Evaluating The Therapeutic Potential of The Oxytocin and Vasopressin Systems in the Treatment of Impulsive Aggression:

A Pre-Clinical Investigation

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Statement of authentication

This thesis is submitted to the University of Sydney in fulfilment of the requirement for the Degree of Master of Philosophy (Science).

The work presented in this thesis is, to the best of my knowledge original except as acknowledged in the text. I declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Signature: Date: 24/09/2019

Co-author declaration

I the undersigned acknowledge the parts of the following statement pertaining to the publications presented in this thesis on which I am a co-author:

Oliver Tan was the primary author of the publications featured in Chapter 2 and Chapter 3 of this thesis. For these publications Mr. Tan took the lead role in: the conception and design of the research; conducting the research; analysis and interpretation of the findings; and writing and critically appraising the manuscripts.

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Publications

The publications that form a major part of the contents of this thesis are:

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    - This paper is presented in Chapter 2.
2. Tan, O., Bowen, M. (under review). Divergent pathways mediate the effects of a 5-HT_{1A} receptor agonist on close social interaction, grooming and aggressive behaviour: exploring involvement of the oxytocin system. *Journal of Psychopharmacology*.
    - This paper is presented in Chapter 3.

Abbreviations

5-HT	5-Hydroxy-tryptamine (Serotonin)
5-HT_{1A}R	5-HT_{1A} receptor
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide
AH	Anterior hypothalamus
AVP	Arginine vasopressin
BNST	Bed nucleus of the stria terminus
CeA	Central amygdala
CSF	Cerebrospinal fluid
DRN	Dorsal raphe nuclei
EC50	Half maximal effective concentration
GABA	Gamma aminobutyric acid
HAA	Hypothalamic attack area
HAB	High anxiety behaviour
IED	Intermittent explosive disorder
LAB	Low anxiety behaviour
LAL	Long attack latency
LS	Lateral septum
MeA	Medial amygdala
mPFC	Medial prefrontal cortex
NAB	Normal anxiety behaviour
OXT	Oxytocin
OXTR	Oxytocin receptor
PAG	Periaqueductal gray
PFC	Prefrontal cortex

PVN	Paraventricular nucleus of the hypothalamus
SAL	Short attack latency
SON	Supraoptic nucleus of the hypothalamus
V1aR	Vasopressin V1a receptor
V1bR	Vasopressin V1b receptor
V2R	Vasopressin V2 receptor
VMHvl	Ventrolateral part of the ventromedial hypothalamus

Abstract

Aggression, the act of hostility with the intent to harm, is present across many different species. Whilst it can be a normal or adaptive behaviour in certain situations, such as to defend oneself or to compete over limited resources, it may become pathological if expressed an inappropriate context, at a disproportionate level leading to excessive damage or injury. Across a wide range of psychological disorders aggression is highly prevalent. Despite this, there are a lack of targeted, efficacious and safe pharmacological treatments that are available.

The current thesis explores the oxytocin (OXT) and vasopressin (AVP) system as a novel therapeutic target for the treatment of aggression within a mouse model of non-territorial, pathological aggression. Decades of previous research have shown that extended social isolation causes mice to exhibit hyper-aggressive behaviour. Thus we sought to evaluate whether the neuropeptides OXT and AVP, frequently associated with positive social behaviour, could inhibit aggression in socially isolated mice. Whilst previous research has examined the role of the neuropeptides OXT and AVP in different rodent models of aggression, this has largely been conducted within models which are reflective of normal or adaptive aggression. As such, previous research has not of yet evaluated these neuropeptides in a model that is more translatable and clinically relevant to pathological human aggression.

Chapter 1 provides an overview of the prevalence and burden of aggression, the underlying neurobiology of aggression and how the involvement of the serotonergic, and oxytocin and vasopressin systems may point towards a novel therapeutic target.

Chapter 2 comprises of 7 experiments that aimed to examine the effects of OXT and AVP within a mouse model of non-territorial, impulsive aggression and to more precisely examine the involvement of the oxytocin receptor (OXTR) and vasopressin V1a receptor (V1aR) in the observed effects. This study demonstrated that OXT, AVP and selective OXTR agonist TGOT had anti-aggressive and pro-social effects. Importantly this study also revealed that these effects are likely mediated through actions at the V1aR as the effects of OXT were blocked by pre-treatment with a selective V1aR antagonist but not an OXTR antagonist. Furthermore, consistent with their known affinities for the V1aR a much lower dose of AVP than OXT, and a much higher dose of TGOT was required to observe the same effects. Taken together the findings suggest that activation of the V1aR is critical for inhibiting aggressive behaviour and promoting pro-social behaviour, as such the V1aR may present a novel therapeutic target in drug discovery for the treatment of aggression.

Chapter 3 presents 4 experiments aiming to determine whether the anti-aggressive effects of a 5-HT_{1A} receptor agonist could be explained the downstream effects of OXT. This was of interest as 5-HT_{1A}R agonists and OXT both inhibit aggression and promote pro-social behaviour wherein stimulation of the 5-HT_{1A} receptor expressed on OXT neurons in the paraventricular nucleus (PVN) of the hypothalamus leads to the release of endogenous OXT. Pre-treatment with an OXTR antagonist did not attenuate the anti-aggressive effects of the 5-HT_{1A}R agonist, but the pro-social enhancing effect was significantly reduced, albeit not completely blocked, and the increase in grooming was completely blocked. Conversely, pre-treatment with a V1aR antagonist did not attenuate any of the aforementioned behaviours. Our study demonstrates that while stimulation of

endogenous oxytocin appears to be involved in the effects of 5-HT_{1A}R activation on pro-social behaviour and grooming, it is not involved in the anti-aggressive effects of 5-HT_{1A}R. Overall, this suggests that while 5-HT_{1A}R agonists and OXT can both inhibit aggression, this likely occurs through divergent pathways.

Overall, the findings suggest that both OXT and AVP act at V1aRs to inhibit aggressive behaviour and promote pro-social behaviour. However, given the poor pharmacokinetic properties of these neuropeptides it is unlikely that they can be used to attain long term clinically relevant outcomes. Rather, this thesis serves to highlight the V1aR as a strong candidate for the development of small molecule therapeutics.

Chapter 1: General Introduction and Literature Review

1.1 Chapter Overview

Aggression can be broadly defined as behaviour directed toward another object or living creature that has the potential to cause harm (Numan, 2015; Rosell & Siever, 2015; Siever, 2008). Abnormal aggression is highly prevalent across a wide range of clinical populations, affecting those with autism spectrum disorder, conduct disorder, substance use disorders, psychotic and personality disorders, neurodegenerative disorders, traumatic brain injury, and stroke. Aggression places an enormous burden on individuals, communities, the healthcare system, the judicial system and the broader economy.

Unfortunately, despite its prevalence and burden there are no effective pharmacological treatments available for aggression. Whilst there have been numerous attempts to develop a ‘serenic’ drug to specifically treat aggression, many are largely inefficacious or come with myriad liabilities.

In rodent studies, 5-hydroxytryptophan (5-HT; serotonin) is able to reduce aggression via its actions at the 5-HT_{1A} receptor (5-HT_{1A}R) (de Boer & Koolhaas, 2005; de Boer & Newman-Tancredi, 2016; Miczek et al., 1998). As a result, there have been numerous attempts at developing 5-HT_{1A}R agonists or to repurpose existing atypical antipsychotic medications with 5-HT_{1A}R activity for the treatment of aggression (Newman-Tancredi & Kleven, 2011; Santa Cruz et al., 2017). Unfortunately, these attempts have failed to deliver a breakthrough treatment for hyper-aggressive behaviour. Attention has thus turned to exploring other systems.

One system that is receiving growing interest as a potential target for treating aggression is the oxytocin system (de Jong & Neumann, 2018). Oxytocin is well known for its role in promoting positive social behaviour in mammalian species

(Gordon et al., 2011; Heinrichs et al., 2003). It is thus of interest to ponder whether activation of brain oxytocin pathways might change the nature of the social interaction from aggressive to pro-social.

