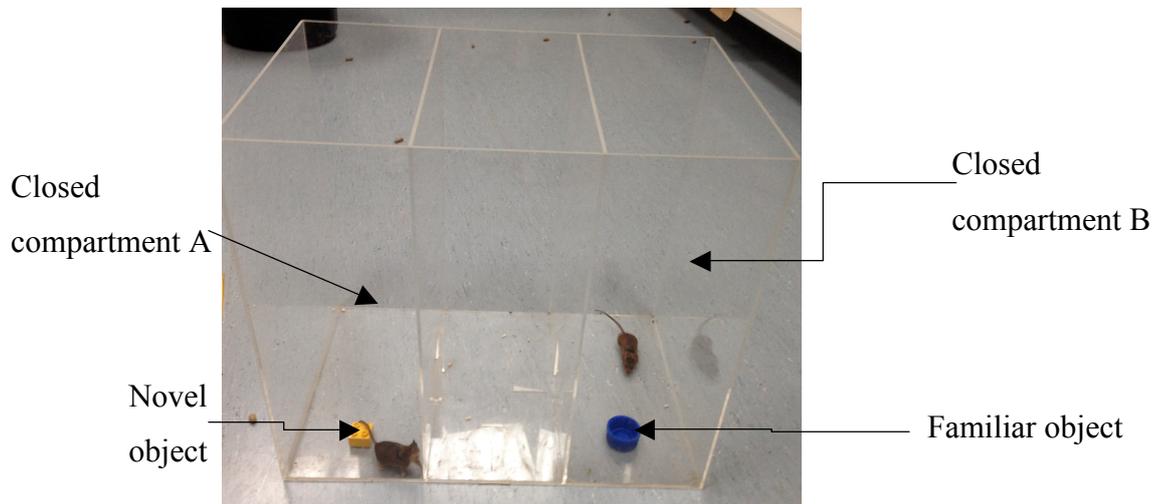
#### **2.1 Animals**

All experiments were approved by the University of Waikato's Ethics Committee and adhered to all policies put in place by the Animal Welfare Act 1999.

Female mice, strain C57BL/6, were housed in a 12 hour light/dark cycle (lights on at 07:00) and were 12 weeks old. The mice were housed with two other females in a 23°C room. The animals weighed an average of 25 g. Mice were provided with ad libitum access to standard rodent chow (Teklad) and tap water until the beginning of the experiment. Experiments were performed between 9am and 12pm.

#### **2.2 Experimental Arena**

The experimental arena consisted of a large, transparent plexiglass container, which was separated into three compartments. The compartments were equally sized and were separated by two plexiglass walls. The mice were not able to move between compartments. In the left and right compartments, not in the middle neutral zone, a mouse was placed with either a familiar blue bottle lid or a novel yellow plastic brick. These objects were taped to the floor of the compartment to ensure the object was not moved by the individual. This experimental area is shown in Fig 2.1.



**Figure 2.1.1 Experimental set up of the arena for the novel vs familiar exposure of an object.** This experimental arena consists of three plexiglass compartments. The middle compartment remained neutral, or without an individual placed in it while the right (compartment B) and left (compartment A) compartments were used for the study. The novel brick was placed in compartment A and the familiar object was placed in compartment B.

### **2.3 Experiment 1: OT receptor antagonist L-368,899 and its effect on time spent exploring familiar and novel objects**

Each animal was subjected to habituation, familiarization and testing sessions. Two habituation sessions (10 min each) in which animals were placed in the testing apparatus (60x60 cm arena; grey floor, camera placed 90 cm above) were administered 1 and 2 days before the familiarization session. In the familiarization session (5 min), mice were allowed to investigate two identical objects in the apparatus; the two objects presented at that time were not used in subsequent testing.

The test objects were glued to the bottoms of the arena at opposing corners. The test objects were blocks varied in height and width (6x3x2 and 10x3x2 cm). The objects were attached onto the flooring at 5 cm from opposing corners. Familiar objects were placed for 24 h before the test in the cage.

Injections: Each mouse was accustomed to receiving intraperitoneal (IP) injections. IP drug administration was performed 5-10 minutes prior to the beginning of each test. L-368,899 (Tocris) was dissolved in isotonic saline just prior to use and injected at the dose of 1mg/kg body weight.

Novel object preference was assessed 30 min after the familiarization session. All mice received both treatments (saline or L-368,899), but on different testing days. As such, each mouse was subjected to two testing sessions. Each session was recorded and object preference was determined as time investigating the novel object divided by total time investigating both objects. A mouse was considered to be investigating an object when its head was oriented within 45° and 1 cm from the object. Rearing with the head oriented upward was also considered, if at least one paw was placed on the object. Climbing on the object or sitting on it was excluded. A t-test was used to test for significance. Results were significant with a p value less than or equal to 0.05.

## **2.4 Experiment 2: Activation of OT and AVP neurons in the PVN and SON following exposure to a familiar or novel object in a familiar environment.**

Mice were group housed in cages. The groups consisted of five mice. Cage 1 housed the five mice exposed to a familiar stimulus (blue lid) and cage 2 housed the five mice exposed to a novel stimulus (yellow plastic brick). Each day for three days prior to the experiment, all experimental mice were individually placed in the arena for 30 minutes to ensure a reduction in any change of neuronal activity that may result from exposure to the new arena. The order of the pre-experimental exposure was random.

Five mice, from cage 1, were individually placed in the left compartment of the arena with a familiar blue bottle lid. This lid was taken from their cage. The lid had been left in their cage for three days prior to the experiment to maximise exposure. Five mice, from cage 2, were individually placed in the right compartment of the experimental arena with a novel yellow plastic brick. Both sets of mice, the familiar and the novel exposure mice, were left in the arena for 30 minutes with their test object. The test subject was then removed, their tail was marked and they were put back in their cage for 30 minutes.

30 minutes after exposure to the test stimulus, to allow time for neuronal activity response, animals were deeply anesthetized with pentobarbital and perfused through the aorta with 5 ml of 0.9% saline followed by 50 ml of 4% paraformaldehyde (PFA) in 0.1 m phosphate buffer (pH 7.4). Brains were dissected out and post-fixed overnight in the same PFA fixative at 4°C.

### 2.4.1 Sectioning and staining

Coronal 50- $\mu$ m brain coronal sections were then cut using a Vibrotome (Leica). Sections were then processed for single and double immunohistochemistry. Every fourth brain section was used in the single c-Fos staining, and every fourth one containing the PVN was stained for c-Fos and OT. There were equal distances between sections used in the immunohistochemistry experiments.

Sections were treated for 10 min in 3% H<sub>2</sub>O<sub>2</sub> and 10% methanol [diluted in Tris-buffered saline (TBS), pH 7.2] and incubated for 48 h at 4°C in primary goat anti-Fos antibody (1:1100; Santa Cruz Biotechnology, Santa Cruz, CA). Subsequently, tissue was incubated for 1 h in rabbit anti-goat antibody (1:400; Vector Laboratories, Burlingame, CA). After a 1-h incubation in avidin-biotin complex (ABC, 1:800; Vector), peroxidase was visualized with 0.05% 3,3'-diaminobenzidine tetrahydrochloride (Sigma Diagnostics, St. Louis, MO), 0.01% H<sub>2</sub>O<sub>2</sub>, and 0.3% nickel sulfate. All incubations in antibodies were performed in the TBS-based mixture of 0.5% Triton X-100 (Sigma Diagnostics) and 0.25% gelatin (Sigma Diagnostics). Intermediate rinsing steps were done in TBS.

After the completion of c-Fos staining, PVN and SON sections were further processed for double staining and the visualization of OT and AVP. The procedure was similar to that described above. However, rabbit anti-OT or goat anti-AVP was used as the primary antibody (1:17,000; Millipore, Temecula, CA), and nickel sulfate was not added to the 3,3'-diaminobenzidine tetrahydrochloride solution to obtain the brown instead of black color.

Sections were then mounted on gelatin-coated slides and dried in a fume hood. After 24 h of drying, sections were dehydrated in ascending concentrations of

alcohol, soaked in xylene, and embedded in DPX (Fluka, Steinheim, Germany) and analyzed using light microscopy.

In the double staining, the following estimates were assessed per section and then per PVN and SON: the total number of OT or AVP positive neurons and the total number of neurons positive for c-Fos. Cells were counted bilaterally, and the percentage of neurons containing c-Fos-positive nuclei was tabulated.

Statistical analysis was done using an unpaired Student's t-test and values were considered significantly different when  $P < 0.05$ .

# 3 Chapter 3

## Results

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### Experiment 1: OT receptor antagonist L-368,899 and its effect on time spent exploring familiar and novel objects

**Table 3.1 Novel versus familiar object investigation following oxytocin receptor (OTR) antagonist intraperitoneal injection.**

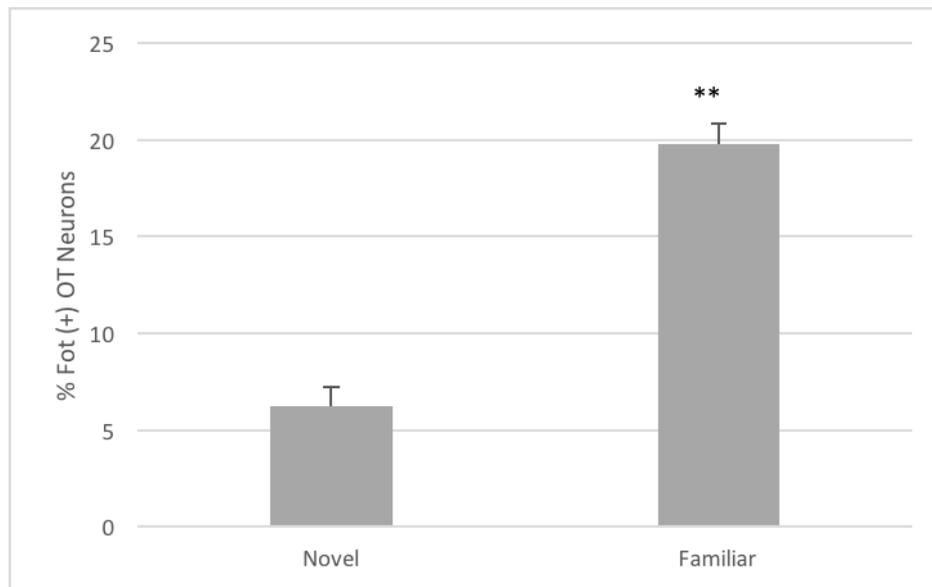
	Novel object investigation	Familiar object investigation	Novel/familiar object investigation ratio
saline	61.5 ± 5.0	45.2 ± 5.2	1.33 ± 0.14
L-368,899	40.6 ± 4.8*	73.1 ± 5.7*	0.55 ± 0.06*

Following injection, mice were exposed to a familiar and a novel object in a familiar environment. The amount of time spent investigating the novel vs familiar object was determined. \*P<0.05.