Interestingly, stimulation of the 5-HT_{1A}R has been shown to cause a downstream release of oxytocin. Hence, it is possible that the anti-aggressive effects of 5-HT_{1A}R stimulation may be driven by its downstream effects on oxytocin. This possibility must be explored in order to determine whether oxytocin pathways offer a truly different pathway for modulating aggression to that targeted by 5-HT_{1A}R agonists, or if oxytocin is simply part of the same pathway with respect to aggression.

The experimental work presented in this thesis first sought to examine whether administration of oxytocin can reduce maladaptive aggression within a pre-clinical mouse model of pathological hyper-aggressive behaviour. We then explored the involvement of the OXT receptor (OXTR) and vasopressin V_{1A} receptor (V_{1A}R) in anti-aggressive effects observed with oxytocin. In the next set of experiments, we aimed to examine whether the utility of 5-HT_{1A}R agonists for reducing aggression and promoting pro-social behaviours is driven by downstream effects on oxytocin.

It is hoped that this work will help stimulate and guide future drug discovery and development for aggression and ultimately help improve patient outcomes.

1.2 Aggression

Aggression is defined as behaviour that is directed toward an object or living being with the potential of causing harm (Baron & Richardson, 1994; Numan, 2015; Rosell & Siever, 2015; Siever, 2008). In a range of contexts, aggression can be viewed as an adaptive behaviour and a key component of mammalian social behaviour, but it can also be maladaptive. It is also important to distinguish between impulsive aggression and instrumental aggression.

Adaptive aggression often serves to maximise one's evolutionary fitness or to protect oneself such as in competition for resources and in establishing and maintaining position within dominance and social hierarchies (Lund, 1975; Nelson & Trainor, 2007). Other examples of adaptive aggression include maternal and paternal aggression, which is critical for protecting offspring (Bosch, 2013; Bosch & Neumann, 2012; Svare, 1981; Trainor et al., 2008), and anti-predator aggression, which is an important survival mechanism (Huntingford, 1976).

At the simplest level, aggression can be considered to be maladaptive if it does not serve to maximise evolutionary fitness (Nelson & Trainor, 2007), or if it does not provide any additional value than typical functional aggressive behaviour (Fanning et al., 2019; Haller & Kruk, 2006; Miczek et al., 2013). Moreover, aggression can be considered to be maladaptive if it is expressed within an inappropriate context, if it is expressed at a disproportionate level, or is directed toward someone inappropriate (Connor, 2002).

Impulsive aggression is aggression in response to some sort of provocative stimulus and is driven primarily by an impulsive emotional response such as anger or fear (Coccaro et al., 2015; Dodge, 1991; Fanning et al., 2019). Moreover, impulsive

aggression occurs in the pursuit of immediate relief and without consideration of potential consequences (Ramirez, 2009). Of note, intermittent explosive disorder (IED) is characterised by frequent acts of impulsive aggression unsuitable for the context in which it is expressed (Coccaro, 2012; Fanning et al., 2019). In addition, maladaptive impulsive aggression presents across dementia, Alzheimer's disease and ASD (Haller & Kruk, 2006; Hodgetts et al., 2013).

In contrast, instrumental aggression is the intentional use of aggression to attain an external goal (Fanning et al., 2019; Miller & Lynam, 2006) such as a reward or advantage. Instrumental aggression is often planned or premeditated and causing harm is secondary to the primary goal (Fanning et al., 2019; Glenn & Raine, 2009). In contrast to impulsive aggression, there is a consideration for the potential consequences of an act of aggression rather than it being solely emotionally driven (Fanning et al., 2019; Ramirez, 2009). Instrumental aggression is linked to psychopathy and is prevalent across personality disorders such as antisocial personality disorder with psychopathic features and in children with conduct disorders high on callous and unemotional traits (Fanti et al., 2008; Glenn & Raine, 2009). Importantly, there are differences in the neural and molecular substrates that underlie impulsive versus instrumental aggression, as well as adaptive versus maladaptive aggression (Ramirez, 2009).

Whilst aggressive behaviour and violence are closely connected concepts there is an important distinction. Aggressive behaviour is an overarching construct seen as the observable manifestation of aggression. This can include various means of causing harm to others and may be verbal, physical or psychological (de Boer, 2018; Haller & Kruk, 2006). Violence, on the other hand, is a specific form of aggression

which requires some type of physical assault (de Boer, 2018; Haller & Kruk, 2006). This is an important distinction as non-violent aggression is still able to produce negative consequences for others. As such, human studies of aggression tend to focus on the non-violent manifestations of aggression whereas rodent studies of aggression focus on the physical aspects of aggression (de Boer, 2018; Haller & Kruk, 2006). Regarding studies using rodent models of aggression, however, there is a greater breadth of research into differing forms of aggression; which will be discussed later.

1.2.1 The prevalence and impact of aggression

This section will discuss the burden of aggression. It will focus on the prevalence of aggression in psychiatric and neurological conditions and disorders, the impact of aggression on the individual, impact on others, and its economic burden. Particular focus will be given to challenges faced by caregivers of individuals with ASD or Alzheimer's/dementia; disorders in which aggression is highly prevalent and poorly managed by existing treatments.

Prevalence of aggression

Aggression is highly prevalent across a wide range of disorders that occur at various stages of life (Liu et al., 2013). Clinically significant aggression is present in over 50% of pre-adolescents with attentional deficit hyperactive disorder (ADHD) (Saylor & Amann, 2016) whilst 53.7%-56% of children and adolescents with ASD exhibit aggressive outbursts (Kanne & Mazurek, 2011; Mazurek et al., 2013). A meta-analysis of 35 studies found that 17% of psychiatric in-patients had committed at least one violent act during their time as an in-patient (Iozzino et al., 2015). In cases following traumatic brain injury the prevalence of aggression was reported to

be 25%-28.4% (Baguley et al., 2006; Rao et al., 2009), while a meta-analysis of 48 studies (Zhao et al., 2016) reported a 40% prevalence of aggression in Alzheimer's disease. Finally, the estimated 12 month prevalence and lifetime prevalence of IED were 3.9% and 7.3% (Kessler et al., 2006).

Impact on the individual

Due to the lack of effective treatments, inappropriate strategies can sometimes be deployed by carers to try and manage aggression. These can include physical restraints (Hamers & Huizing, 2005; Robbins et al., 1987; Sourander et al., 2002; Westling et al., 2010), social exclusion (Rotheram-Fuller et al., 2010) and the use of high doses of antipsychotic medication to induce sedation (Arai et al., 2016; Corbett et al., 2014; Posey et al., 2008). All of these approaches can have a major negative impact on the individuals' quality of life. In Australia, a Royal Commission into Aged Care was recently launched to examine all forms of Commonwealth funded aged care services. The Commission will examine numerous aspects of aged care, including the quality and safety of aged care services, the systems that are in place to ensure high quality care is delivered, and capacity to deal with a range of complex factors such as dignity, choice and control, clinical care, medication management, mental health, personal care, nutrition and end of life care. One of the major focuses of the Commission is to identify a means to reduce or eliminate the use of restrictive practices in the management of aggression in elderly patients (Administrator of the Government of the Commonwealth of Australia, 2019).

Impact on others

Kanne and Mazurek (2011) report that 68% of caregivers had aggression directed toward them from children with ASD. Aggression is particularly challenging

for family members and teachers of children with ASD. Parents of children with ASD who exhibit aggressive behaviour have higher stress levels, highlighting the particularly challenging nature of this symptom (Donenberg & Baker, 1993). Aggression can manifest in numerous ways in ASD, for instance, during mealtimes children may spit out food or knock food off of the table (Lukens & Linscheid, 2008). Children with ASD may also routinely fight with siblings (Meadan et al., 2010; Ward et al., 2016). Consistent with this, Ross and Cuskelly (2006) found that aggression was the most common stressor for siblings of children with ASD, and importantly these siblings were at increased risk of developing internalizing behaviour problems. Within the school environment, children with ASD who display aggression may need to be moved to a more restrictive setting which further complicates their ability to develop friendships with other children (Rotheram-Fuller et al., 2010). In classroom settings, aggressive behaviour may disrupt other students' learning whilst teachers may also experience physical injuries, such as scratches and bites, to the point that they may require protective clothing and equipment in certain situations (Lin et al., 2012).