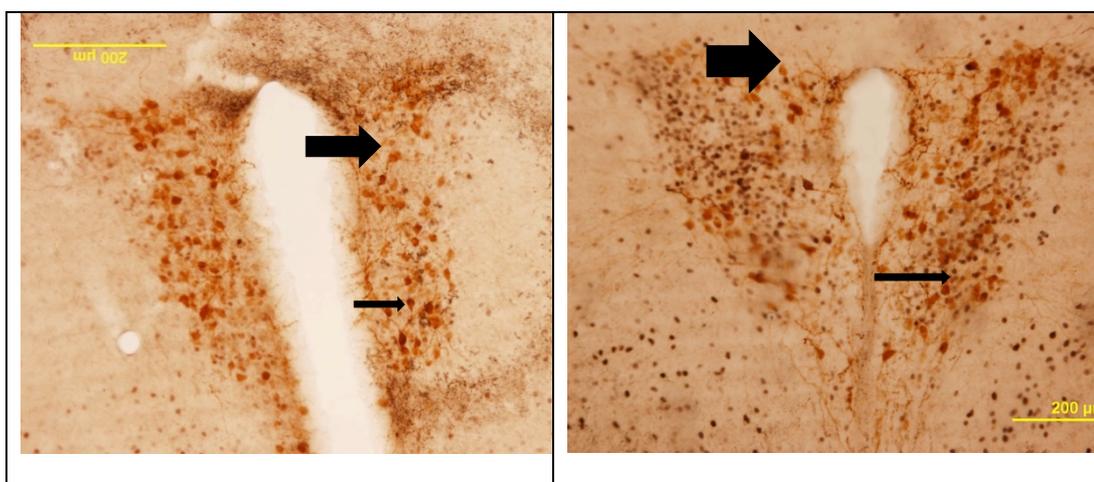
### Experiment 2: Activation of OT and AVP neurons in the PVN and SON following exposure to a familiar or novel object in a familiar environment

Exposure to a familiar object in a familiar environment significantly increased the density of c-Fos positive-OT positive neurons in the PVN compared to the percentage of OT neurons activated following exposure to a novel object in the same familiar environment (Figure 3.1). An example of c-Fos positive-OT

positive and c-Fos negative-OT positive immunoreactivity in the PVN is provided in Figure 3.2.



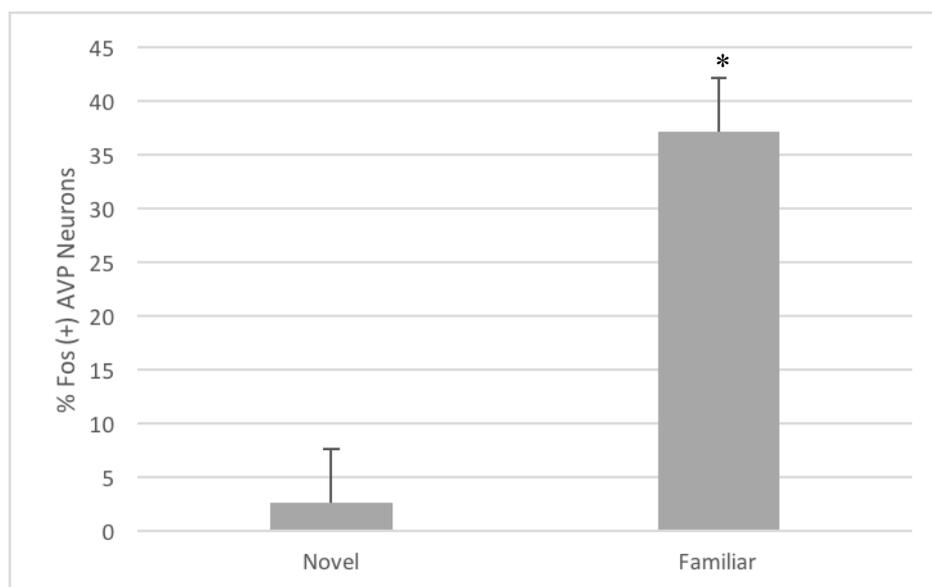
**Figure 3.1. Effect of exposure to a familiar versus novel object on the activation of oxytocin (OT) neurons within the paraventricular nucleus of the hypothalamus (PVN).** The activation of OT neurons within the PVN was analysed following the introduction of the individual to a novel object for 30 min in a familiar environment. \*\* $P < 0.01$ .



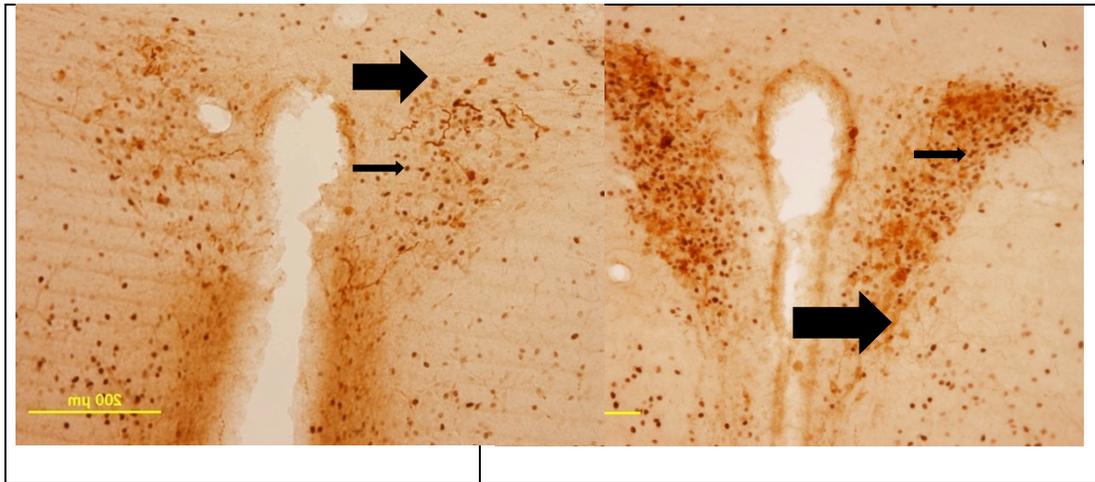
**Figure 3.2. Photomicrograph showing c-Fos positive-oxytocin (OT) cells stained in the paraventricular nucleus of the hypothalamus (PVN).** These

cells were stained following exposure to a novel object (L) versus c-Fos positive OT cells stained following exposure to a familiar object (R). Narrow arrows indicate Fos-positive OT neurons and wide arrows indicate Fos-negative OT neurons.

Exposure to a familiar object in a familiar environment significantly increased the density of c-Fos positive-AVP positive neurons in a familiar environment in comparison to the density of c-Fos positive-AVP positive neurons following exposure to a novel object in a familiar environment (Figure 3.3). An example of c-Fos negative-AVP positive and c-Fos positive-AVP positive immunoreactivity in the PVN is provided in Figure 3.4.

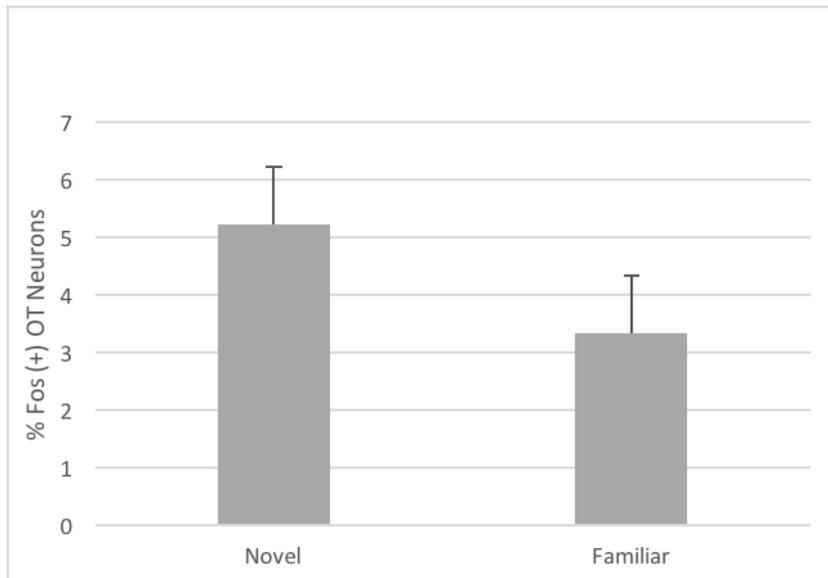


**Figure 3.3. The effect of exposure to a familiar versus novel object on the activation of vasopressin (AVP) neurons within the paraventricular nucleus of the hypothalamus (PVN).** The activation of AVP neurons within the PVN was determined following the introduction of the individual to a novel object for 30 min in a familiar environment. \* $P < 0.05$ .

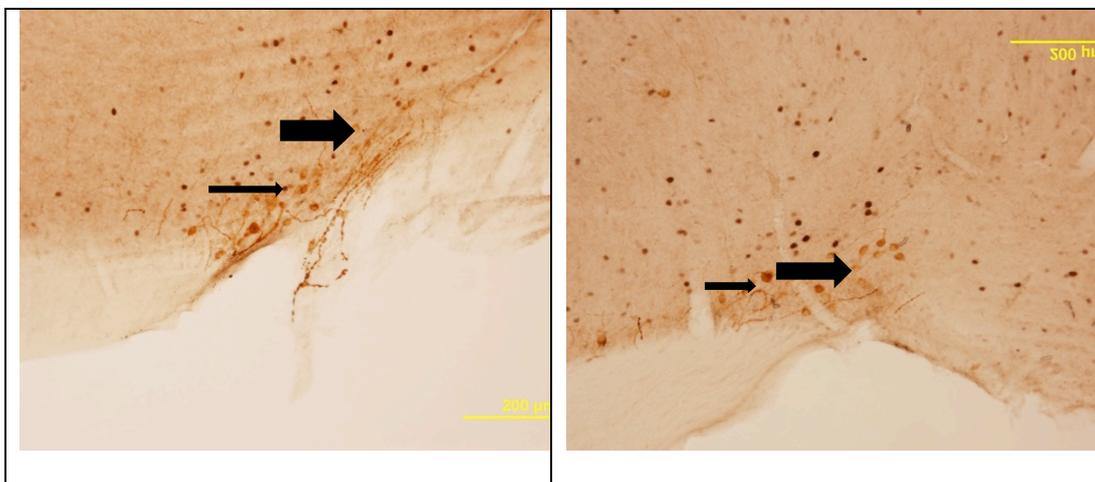


**Figure 3.4. Photomicrograph showing c-Fos positive vasopressin (AVP) cells stained in the paraventricular nucleus of the hypothalamus (PVN).** These cells were stained following exposure to a novel object (L) and c-Fos positive stained AVP cells following exposure to a familiar object (R). Narrow arrows indicate Fos-positive AVP neurons and wide arrows indicate Fos-negative AVP neurons.

Interestingly, exposure to a known object in a familiar environment decreased the density of OT-c-Fos positive neurons in the SON compared to the density of OT neurons activated following exposure to a novel object in a familiar environment (Figure 3.5). An example of c-Fos negative-OT positive and c-Fos positive-OT positive immunoreactivity in the SON is provided in Figure 3.6.

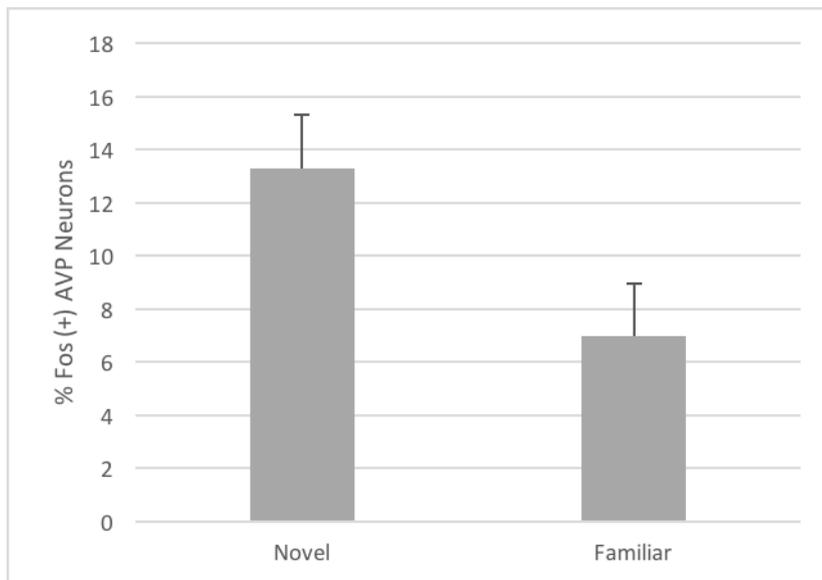


**Figure 3.5.** The effect of exposure to a familiar versus novel object on the activation of oxytocin (OT) neurons within the supraoptic nucleus of the hypothalamus (SON). The activation of OT neurons within the SON was determined following the introduction of the individual to a novel object for 30 minutes in a familiar environment.