Among those suffering from dementia, close to 70% will remain living at home being looked after by their family (Schneider et al., 2002). Given the high prevalence of agitation and aggression in this population, family members can experience considerable distress (Coen et al., 1997). Hoe et al. (2017) highlighted the difficulties faced by family members caring for an elderly relative with agitation and aggression. Family members would regularly experience verbal aggression (including threats), disrupted sleep, and would witness and experience acts of physical aggression towards other people, themselves or objects, including, in extreme cases attempted

strangulation. Carers found it very difficult to cope, with their caring responsibilities negatively impacting their own health.

The challenge of managing agitation and aggression is often a major factor contributing to elderly patients moving to a specialised aged-care facility (Coen et al., 1997). Aggression is thus also a major challenge for carers in these facilities (Talerico et al., 2002). Zeller et al. (2009) note that caregivers in specialised aged-care facilities were confronted with a range of aggressive behaviours, including verbal and physical aggression (including kicks, bites and scratches). Aggression was reported to frequently occur during personal care activities such as feeding, dressing or bathing. Evers et al. (2002) found that the degree of psychological and physical aggression experienced by the carer was significantly correlated with their level of stress.

Economic Burden

The management of aggression has a direct economic burden through the strain it places on the healthcare and justice system as well as indirect downstream costs from factors such as lost productivity. Only a handful of studies, however, have sought to quantify the direct and indirect costs of aggression in dollar-terms. Miller et al. (1993) estimated the direct economic impact from the potential health-related costs from physical injury to those aged twelve years and older resulting from crimes such as assault and murder at $10 billion. Indirect costs from lost productivity were estimated at $23 billion and $145 billion due to reduced quality of life stemming from nonfatal physical or psychological injury with the lifetime cost of all injuries totalling $178 billion between 1987-1990. Similarly, a study by Corso et al. (2007) estimated the direct healthcare costs caused by violence in the United States of America to be

$5.6 billion and indirect costs of $64.4 billion from lost productivity. The overall costs for dementia in the United States of America (Hurd et al., 2013) were estimated to be between $157 billion and $215 billion, and the estimated costs for ASD in the United States of America to be between $268 billion and $461 billion for 2015-2025 (Leigh & Du, 2015).

1.2.2 The neurobiology of aggression

This section will first provide an overview of the different brain regions, neural circuits and some key pharmacological targets involved in the control and expression of aggressive behaviour. This is followed by a more detailed examination of the serotonergic system, which has been the neurotransmitter system most implicated in the pathophysiology of aggression. Discussion of the literature on the involvement of the oxytocin and vasopressin systems in aggression will be presented later in this chapter in the sections dedicated to these neuropeptides, which are central to this thesis.

Neurocircuitry of aggression

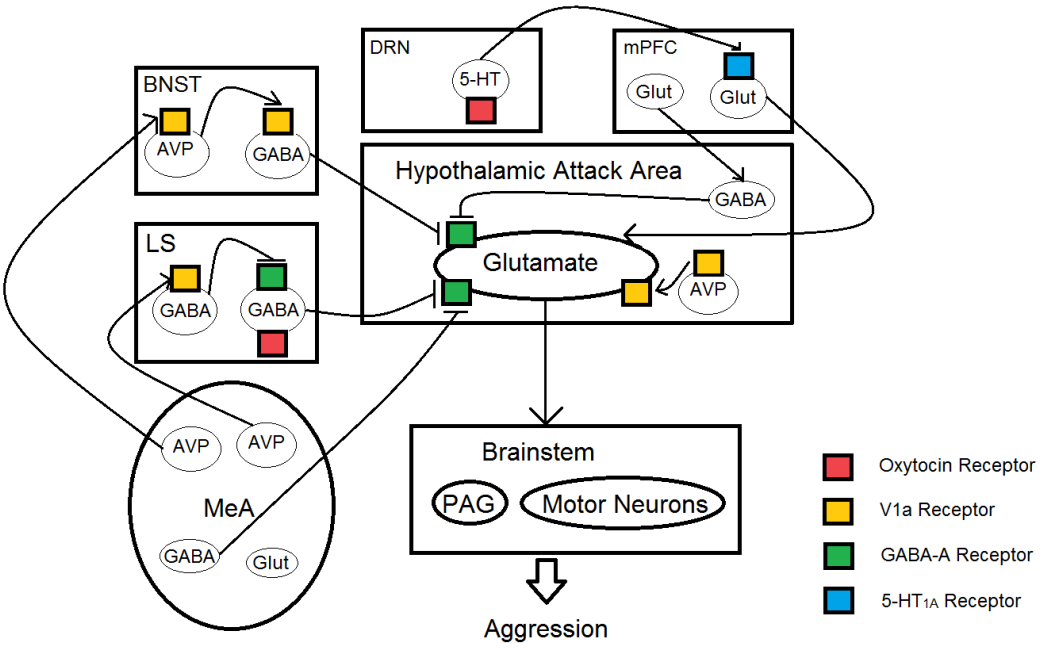

Figure 1. Proposed aggression circuits displaying the main brain regions and some key receptors involved in the regulation of aggression. Note that these drawings are compiled based on studies conducted on rats, mice and golden hamsters and not all of these species will necessarily express all components of these circuits. AVP fibres originating from the MeA project to both the BNST and the LS. Activation of V1aRs expressed on GABAergic interneurons within the LS causes the release of GABA which binds to GABA receptors expressed on GABAergic neurons which project to glutamatergic neurons in the HAA resulting in a net increase in aggression through a disinhibition of GABA signalling to the HAA. Conversely, activation of V1aRs expressed on AVP neurons in the BNST causes release of AVP which binds to V1aRs expressed on GABA neurons synapsing onto GABA receptors expressed on glutamatergic neurons in the HAA, thus reducing aggression through enhancing GABAergic signalling to the HAA. 5-HT neurons from the DRN project to the mPFC wherein activation of 5-HT_{1A}Rs inhibits mPFC projection neurons in the HAA resulting in an inhibitory effect on aggression. Adapted from (Canteras et al., 1995; Numan, 2015; Roeling et al., 1994; Toth et al., 2010; Veenema et al., 2010; Wong et al., 2016).

Overview of aggression circuit

There have been numerous attempts to characterise a circuit for the control and expression of aggression (Numan, 2015; Veenema & Neumann, 2007). At the core of this circuit are the hypothalamic aggression nuclei. In mice, the most important part of this region is the ventrolateral part of the ventromedial hypothalamus (VMHvl), the anterior hypothalamus (AH) in golden hamsters, and the region extending from the anterior hypothalamus to the ventromedial hypothalamus in rats. In humans, the equivalent region appears to involve the mediobasal hypothalamus. Henceforth, when we refer to the hypothalamic aggression area (HAA) we are referring to the aforementioned region(s) relevant for the particular species being discussed. The HAA receives direct input from both the medial amygdala (MeA) and lateral septum (LS) and subsequently sends outputs to brainstem regions such as the periaqueductal gray (PAG) and motor neurons which play a critical role in eliciting the motoric aspects of aggressive behaviour. It is important to note however, that the speculative proposed aggression circuits in Figure 1. draws from studies conducted in mice, rats and golden hamsters, wherein there are species differences in both the aggression phenotype and expression of OXTRs and V1aRs.

Attack nuclei

Across a range of species, stimulation of the HAA elicits attack responses. Activation of the AH has been associated with heightened aggression in rats (Toth et al., 2010), with stimulation of the AH either hormonally (Kruk et al., 2004), or electrically (Halasz et al., 2002; Siegel et al., 1999), resulting in increased aggressive behaviours. Similarly, chemical stimulation of the AH in Golden hamsters, (Ferris &

Delville, 1994; Ferris et al., 1997) also results in increased aggression In mice, the VMHvl appears to play a similar role. Specifically, silencing the VMHvl or killing progesterone receptor expressing cells blocked intermale aggression (Lin et al., 2011), while optogenetic stimulation of the VMHvl elicited immediate attack toward males, females, and inanimate objects (Yang et al., 2013). Moreover, in a task where male mice could nose-poke for access to a submissive male conspecific, using single-unit electrophysiology and population optical recording, the VMHvl was found to be active during nose-pokes, demonstrating that it is also involved in the act of aggression seeking (Falkner et al., 2016). In humans, a form of hypothalamic aggression can be observed wherein patients with abnormalities, such as hypothalamic hamartomas, in the mediobasal hypothalamus exhibiting increased aggressive behaviour (Weissenberger et al., 2001). Surgical removal of the mediobasal hypothalamus reduces aggression in these patients (Ramamurthi, 1988; Sano & Mayanagi, 1988). Finally, preliminary evidence showed that inhibition of the HAA via deep brain stimulation of the hypothalamus significantly reduced the occurrence of outbursts of impulsive aggression in highly aggressive male patients (Rosa et al., 2012).

Inputs to attack nuclei

The LS is an important region providing input to the attack nuclei. Early studies demonstrated an effect known as septal rage, wherein lesions to the LS in rodents led to heightened aggression (Albert & Chew, 1980; Potegal et al., 1981; Slotnick et al., 1973). Similarly, humans with septal tumours were observed to be hyper-aggressive (Zeman & King, 1958). Pharmacological inactivation of the LS via infusion with GABA-A receptor agonist muscimol has been shown to greatly increase

aggressive behaviour in rodents (McDonald et al., 2012). In contrast, electrical stimulation of the LS suppresses aggressive behaviour (Potegal et al., 1981). More recently, Wong et al. (2016) showed that optogenetic activation of the LS decreased aggression whereas inhibition increased aggression. Importantly, the majority of projections from the LS end in the medial hypothalamus (Risold & Swanson, 1997), where they strongly affect the activity of medial hypothalamic neurons (Blume et al., 1982).

The LS-VMHvl pathway seems to be particularly important in the regulation of aggression. In mice, optogenetic activation of the LS-VMHvl pathway suppressed aggressive behaviour, and optogenetic activation of the LS inhibited the activity of attack-excited cells but activated attack-inhibited cells (Wong et al., 2016). In addition, the facilitation of excitatory synaptic transmission by activation of vasopressin V1b receptors (V1bR) expressed on CA2 presynaptic terminals in the LS promotes aggression in mice which may be due to disinhibition of a subnucleus within the VMH (Leroy et al., 2018).

The HAA also receives direct inhibitory input from the MeA to regulate aggression (Numan, 2015). The MeA integrates olfactory information (Swanson & Petrovich, 1998), and projects to the HAA via the BNST (Canteras et al., 1995). Different neurons within the MeA of male mice respond to different odors such as in response to an odour from a predator or an odour from a reproductive female (Choi et al., 2005). Recently it was shown that optogenetic stimulation of GABAergic neurons within the MeA promoted aggression whilst the stimulation of glutamatergic neurons in the MeA suppressed aggression (Hong et al., 2014). Here, the authors speculate that aggression is promoted through the disinhibition of glutamatergic

VMHvl neurons (Choi et al., 2005; Lin et al., 2011) possibly via GABAergic MeA neurons.

In addition, there are glutamate neurons from the medial prefrontal cortex (mPFC) projecting to the HAA which may synapse onto GABAergic neurons within the HAA to decrease aggression (Toth et al., 2010). Takahashi and Miczek (2014) have shown that the optogenetic stimulation of excitatory neurons in the mPFC inhibits aggressive behaviour whereas the suppression of those neurons promotes aggression.

Outputs from attack nuclei

Injection of an anterograde tracer into the HAA revealed projections to various brainstem regions including the PAG, the mediodorsal thalamus and the LS (Roeling et al., 1994). There also appears to be a reciprocal relationship with the outputs to the LS and between the mPFC via the mediodorsal thalamus (Toth et al., 2010). These reciprocal relationships between the HAA and the mPFC and LS suggest that these regions modulate output of the HAA (Numan, 2015). It appears that the various inputs to the HAA stimulate aggressive behaviour, via outputs to the PAG and lower brainstem regions which ultimately project to downstream motor neurons that are responsible for the physical display of aggression (Numan, 2015).

Serotonin Circuitry

The serotonergic neurotransmitter system has been heavily implicated in the underlying pathophysiology of aggression (Rosell & Siever, 2015; Siever, 2008) and is the most extensively studied. The serotonin deficiency hypothesis holds that reduced activity in the serotonin system plays a major role in promoting aggressive

behaviour (de Boer & Koolhaas, 2005). Specifically of relevance to this thesis are the 5-HT_{1A}Rs, which have been shown to play an especially important role in regulating aggression (Rosell & Siever, 2015).

5-HT_{1A}Rs are expressed both pre-synaptically as autoreceptors on serotonergic neurons in the dorsal and median raphe nuclei (Polter & Li, 2010) and postsynaptically in the hippocampus (Varnäs et al., 2004). 5-HT_{1A}R agonists have been shown to reduce aggression in rodents (de Boer et al., 2000; Miczek et al., 1998; Moechars et al., 1998), and de Boer and Koolhaas (2005) demonstrated that administration of S-15535, a presynaptic 5-HT_{1A} autoreceptor agonist and competitive postsynaptic 5-HT_{1A}R antagonist reduced aggression in rats in the resident intruder test. Here, one possibility is that inhibitory actions on 5-HT interneurons would inhibit 5-HT neurons projecting to the forebrain resulting in an increase of 5-HT into the forebrain consistent with the serotonin deficiency hypothesis.

An early study by Valzelli (1973) examined differences in 5-HT turnover measured by 5-HIAA/5-HT ratio across different mouse strains within the resident intruder paradigm following social isolation and found that both socially isolated mice (who show heightened aggression) and more aggressive strains have reduced 5-HT turnover relative to their less aggressive counterparts. Selective-serotonin-reuptake-inhibitors (SSRIs), which increase 5-HT concentration in the synaptic cleft by blocking the serotonin transporter, have also been shown to be anti-aggressive in both humans and rodents (Mitchell & Redfern, 2005; Walsh & Dinan, 2001). A similar effect was observed via genetic manipulation, with knockout of the 5-HT transporter

reducing aggression in mice (Holmes et al., 2002). TPH2 knockout mice, which have lower serotonin levels, displayed much greater aggression than wildtypes (Mosienko et al., 2012). In addition, Peeters et al. (2019) demonstrated that higher doses of a selective 5-HT_{1A}R full agonist were needed to reach the equivalent reduction in aggression in TPH2 KO rats compared to corresponding wildtypes and the highly aggressive wild type Groningen (WTG) rats, suggesting that this heightened aggression is related to diminished 5-HT_{1A}R sensitivity. This is in contrast to previous studies which had instead found increased 5-HT_{1A}R sensitivity in TPH2 KO mice (Araragi & Lesch, 2013; Mlinar et al., 2017) and is not likely explained by upregulation of 5-HT_{1A}R expression as findings are highly inconsistent in TPH2 KO mice (Araragi & Lesch, 2013; Gutknecht et al., 2012; Jacobsen et al., 2012; Kriegebaum et al., 2010; Mosienko et al., 2014). Findings in humans are consistent with those in rodents, with criminals who had committed violent crimes classified as impulsive found to have significantly lower CSF 5-HIAA than healthy controls (Virkkunen et al., 1995).

Both V1aRs and 5-HT_{1A}Rs are expressed in the AH of hamsters, wherein injection of AVP into the AH enhances resident intruder aggression which is able to be blocked with administration of a 5-HT_{1A}R agonist into the same region (Ferris et al., 1997). This suggests that stimulation of 5-HT_{1A}Rs can suppress aggression by inhibiting the neurons that are activated by AVP which project to the HAA (although see more on AVP and aggression, below) (Ferris et al., 1997). Whilst it is also possible that another mechanism may be that stimulation of 5-HT_{1A}R containing pyramidal output neurons in the mPFC may suppress aggression (Puig et al., 2005), this has yet to be directly tested.