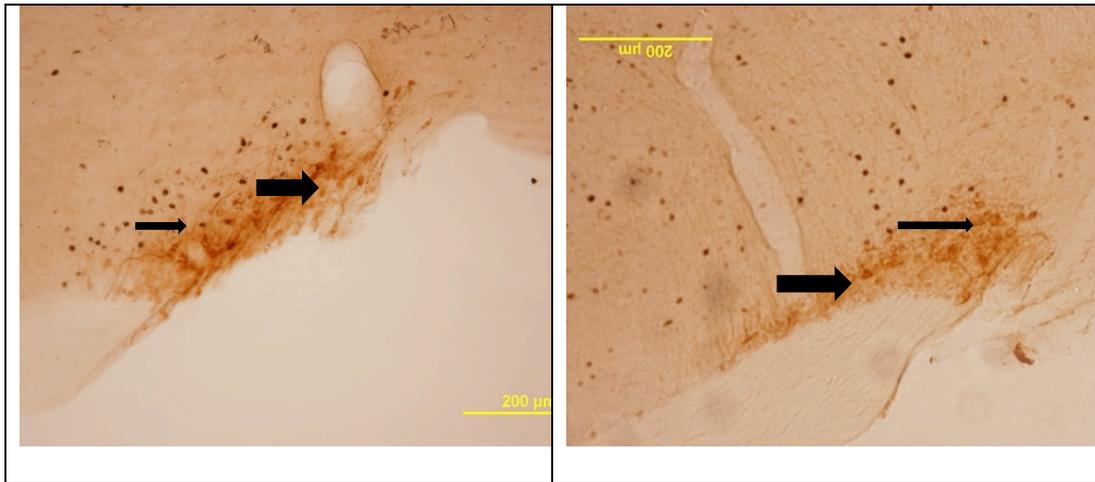


**Figure 3.6.** Photomicrograph showing c-Fos positive oxytocin (OT) stained cells in the supraoptic nucleus of the hypothalamus (SON). These cells were stained following exposure to a novel object (L) and c-Fos positive stained OT cells following exposure to a familiar object (R). Narrow arrows indicate Fos-positive OT neurons and wide arrows indicate Fos-negative OT neurons.

Exposure to a known object in a familiar environment also decreased the percentage of AVP positive neurons in a familiar environment in comparison to the number of AVP positive neurons when exposed to a novel object (Figure 3.7). An example of c-Fos positive-AVP positive and c-Fos negative-AVP positive immunoreactivity in the SON is provided in Figure 3.8.



**Figure 3.7.** The effect of exposure to a familiar versus novel object on the activation of vasopressin (AVP) neurons within the supraoptic nucleus of the hypothalamus (SON). The activation of AVP neurons within the SON was determined following the introduction of the individual to a novel object for 30 minutes in a familiar environment.



**Figure 3.8. Photomicrograph showing c-Fos positive vasopressin (AVP) cells stained in the supraoptic nucleus of the hypothalamus (SON).** These cells were stained following exposure to a novel object (L) and c-Fos positive stained AVP cells following exposure to a familiar object (R). Narrow arrows indicate Fos-positive AVP neurons and wide arrows indicate Fos-negative AVP neurons.

## Discussion and Conclusions

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The current set of experiments was performed to determine whether changes in OT and AVP neuronal activity are associated with the introduction of an individual to a novel versus a familiar stimulus. I wished to determine whether an injection of an OTR antagonist (thus, a blockade of the OT receptor) would have an effect on the amount of time spent with a novel object, whether OT neuronal activity would be changed in the PVN or SON of the hypothalamus upon introduction of an individual to a novel versus a familiar object, and finally whether there would be any change in the neuronal activity of AVP cells within the PVN or SON upon introduction of an individual to a novel object.

These investigations showed that an OTR antagonist does, in fact, change the amount of time an individual spends with a novel object. When the OTR antagonist is injected IP, the individual spends less time with a novel object and more time with a familiar object. We also found that the activity of OT and AVP neuronal cells is increased when an individual is exposed to a familiar object versus a novel object. Trends in the neuronal activity of OT and AVP neuronal cells in the SON were seen when an individual was exposed to a novel versus a familiar object. These trends showed that OT and AVP neuronal activity in the SON were increased when an individual was exposed to a novel object instead of when exposed to a familiar object.

The OTR antagonist, L-368,899, was injected IP to investigate changes in the amount of time spent with a familiar versus a novel object after OT receptor blockade. L-368,899 is a unique, high affinity, non-peptide OTR antagonist that is able to cross the blood brain barrier (BBB) (Olszewski et al., 2010). It was originally developed for the treatment, via blockade of uterine OTRs (Boccia, Goursaud, Bachevalier, Anderson, & Pedersen, 2007), of premature labour but failed in clinical development trials (Manning et al., 2012). While L-368,899's effects on preterm labour were being studied, the ability of the OTR antagonist to cross the BBB was observed (Pettibone & Freidinger, 1997). One study conducted on rhesus monkeys given an intravenous (iv) injection of the OTR agonist L-368,899 found that L-368,899 was present in cerebral spinal fluid (CSF) 40 min after injection with the antagonist. This study also found that the antagonist accumulated in the hypothalamus, septum, orbitofrontal cortex, amygdala and hippocampus, but was not found in other areas of the brain (Boccia et al., 2007). Based on L-368,899's ability to penetrate the BBB and its high affinity for the OTR (Vrachnis, Malamas, Sifakis, Deligeoroglou, & Iliodromiti, 2011), I can conclude that the antagonist was in fact targeting the receptors within the CNS.

When investigating the amount of time spent with a novel versus a familiar object following the IP injection of the OTR antagonist L-368,899, we found that time spent with the novel object was decreased using the L-368,899 OTR antagonist. When an individual was injected IP with saline, the individual spent an increased amount of time with the novel object. These findings suggest that the blockade of

OT ligand binding may result in changes to exploratory behaviour in relation to the novel stimulus. The findings are supported by a study using male prairie voles and the OTR antagonist L-368,899. This study also found that when an individual was injected IP with the OTR antagonist, it spent a reduced amount of time exploring the novel inanimate object, in this case a toy (Madularu, Athanassiou, Yee, Kenkel, et al., 2014). The preferences for novel objects following IP saline injections was also supported in a study using female rats (Madularu, Athanassiou, Yee, Kenkel, et al., 2014). As discussed previously, OT has an anxiolytic effect when an individual is exposed to a novel or stress inducing stimulus (Gibbs, 1984; Lukas & Neumann, 2013). As a result of the stress experienced by encountering a novel object, coupled with the reduced anxiolytic effects of OT due to the OTR antagonist, the novel object may seem increasingly stressful to the individual (Tops et al., 2013). This increased stress results in the individual spending much less time with the novel object than it would if it had normal neuronal activity of its OT circuit. In a situation without the use of the OTR antagonist, it is assumed that the stress response produced as a result of novelty would induce the anxiolytic effects of the OT circuit (I. D. Neumann & Slattery, 2016; Sutherland & Tops, 2014). This increased OT neuronal activity would allow the individual to be calmed by the anxiolytic effects of OT and approach the novel and stressful stimulus in order to assess its risk potential. This stressful stimulus approach is an important hurdle to overcome as it has important implications in an individual's ability to approach novel conspecifics and novel objects. The ability to approach novel social conspecifics is imperative in forming social groups and also forming social memories (Lukas & Neumann, 2013). This ability for OT to aid social approach and form social memories may also be important in the approach and memory formation of a novel object. There

are also studies that suggest the OT antagonist may affect locomotion and hence may have resulted in my finding of a decreased amount of time spent with the novel object. One study using female and male voles found that when OT was administered there were no changes in total locomotion but when the OTR antagonist was used, locomotion was reduced (Yu, Zhang, & Tai, 2016). A similar study using rats and icv injection of OT and OTR antagonist showed that OT injection increased the amount of locomotion in the rat while the OTR antagonist decreased locomotion (Yan et al., 2014). In light of these studies, it is possible that the effect of time spent with the novel versus familiar object could have been due to the OTR antagonist and its effects of decreased locomotion.

When analysing the amount of time spent with a familiar object versus a novel object following the injection of an OTR antagonist, we found that time spent with the familiar object is increased following the injection of the L-368,899 OTR antagonist suggests that the individual may no longer view the object as familiar. As explained previously, OT plays a significant role in an individual's ability to recall prior exposure to objects and/or conspecifics. Abolishing or attenuating these abilities via an OTR antagonist may effect an individual's ability to recall prior exposure to the object. An experiment using rats and an OTR antagonist showed that the antagonist diminished the rat's ability to establish and therefore recognise a conspecific (Engelmann et al., 1998). Although this study was performed using social recognition of conspecifics, we can infer that the same OT system would be needed to recall prior exposure to a novel object. In the current experiment using a familiar object, the data would suggest that the object is not remembered and therefore is seen as novel. Similar studies used in social situations supported these findings. Both OT and AVP are known to be involved

in social memory and social recognition (Albers, 2012). OT knock out mice have impairments in their ability to recognize other individuals (Ferguson et al., 2000) while centrally and peripherally injected OT and AVP increase social memory in rats (Le Moal, Dantzer, Michaud, & Koob, 1987; Song, Larkin, Malley, & Albers, 2016). It is possible that because the OTR antagonist reduces the familiarity of the object by reducing the individual's ability to recall prior experience (Song et al., 2016) and makes the individual see the familiar object as novel, the individual spends an increased (compared to the saline group) amount of time investigating the familiar object.

The neuronal activity of OT and AVP in the PVN and SON were investigated following the introduction of an individual to a novel or a familiar object. In four sets of experiments, individuals were exposed to a novel or a familiar object and immunohistochemical data were analysed in order to find any changes in OT or AVP activity within these areas of the brain. Immunohistochemistry was used to visualise changes in OT and AVP neuronal activity. Using double staining techniques allowed us to see how many OT and AVP neurons were in the PVN or SON, and how many of these OT and AVP neurons were positively stained for c-Fos; and therefore “activated”. The current set of experiments was a follow-up for a previous Master of Science thesis project completed by Ron Blaza, which found that the OT neuronal activity is very low in a familiar empty arena (in the absence of any additional objects – familiar or non-familiar that constitute the enrichment of the environment to which an animal is exposed) (Blaza, 2015). This evidence allowed us to proceed with the studies described herein, in which an individual was exposed to a novel versus familiar object introduced to such a familiar arena. Thus, instead of a “social enrichment” of a familiar experimental

apparatus, the current set of experiments focused on object enrichment and a potential effect it might have on the activation of the hypothalamic OT/VP neurons (and, consequently, the neuroendocrine balance imposed upon the release of these neuropeptides within the brain as well as into the general circulation).