Interestingly, however, there also appear to be contradictory findings with some studies instead showing a positive correlation between aggression and 5-HT levels (Kulikov et al., 2012; van der Vegt et al., 2003). For example, administration of the 5-HT precursor L-tryptophan, which increases 5-HT levels, heightening aggression and administration of a TPH2 inhibitor, which decreases of 5-HT levels, reducing aggression (Kulikov et al., 2012). Similarly, van der Vegt et al. (2003) found a positive correlation between aggression and CSF 5-HT and 5-IAA in rats. Furthermore, in a review of studies examining the relationship between 5-HT activity and aggression in humans, de Boer and Koolhaas (2005) noted that a negative correlation and 5-HT activity was only present in individuals displaying impulsive pathological aggression whilst conversely a positive correlation was present in subjects displaying functional forms of aggression. Similarly, whilst a recent meta-analysis examining the relationship between serotonin and aggression across human studies only found a weak correlation (Duke et al., 2013), it is likely that it may be underestimating the strength of the effect as it did not distinguish between different types of aggression.

One way of resolving this apparent contradiction is that studies with a negative correlation between 5-HT activity and aggression were in animals that had some form of manipulation such as gene knockouts or social isolation which may produce a phenotype that represents an impulsive or pathological form of aggression (Holmes et al., 2002; Mosienko et al., 2012; Olivier et al., 1989). Studies where a positive correlation between 5-HT activity and aggression was observed were conducted in healthy rodents (Kulikov et al., 2012; van der Vegt et al., 2003). This may reflect a differential role of 5-HT in functional versus escalated aggressive behaviour, such that the serotonin deficiency hypothesis may only hold in cases of

impulsive pathological forms of aggression. Thus, perhaps it is more accurate to think of 5-HT as playing more of a homeostatic role with regards to aggression: promoting functional aggression and inhibiting maladaptive impulsive aggression.

Relevant to the model that is central to the experimental work in this thesis, Liu et al. (2019) demonstrated changes in central serotonergic neurons play an important role in heightened aggression that emerges following social isolation. TPH2 KO mice and Lmx1b KO mice, which are deficient in central serotonin, displayed elevated aggression and administration of 5-HT to Lmx1b KO mice blocked the heightened social isolation induced aggression. In humans, Klasen et al. (2019) demonstrated that acute tryptophan depletion was associated with greater aggression during a virtual violence scenario. Moreover, subjects with the low expressing monoamine oxidase A allele were more susceptible to the effects of acute tryptophan depletion on PFC-amygdala connectivity than those with the high expressing allele. Moreover, acute tryptophan depletion reduced aggression-specific amygdala connectivity with the bilateral supramarginal gyrus. Moreover, acute tryptophan depletion reduced aggression-specific amygdala connectivity with the bilateral supramarginal gyrus. The supramarginal gyrus has been implicated within the empathy network (Shamay-Tsoory, 2011), with functional impairments linked to a reduction in empathic responses to pain in others (Coll et al., 2017) and reduced activity in this region associated with a blunted emotional response toward violent videos (Strenziok et al., 2011). Hence, this reduced connectivity to a possible empathic network may underpin the increased aggression following acute tryptophan depletion.

More recently, (Hemmings et al., 2018) aimed to examine genetic variations in the 5-HT transporter and monoamine oxidase A genes that may underpin appetitive aggression. They discovered that the STin2 VNTR12 allele was positively associated with appetitive aggression, but negatively associated with reactive aggression which highlights that there may be an underlying genetic basis that distinguishes the two forms of aggression. Alekseyenko et al. (2019) has identified cholinergic and GABAergic pathways descending from optic glomerulus LC12 are involved in the downstream actions of 5-HT.

1.2.3 Current treatments for aggression

As aforementioned, a common strategy to manage aggression in ASD and in the elderly is to administer antipsychotic medication at high, sedative doses (Liu et al., 2016; Posey et al., 2008; Talerico et al., 2002). Given the problems with this strategy, there has been considerable interest for some time in developing a serenic drug (Olivier & Mos, 1986) that selectively reduces aggression. Unfortunately, no serenic drug has yet reached the market (Verhoeven & Tuinier, 2007).

Many attempted serenic drugs have targeted the serotonin system; the most common being 5HT1$_A$R agonists (Verhoeven & Tuinier, 2007). Activation of the 5-HT$_{1A}$R by 5-HT$_{1A}$R agonists, such as 8-OH-DPAT, S-15535 and Buspirone is effective in reducing aggression in rodents (Centenaro et al., 2008; de Boer & Koolhaas, 2005; de Boer et al., 2000; Sanchez & Hyttel, 1994), with preliminary evidence demonstrating efficacy of 5-HT$_{1A}$R agonist buspirone in humans (Santa Cruz et al., 2017). However, 5-HT$_{1A}$R agonists can lead to numerous dangerous side effects, such as serotonin syndrome, if administered at high doses (Bartlett, 2017; Buckley et al., 2014; Ener et al., 2003; Volpi-Abadie et al., 2013) .

As mentioned above, there have been numerous attempts at repurposing anti-psychotic drugs, as these target the serotonin and dopaminergic systems, both thought to be involved in the aetiology of aggression (Nelson & Trainor, 2007; Seo et al., 2008). For example, risperidone has been considered for the treatment of agitation and aggression in neurodegenerative conditions in elderly populations such as Alzheimer's disease and dementia (Liu et al., 2016; Santa Cruz et al., 2017). Epidemiological studies have demonstrated that the use of anti-psychotics in the elderly is associated with increased rates of mortality (Arai et al., 2016; Koponen et al., 2017; Zhai et al., 2016). As a result, the use of anti-psychotics for the treatment of aggression has been contraindicated in this population (Corbett et al., 2014).

Currently, two atypical antipsychotics, risperidone and aripiprazole, have been approved by the FDA for the treatment of aggression and irritability in youths with ASD (LeClerc & Easley, 2015; Posey et al., 2008). A recent meta-analysis of RCTs examining the efficacy of antipsychotics for the treatment of irritability and aggression in ASD by Fung et al. (2016) revealed that both risperidone and aripiprazole produced significant reductions in irritability and aggression with large effect sizes ($d = 0.9$ and $d = 0.8$, respectively). Importantly, however, the meta-analysis indicated that risperidone and aripiprazole were also found to cause serious adverse effects in the therapeutic dose range, including sedation, weight gain and extra-pyramidal symptoms (Fung et al., 2016).

1.2.4 Rodent models of aggression

Rodents exhibit aggressive behaviour in different contexts and for different reasons. This includes defence of territory, competition for resources, protection of offspring, and establishment of positions within a dominance hierarchy (Blanchard &

Blanchard, 1977). The act of aggression may lead to harm to the aggressor as well as the conspecific being confronted. As such, inhibitory control mechanisms exist to minimize the extent of aggression to avoid potentially harmful consequences. For example, initial threatening behaviour may be sufficient to ward off physical conflict (Haller & Kruk, 2006; Miczek et al., 2013). Despite these inhibitory control mechanisms, aggression may still become escalated to the point that it is considered a form of pathological aggression if expressed out of context or serves no further additional value than typical functional aggressive behaviour (Haller & Kruk, 2006; Miczek et al., 2013).

A potential limitation of studying aggression in rodents is that pathological aggression in humans can be clearly diagnosed, while mouse pathological aggression cannot, with the more abstract components of pathological aggression in humans, such as planning a violent act, not easily transferable in rodents (Blanchard et al., 2003). Thus, this thesis will focus specifically on modelling impulsive aggression, an exaggerated form of reactive aggression characterised by an increase in the magnitude of the aggressive response to a frustrating or threatening stimulus and/or an aggressive response to an inappropriate stimulus (Blanchard & Blanchard, 2003).

Resident-Intruder Paradigm

The predominant model used to study aggressive behaviour in rodents is the resident-intruder paradigm. The paradigm involves an ‘intruder’ rodent being placed into the home-cage of a ‘resident’ rodent, typically a male. The behaviour of the resident rodent toward the intruder rodent is analysed with measures such as fight latency (time before first attack by resident towards intruder) and total time spent

fighting (Koolhaas et al., 2013). One limitation of this paradigm is that it is thought to be a measure of territorial aggression, an adaptive defensive response intended to defend territory against an intruder. As such, it has been argued that this is not the best model for studies interested in translatability to clinically relevant pathological aggression (Olivier & Young, 2002).