OT neuronal activity within the PVN was analysed following the exposure of an individual to a novel or a familiar object. We found that there was a significant increase in the activity of OT neurons within the PVN when an individual was exposed to a familiar object opposed to a novel one. Similar studies assessing OT's activity in the PVN following exposure to a familiar conspecific have shown that OT is necessary in recognising a familiar conspecific (Lukas et al., 2013; I. D. Neumann & Landgraf, 2012). Rats examined for their discrimination between known and novel conspecifics have shown that exposure to a previously introduced conspecific results in discrimination between a familiar conspecific and a novel conspecific. Following icv administration of OT, the amount of time an individual was able to recognise a conspecific increased (I. D. Neumann & Landgraf, 2012). OT receptor antagonists have also been shown to effect social memory. When administered immediately after the introduction of the experimental rodent to a conspecific, the OT receptor antagonist interfered with the rat's ability to establish a memory of, and therefore recognize, a conspecific (Engelmann et al., 1998).

The injection of OT into OT knockout mice restored the ability for the individual to recognise a familiar conspecific (Lukas & Neumann, 2013). These findings suggest that social recognition of a familiar conspecific is related to OT neuronal activity. Experiments investigating how an individual reacts to, and recognises, a

familiar object have not yet been thoroughly explored but one study found that female prairie voles prefer anesthetized conspecifics as opposed to novel conspecifics. This finding suggests that OT facilitates a preference for familiarity in the absence of social stimuli (J. R. Williams, Catania, & Carter, 1992). This may allow us to link OT with general familiarity or familiarity in objects instead of in social settings alone. One study examining behaviour in response to objects showed that when rats were injected with OT, they exhibited increased preference for familiar objects (Madularu, Athanassiou, Yee, & Mumby, 2014). No studies, to my knowledge, have been done assessing the specific changes of OT neuronal activity within the PVN. In the current study, we found that OT neuronal activity significantly increased in response to a familiar object opposed to a novel one. This suggests that recognition of a previously explored object may involve activation of OT neurons within the PVN.

Experiments investigating AVP's neuronal activity within the PVN during investigation of a familiar versus novel object found that AVP neuronal activity significantly increased when an individual was presented with a familiar object opposed to a novel one. Studies performed on AVP's importance in social recognition have shown that AVP administration prolongs social memory in both males and females (Bielsky & Young, 2004). AVPR1b has been shown to reduce social avoidance following periods of social defeat (Litvin et al., 2011). As these studies show that AVP is active in forming or recalling social memories, and our current study shows that when an individual is exposed to a familiar object the activity of AVP neurons within the PVN increases, it may be that AVP within the PVN is involved in the recognition and memory processing of a familiar object opposed to a novel one as well as social familiarity (Albers, 2012).

In this set of experiments, we explored OT's activity within the SON upon the introduction of an individual to a novel or a familiar object. Immunohistochemical analysis showed that there was a decrease in OT neuronal activity when an individual was coupled with a familiar stimulus versus a novel one but these findings were not significant. As well as the PVN, the SON is a known site of excretion of OT (I. Neumann et al., 1994), but it is possible that the SON is more heavily involved in social recognition and less involved in inanimate object recognition. As OT within the SON has been shown to be important in processing and recognising olfactory cues (Sanchez-Andrade & Kendrick, 2009) to form social memories, it is possible that because the inanimate object does not possess these olfactory cues, the OT neuronal systems within the SON is activated less when an individual is recalling an inanimate object as opposed to a social conspecific. It is also possible that the study should be repeated with a larger number of individuals as during the immunohistochemical analysis, both sets of samples (familiar and novel object exposure) of the SON had very few positive neurons to count. This led to a high standard error which suggests the experiments may need to be repeated using a larger sample set.

Finally, AVP neuronal activity was assessed in the SON following the introduction of an individual to a novel versus a familiar object. These studies were not significantly supported but trends in the activity of AVP neurons within the SON showed that the number of active AVP neurons decreased when an individual was exposed to a familiar object opposed to a novel one. The P value for this data was 0.08 which is not quite significant but does show that there is a trend in the activity of AVP neurons when exposed to a familiar object opposed to

a novel one. The trend seen in these results allows us to consider that, as well as being important in the recognition of social familiarity (Albers, 2012; Le Moal et al., 1987), AVP may also be important in the SON when recognising a familiar object. A second explanation for this trend may be a link to AVP's role in stress. It is known that the PVN and SON, during times of stress, release AVP. This AVP release triggers the release of adrenocorticotropin from the anterior pituitary and glucocorticoids from the adrenal gland (Engelmann et al., 2004). The release of these glucocorticoids produces an array of effects in response to the stressful stimulus. These responses include mobilising energy, increasing cardiovascular tone and delaying processes in the body that are not critical and controlling osmolality (Fodor et al., 2016). One study using male AVP-deficient rats showed a decrease in anxiety-like behaviours (Balazsfi et al., 2015), another study using female AVP-deficient rats also showed a decrease in anxiety like behaviours (Fodor et al., 2016). The use of AVP deficient rats also showed that the lack of AVP did not reduce the individual's ability to form social memories (Fodor et al., 2016). This suggests that while AVP may be important in the regulation of stress involved with a novel stimulus, it may not be essential in memory formation. In our experiments, the increase in AVP activity in the SON following an introduction to a novel stimulus may be a result of AVP's regulation of the stress response. More investigation is needed in order to obtain significant data and to investigate further links between AVP neuronal activity and neuronal responses to familiar versus novel objects.

The previously described studies targeted OT and AVP neuronal populations within the parvocellular neuronal cells of the PVN and SON. The magnocellular neurons involved in OT and AVP synthesis and transport to the pituitary were not

targeted as my thesis is not focusing on neuronal activity in the periphery, for example the uterus. I am interested in investigating the parvocellular OT neurons as these neurons synthesise and transport OT and AVP which acts within the CNS to control behaviour; such as maternal care, social approach, or behaviours related to novelty (Onaka & Yagi, 1993; Pedersen & Prange, 1979). Although changes in peripheral levels of OT and AVP were not investigated in this thesis, changes in peripheral levels of OT and AVP may be useful in determining total changes in these neuropeptides in response to novelty. Studies investigating combined emotional and physical stressors such as forced swimming have found a changes in both peripheral and central levels of OT and AVP (Wotjak et al., 1998; Wotjak et al., 2001).

The prairie vole is an effective model for investigating the importance of brain regions and their responsiveness to social and inanimate novelty and familiarity. Because some species of the prairie vole are monogamous and form stronger social bonds with regards to familiar conspecifics and novel ones, the patterns in OT and AVP neuronal activity could mediate the development of certain types of affiliation, either with conspecifics or objects. One study investigating differences between the OTRs in the monogamous prairie vole and the polygamous montane vole found that in the prairie vole, the density of OTRs was highest in the prelimbic cortex, bed nucleus of the stria terminalis, NAcc, midline nuclei of the thalamus, lateral septum, and the lateral aspects of the amygdala. The montane vole showed little OTR binding in these regions and OTRs in this species were localised to the ventromedial nucleus of the hypothalamus, and cortical nucleus of the amygdala. These findings suggest that the difference in expression of the OTR in these areas of the CNS may be a link to the differences seen in affiliation

to familiar versus novel conspecifics. Furthermore, AVP infusion directly into the lateral septum improves social memory over time while the use of an AVPR1a antagonist in the lateral septum results in a decrease or complete deficiency in social memory (Lukas & Neumann, 2013). The amygdala is also thought to be important in the mediation of OT and social behaviour. Parvocellular and magnocellular OT neuronal cells project from the hypothalamus to the amygdala (Debiec, 2007). Both OT and OTRs are present in the amygdala of the mouse (Takayanagi et al., 2005). In the mouse, OTR binding in the amygdala is necessary for social bonding. Injection of OT directly into the medial amygdala has been shown to restore the ability of OT knockout mice to develop social recognition behaviours. OT antagonist injection directly to the medial amygdala prevented social recognition behaviours (Ferguson, Aldag, Insel, & Young, 2001).

The mice used in the current studies were females. Although male and female mice naturally display differences in behaviour, mostly involved with sexual responses, scientists have not yet been able to link these differences to unique structures or circuits within the female or male brain (Segovia & Guillamon, 1993; Simerly, 2002). Although brain structures are not yet proven to be different, the brain can be effected by sex steroids leading to differences in behaviour. The number of OTRs expressed in the hypothalamus may be dependent on levels of sex steroids, including estradiol (L. J. Young, Wang, Donaldson, & Rissman, 1998, Gimpl & Fahrenholz, 2001). One study using estrogen receptor knock out mice concluded that without estrogen, OT was not able to bind to the OTR (L. J. Young et al., 1998). The mice used in the current experiments were anoestrous. In mice, circulating levels of estrogen peak just before ovulation between proestrous and estrous and then drop to basal levels (Walmer, Wrona, Hughes, &

Nelson, 1992). Because the animals used in these studies were anoestrous, we can be confident that estradiol had minimal effect on OT binding. Because the structures within the brain of males and females have not been proven to be different, and my mice were in anoestrous, we can be confident that the sex of the mice used in the current studies were not a contributing factor in determining differences in OT and AVP neuronal activity.

Future studies may examine the effects of different doses on the OT antagonist L-368,899 on familiar versus novel object preference. IP injection of OT may also be a useful study to carry out to examine any differential effects an increase in OT levels may have on behaviour of an individual in response to a novel or familiar object. Immunohistochemical assessment of OT and AVP neuronal activity in the amygdala and NAcc may also be helpful in determining OT and AVP's roles in behavioural responses to familiar versus novel stimuli.

The use of AVP agonists/antagonists would be useful in determining effects on time spent with a novel versus a familiar object. As discussed previously, AVP is associated with social memory (Landgraf & Neumann, 2004). One study showed that social memory is prolonged in male and female rats following icv AVP administration and the use of an AVPR1a antagonist diminishes this prolonged social memory (Inga D Neumann et al., 2013). Using an AVP agonist/antagonist may allow the researcher to make conclusions about any differential findings that may be associated with the two peptides and their antagonists.

Another interesting future study may be to investigate the neuronal activity of OT and AVP in relation to affiliation for objects. Affiliative bonds are enduring attachments that allow an individual to function within a social group. These bonds have been described using the term “love” to define the way in which individuals have used affiliative behaviour to evolve into social beings (Feldman, 2012). The first link made between affiliation and OT was as a result of maternal care and the OT mediated affiliation between a mother and her young (F. Champagne, Diorio, Sharma, & Meaney, 2001). Rat mothers who showed higher degrees of maternal care including licking and grooming showed had higher levels of serum OT and showed higher levels of affiliative bonding to young (F. A. Champagne, 2011). OT’s role in affiliative bonding between individuals is linked with the mesolimbic dopamine system and the concurrent release of dopamine in the NAcc (Cameron, Pomerantz, Layden, & Amico, 1992). As well as the ability to become affiliated or attached to social stimuli, individuals can also become affiliated with non-social, inanimate stimuli. Like social familiarity/novelty and object familiarity/novelty, fewer studies have been performed on the neurological aspects affecting an individual’s willingness to bond with an inanimate object than have been performed on social bonding.

## **Conclusion**

In conclusion, differences in OT and AVP neuronal circuits are seen in the PVN in response to familiar versus novel objects. In this set of experiments, there is greater activation of OT and AVP neurons in the PVN of the hypothalamus when an individual is exposed to a familiar versus a novel object. These findings are supported by the use of OTR antagonist L-368,899. These behavioural pharmacology studies showed that following injection of the antagonist, time spent with a novel object decreased and time spent with a familiar object increased. Combined, the two experiments show that OT neuronal activity is varied depending on the familiarity of an object. These studies also showed supporting evidence that AVP, a closely related neuropeptide to OT, neuronal activity is varied depending on the familiarity of an object. These findings are important in better understanding neuroendocrine responses to familiar versus novel stimuli. An increased understanding in these responses will assist further research on health pathologies associated with abnormal behaviours in novel social environments and environments with novel inanimate objects.

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