Maternal Aggression

Another adaptive form of aggression is maternal aggression (Haller & Kruk, 2006; Miczek et al., 2013). Pregnant female rodents express a broad range of maternal behaviours to ensure the development and survival of their offspring. An important component of this behavioural repertoire is maternal aggression. In maternal aggression, aggressive behaviour is used to defend offspring from intruders, especially male intruders who are prone to non-parental infanticide (Svare, 1981). During the course of maternal aggression, the mother will direct attacks toward the vulnerable body parts of the male intruder such as the neck, back and genitals (Svare, 1981).

Ultimately, maternal aggression serves to ensure transmission of genes through the offspring. In line with this, virgin females rarely display aggression toward intruders (Svare, 1981). Hence, maternal aggression can also be seen as an adaptive, rather than a pathological response (Bosch, 2013).

Highly aggressive strains

Rodents may be selectively bred for extremes in aggression to reflect the natural genetic variability in aggression observed within human populations. An example of this is the short attack latency (SAL) and long attack latency (LAL) mice

(van Oortmerssen & Bakker, 1981). SAL mice show much higher levels of aggression compared to standard strain mice, whereases LAL mice show much lower levels of aggression (Haller & Kruk, 2006). In rats, Low anxiety behaviour (LAB), high anxiety behaviour (HAB) and normal anxiety behaviour (NAB) rats were initially selectively bred to examine differences in anxiety phenotypes (Neumann et al., 2010). However, in addition to differences in anxiety-like behaviour, it was identified that LAB rats exhibit not only higher levels of aggression but also more extreme forms of aggression. For instance, in the resident-intruder paradigm a much higher proportion of LAB rats direct attacks toward the vulnerable body parts of a male intruder (Neumann et al., 2010). Moreover, they exhibit aggressive behaviour toward a non-oestrus female intruder and aggressive behaviour toward an anesthetised male intruder, behaviours that are not observed in the HAB or NAB rats (Neumann et al., 2010). As such, strains such as the SAL mice and LAB rats have become important tools for studying aggression.

Social Isolation-Induced Aggression

The social isolation of rodents has been used as a means of a prolonged stressor. In mice, extended social isolation has been shown to lead to a multitude of behavioural deficits such as learning and memory deficits but also hyper-locomotion, anxiety and impulsivity (Koike et al., 2009). Of relevance to this thesis, isolation reared mice exhibit heightened aggression (Brain, 1975). Importantly, it has been shown that the behavioural alterations from social isolation are accompanied by disturbances to the serotonergic system. Specifically, isolated mice display heightened levels of offensive aggression, which is thought to be most closely aligned to reactive aggression in humans (Blanchard, 2017; Blanchard & Blanchard,

2003). For example, 4 weeks of isolation has been shown to lead to a significant reduction in 5-HT_{1A}R expression (Schiller et al., 2003), reductions in 5-HT_{1A}R binding site density (Preece et al., 2004), increased binding at the 5-HT_{1A}R which may be attributed to either reduced 5-HT availability or greater 5-HT_{1A}R expression (Schiller et al., 2006), and reductions in the transcription of all postsynaptic 5-HT receptors in the prefrontal cortex (Bibancos et al., 2007). In addition, 5 weeks of social isolation has also been shown to decrease mRNA transcription of OXTRs but not V1aRs in the central amygdala (Han et al., 2018).

While both the selective breeding models and the isolation induced aggression models are believed to be more relevant to pathological aggression in comparison to standard resident-intruder and maternal aggression models, as this thesis aims to serve as a proof of concept for future drug discovery, a significant advantage of the isolation induced aggression models over the selective breeding models is the extent of drug screening that has been performed in the former, establishing the predictive validity of the model. Drug screening utilising the paradigm is predictive of both the atypical anti-psychotics and 5-HT_{1A}R agonists (eg. Malick & Barnett, 1976; Olivier et al., 1989; Sanchez et al., 1993; White et al., 1991; Wongwitdecha & Marsden, 1996). Thus, the isolation-induced aggression model with testing in a neutral context is a more appropriate model to examine aggression relevant to human clinical populations (Olivier & Young, 2002). Moreover, the model provides greater predictive validity and is the preferred model for initial serenic drug-screening (Olivier & Young, 2002).

1.3 Oxytocin and Vasopressin

This section will cover the oxytocin and vasopressin systems, their involvement in aggression and their potential to serve as novel therapeutic targets for aggression. This section will begin by first providing an overview of the neuropeptides, the central oxytocin system and its activity at differing receptor targets. Subsequently this will be followed by an examination of oxytocin and vasopressin in aggression and their potential to serve as novel therapeutic treatments.

Structure and Function

Oxytocin (OXT) and arginine vasopressin (AVP) are nine amino acid neuropeptides with a high level of structural similarity, differing by only two amino acids (see Figure 2). OXT and AVP are primarily synthesised in the magnocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus, with AVP also being synthesised in the suprachiasmatic nucleus. Both hormones are first released centrally and subsequently transported to the posterior pituitary where they are then released into the periphery. In the periphery, OXT is known for its role in stimulating uterine contractions and lactation (Jurek & Neumann, 2018), whereas AVP, also known as anti-diuretic hormone, has an important role in kidney function in regulating water resorption and controls vascular smooth muscle cell constriction (Koshimizu et al., 2012).

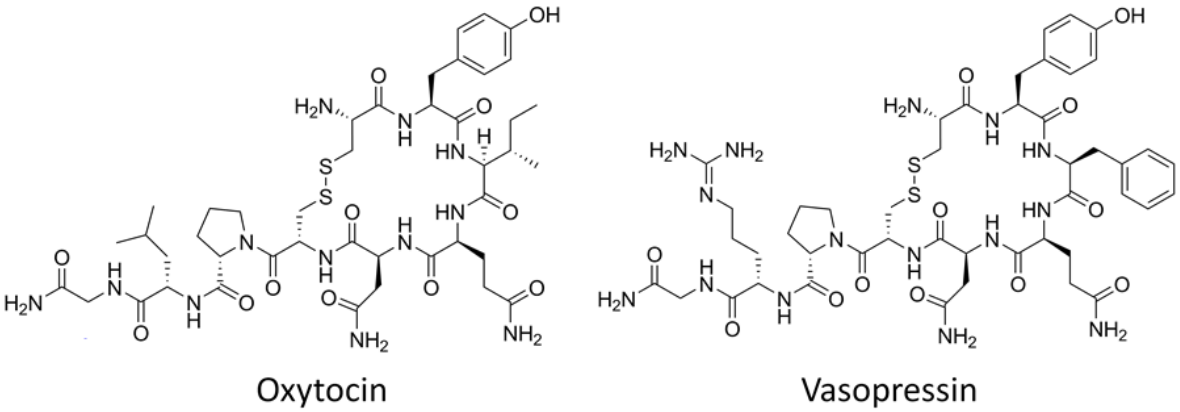

Figure 2. Diagrams of the hypothalamic neuropeptides oxytocin and vasopressin. Due to the high level of structural similarity, there is significant cross-reactivity between the two neuropeptides.

Central Oxytocin

OXT and AVP are released both denritically and axonally. Within the PVN, OXT and AVP are released from dendrites of magnocellular neurons (Ludwig & Leng, 2006). The somato-dendritic release of OXT within the SON and PVN may occur through extended dendritic trees of OXT neurons (Ludwig & Leng, 2006; Neumann, 2007) to facilitate autocrine and paracrine regulation of OXT neurons such as during lactation (Neumann et al., 1994; Neumann et al., 1993). Magnocellular OXT neurons originating from the PVN or SON project to various forebrain regions, including the prefrontal cortex (PFC), nucleus accumbens (NAcc), LS, hippocampus, MeA and CeA where axonal peptide release occurs (Dolen et al., 2013; Grinevich et al., 2016; Knobloch et al., 2012; Menon et al., 2018; Sofroniew, 1983). Parvocellular OXT neurons also project from the PVN to the brainstem and spinal cord where they play a role in autonomic functions and pain regulation (Eliava et al., 2016).

OXT is a highly evolutionarily conserved neuropeptide (Gimpl & Fahrenholz, 2001; Jurek & Neumann, 2018) that has been implicated in a wide range of social

behaviours across species. OXT has been shown to play a role in mating and pair bonding (Young & Wang, 2004), maternal-infant attachment (Insel & Young, 2001), pro-social behaviour (Lukas et al., 2011), stress and anxiety (Neumann & Slattery, 2016; Slattery & Neumann, 2010a; Slattery & Neumann, 2010b), and the regulation of reward and motivation (Bowen & Neumann, 2017b). Human studies have also demonstrated OXT's importance in social cognition (Keech et al., 2018) and social communication (Ditzen et al., 2009). In healthy adults even a single dose of intranasal OXT can produce pro-social and anxiolytic effects (Bos et al., 2012). For example, a single dose of intranasal OXT has been shown to increase trust (Kosfeld et al., 2005), generosity (Zak et al., 2007) and lessen the response to a social stressor (Heinrichs et al., 2003). However, it should be noted that numerous human studies of intranasal oxytocin have been criticised for methodological issues, including lack of power, and thus findings should be interpreted with some caution until larger, more definitive trials are completed (Leng & Ludwig, 2016).

Likely due to their close structural similarity, OXT and AVP exhibit a high degree of cross reactivity with regards to their binding sites. For OXT the primary receptor is the OXT receptor (OXTR) whereas AVP has three main receptors. The V1a receptor (V1aR) and V1b receptor (V1bR) are the main binding sites for AVP in the central nervous system, and the vasopressin V2 receptor (V2R) is the main binding site for AVP in the peripheral nervous system (Manning et al., 2012). However, recent work has demonstrated that OXT also has relatively high to high (depending on the species) affinity for the V1aR (Busnelli et al., 2013; Manning et al., 2012).

For instance, when examining the mouse variants of the aforementioned receptors, OXT had 0.83 nM affinity for OXTR and 20.38 nM affinity for the V1aR whilst AVP had 0.87 nM affinity for the OXTR and 1.11 nM affinity for the V1aR. Pharmacological doses of peripheral OXT and AVP used in mouse studies are likely to have actions at both receptors (Bowen, 2019; Busnelli et al., 2013; Manning et al., 2012). It is thus difficult to determine, at least when considering the effects of exogenously administered oxytocin, whether the effects of OXT are mediated via actions at the OXTR and/or the V1aR given significant activity at both receptors (Song & Albers, 2018).

Consistent with OXT's considerable affinity for both the V1aR and OXTR, a growing body of literature demonstrates several effects of exogenous OXT are mediated via actions at the V1aR. Hicks et al. (2014) showed that peripheral administration of OXT and AVP led to dose-dependent reductions in body temperature that were blocked by pre-treatment with the selective V1aR antagonist SR49059. Ramos et al. (2013) reported that peripheral OXT and AVP increased social interaction, which was blocked by pre-treatment with the V1aR antagonist SR49059, but not the OXTR antagonist C25. Similarly, peripheral OXT increased social huddling in response to threat, and this was blocked by pre-treatment with SR49059 (Bowen & McGregor, 2014). Importantly, given that AVP has a much greater affinity for V1aRs than OXT (Manning et al., 2012), the effects of AVP were more potent than OXT (Bowen & McGregor, 2014; Ramos et al., 2013). Interestingly, OXT administration in OXTR KO mice was able to increase social behaviour and reduce aggression and these effects were blocked by pre-treatment with SR49059 (Sala et al., 2011). Everett et al. (2018) demonstrated that methamphetamine-primed reinstatement could be prevented by both systemic OXT injection and microinjection into the nucleus accumbens core, and OXT effects were attenuated by pre-treatment with a V1aR antagonist. Taken together, these aforementioned studies highlight that many of the therapeutically relevant effects of exogenous OXT may be mediated through actions at the V1aR.

1.3.1 Blood brain barrier penetration following peripheral oxytocin administration

One potential drawback of OXT as a therapeutic is that it may not cross the blood brain barrier (BBB) at sufficient concentrations to cause changes in behaviour (Leng & Ludwig, 2016). To investigate this further, Smith et al. (2019) examined differences in brain and plasma OXT concentration in oxytocin null mice that do not

produce any endogenous OXT following intranasal administration. The authors demonstrated that OXT could be detected in extracellular fluid from the amygdala and hippocampus of the oxytocin null mice following intranasal OXT administration. The results were highly consistent with earlier research demonstrating increased brain and plasma OXT concentrations following i.p. and intranasal administration in rats and mice (Neumann et al., 2013) and confirmed that it was indeed peripherally administered oxytocin entering the brain as opposed to stimulation of endogenous release. Furthermore, an earlier study demonstrated that OXT was detectable in the CSF following intranasal and intravenous administration to rhesus monkeys (Lee et al., 2018). Given the EC50 for oxytocin at the mouse oxytocin receptor is close to 4.5 nM (Busnelli et al., 2013), Bowen (2019) estimated the brain concentrations of OXT in the (Smith et al., 2019) study (which were on the lower end of what is typically used in peripheral administration studies) as close to the EC50; demonstrating that the often high peripheral doses used in rodent studies are highly likely to lead to pharmacologically relevant brain concentrations. Whilst this demonstrates that peripheral administration of OXT can cross the BBB at behaviourally relevant concentrations, this cannot necessarily be generalised to humans (Bowen, 2019). The EC50 for the OXT at the human OXTR is close to 10 nM (Passoni et al., 2016). Conversely to the findings in the rodent literature intranasal administration of OXT in non-human primates (Lee et al., 2018; Modi et al., 2014) and humans (Striepens et al., 2013) have shown much lower peak CSF concentrations which would be expected to only cause marginal occupancy and activation of the OXTR.

1.3.2 The Therapeutic Potential of the Oxytocin System

Given the important role oxytocin plays in social behaviour across species, (Gordon et al., 2011) there is growing interest in the brain oxytocin system as a

potential novel therapeutic target for the treatment of a number of psychiatric disorders that feature social deficits as either core or secondary symptoms (Cochran et al., 2013; Matsuzaki et al., 2012; Neumann & Slattery, 2016). This includes ASD (Guastella et al., 2010a), social anxiety disorder (Neumann & Slattery, 2016), schizophrenia (Dagani et al., 2016), major depressive disorder (Slattery & Neumann, 2010b) and substance-use disorders (Bowen et al., 2016; Bowen & Neumann, 2017a; Bowen & Neumann, 2017b; McGregor & Bowen, 2012). Social deficits in all of these disorders are a significant barrier to recovery, negatively impacting treatment seeking and engagement in social support (Kalin et al., 2015; White & Roberson-Nay, 2009).

1.3.3 Oxytocin and Aggression

In addition to the previously discussed disorders, there is increasing interest in the brain OXT system as a novel treatment target for aggression (de Jong & Neumann, 2018). Numerous lines of evidence point to involvement of the OXT system in regulating aggressive behaviour in humans and other species, with the prevailing hypothesis that low OXT activity may be linked with elevated aggression (de Jong & Neumann, 2018). For instance, aggression has been shown to be negatively correlated with activation of OXT neurons in rats (Calcagnoli et al., 2014). In WTG rats, high inter-male aggression was associated with reduced OXT synthesis in the PVN, but not the SON, and increased OXTR binding in the CeA and BNST (Calcagnoli et al., 2014). Here, the increased binding may be explained by reduced availability of the neuropeptide. Another possibility is that OXTR expression may be upregulated in these regions to act as a compensatory mechanism in response to the low OXT availability resulting from the reduced synthesis of OXT in the PVN, yet the authors suggest that increased OXTR density may conversely result in a

downregulation of hypothalamic OXT synthesis. Moreover, maternal separation, which is known to result in increased aggression (Haller et al., 2014), caused reduced OXT binding in the LS and caudate putamen and greater binding in the medial preoptic area of adult male Wistar rats, but did not have an effect on hypothalamic OXT synthesis (Lukas et al., 2010). Conversely, when virgin female rats, which typically exhibit low levels of aggression, were trained to fight increases in OXT release were associated with reductions in aggression (de Jong & Neumann, 2018). OXTR KO mice exhibit heightened aggression (Sala et al., 2011).

In rodents it has been shown that administration of OXT can have anti-aggressive properties, where both acute (Calcagnoli et al., 2013) and chronic (Calcagnoli et al., 2014) intracerebroventricular infusion of OXT, intranasal administration of OXT (Calcagnoli et al., 2015a), and infusion of OXT directly into the CeA (Calcagnoli et al., 2015b) successfully inhibited inter-male territorial aggression in the resident intruder test. In two of these studies (Calcagnoli et al., 2013; Calcagnoli et al., 2015b) the anti-aggressive effects of OXT were blocked with an OXTR antagonist. However, it should be noted that the peptidergic antagonist used, desGly-NH2,d(CH2)5[Tyr (Me)2 ,Thr4]OVT lacks considerable selectively for the rat OXTR over the rat V1aR (Manning et al., 2012). In addition, the heighted aggression in OXTR KO mice was shown to be reduced with OXT administration, an effect blocked by pre-treatment with a V1aR antagonist (Sala et al., 2011). This suggests that despite the absence of OXTRs, OXT may exert its effects on aggression at least partly through the V1aR. Of relevance to the model of aggression used in this thesis, 5 weeks of social isolation led to a decrease in mRNA transcription of OXTRs but not V1aRs in the central amygdala (Han et al., 2018). Similarly, increased OXT mRNA expression in the PVN and decreased OXTR binding in the anterior portion of the

NAcc across both genders was found in rats following 7 weeks of post weaning social isolation (Oliveira et al., 2019).

Human studies

Human studies have identified relationships between peripheral OXT levels and aggression. For example, plasma OXT concentrations were negatively correlated with aggression in boys with ADHD (Demirci et al., 2016). In addition, basal saliva OXT concentrations were negatively correlated with the degree of callous and unemotional traits in young boys with conduct problems (Levy et al., 2015). Similarly, Dadds et al. (2014) found that methylation of the OXTR was positively correlated, whereas plasma OXT concentration was negatively correlated with callous and unemotional traits in boys with conduct problems. Young adult females diagnosed with bipolar disorder had lower levels of OXT and OXT levels were negatively correlated with trait aggression measures (Bertsch et al., 2013). Interestingly, Jokinen et al. (2012) found that in females, but not males, who had attempted suicide, low OXT was associated with heightened aggression.

Considering these studies finding an association between low OXT levels and heightened aggression, other work has examined if administration of intranasal OXT might reduce aggression. Most of these studies have measured aggressive behaviour with computer-based tasks such as the Point Subtraction Aggression Paradigm (PSAP), which uses the number of times a participant selects an aggressive response choice as a measure of aggression. These studies found that acute intranasal administration of OXT did not affect measures of aggression (Alcorn et al., 2015a; Alcorn et al., 2015b) in healthy males nor males with anti-social personality disorder. Conversely, in women with higher state anxiety intranasal OXT

was found to reduce aggression (Campbell & Hausmann, 2013). Evidence from a clinical sample of young adult female patients with bipolar disorder and high trait aggression found that intranasal OXT was able to reverse their high threat sensitivity in response to angry faces (Bertsch et al., 2013), suggesting it may inhibit processes central to impulsive aggression in this population (Mancke et al., 2015).

Conversely, some studies have found OXT can instead produce effects that are inherently antisocial and may promote aggressive behaviour (Beery, 2015). For example, OXT has been shown to induce feelings of envy and mistrust (Shamay-Tsoory et al., 2009) heighten out-group bias de (De Dreu et al., 2011) and heightened anxiety elicited by unpredictable threats (Grillon et al., 2013). Intranasal OXT has been shown to increase in-group conformity while increasing anti-social tendencies toward members of the out-group (De Dreu & Kret, 2016). Importantly, the findings from these human studies which show that OXT administration may promote aggressive behaviour were conducted in healthy participants. Conversely, the studies discussed above demonstrated OXT was negatively correlated with aggression in clinical populations, and that administration of OXT can reduce aggression in relevant clinical populations. Moreover, these studies more frequently directly assessed measures of aggression. We thus believe the human literature, on a whole, supports the potential utility of OXT for reducing pathological aggression, although note the comments made above highlighting the caveats it is important to be cognisant of when interpreting the findings of intranasal oxytocin studies.

1.3.4 Interactions between oxytocin and serotonin

Serotonergic fibres originating from the dorsal and medial raphe nuclei of the brainstem project to the PVN and SON where 5-HT_{1A}Rs are expressed on

postsynaptic OXT neurons (Larsen et al., 1996; Sawchenko et al., 1983). Interestingly, stimulation of these 5-HT_{1A}Rs leads to a downstream release of endogenous OXT (Bagdy & Kalogeras, 1993; Jørgensen et al., 2003). This release of OXT is blocked by pre-treatment with the selective 5-HT_{1A}R antagonist WAY-100635 (Chemel et al., 2006; Hunt et al., 2011; Martel et al., 2007; Petrunich-Rutherford et al., 2018).

Like OXT, MDMA enhances sociability. Moreover, it appears to do so via 5-HT_{1A}R mediated stimulation of OXT release. Heightened social interaction between two unfamiliar Wistar rats was observed following administration of MDMA (Morley & McGregor, 2000), an effect reversed by co-administration of the 5-HT_{1A}R antagonist WAY-100635 (Morley et al., 2005). In subsequent work, Thompson et al. (2007) reported increased activity of OXT neurons in the PVN and SON following MDMA administration in rats and blocked the pro-social effects of MDMA with OXTR antagonist pre-treatment. Chronic treatment with the 5-HT1aR agonist 8-OH-DPAT during the neonatal period in a genetic mouse model of autism was able to restore normal sociability, the effect of which was blocked if 8-OH DPAT was co-administered with OXTR antagonist L-368,899 (Nagano et al., 2018).

Convergent findings have been reported in humans. Wolff et al. (2006) found elevated blood OXT concentrations amongst dance party attendees who had taken MDMA. Similarly, Hysek et al. (2012) reported increased circulating OXT in healthy volunteers following MDMA administration. Importantly, blood OXT levels following MDMA are significantly correlated with subjective self-reported feelings of sociability (Dumont et al., 2009).

There is also evidence of OXT regulating 5-HT release. In the PVN and SON 5-HT fibres follow and overlap OXT cells (Emiliano et al., 2007). Serotonergic neurons in the raphe nuclei express OXTRs which modulate 5-HT release (Yoshida et al., 2009). Moreover, greater 5-HT neuron axon length was observed following postnatal OXT administration in male prairie voles (Eaton et al., 2012).

1.3.5 Vasopressin and Aggression

In contrast to the myriad studies showing the anti-aggressive properties of OXT, the role of AVP in aggression appears considerably more complex and appears to be moderated by a range of factors. For example, the species, brain regions of interest, receptors and type of aggression being assessed all appear to moderate effects of AVP on aggressive behaviour.

A number of early studies showed that AVP had a stimulatory effect on aggressive behaviour. For example, when Ferris and Potegal (1988) microinjected a peptidergic V1aR antagonist into the AH of Syrian Golden Hamsters it reduced aggression in the resident intruder paradigm. In other work in Syrian Golden Hamsters, microinjection of AVP into the ventrolateral hypothalamus (Delville et al., 1996) and the AH (Ferris et al., 1997) resulted in increases in aggression in the resident-intruder test. In a more recent study, Bosch and Neumann (2010) showed that higher AVP mRNA expression was found in the PVN of HAB rats which exhibit a high level of maternal aggression. Elevation of AVP release within the CeA during bouts of maternal aggression in HAB dams was also found to correlate with the amount of offensive aggression expressed, which was subsequently blocked by bilateral administration of a V1aR antagonist into the CeA. However, this elevation of

AVP release was not observed within the PVN, and AVP binding within the CeA and PVN did not differ between female HAB and LAB rats.

Conversely, there is evidence demonstrating that AVP can inhibit aggression. Vasopressin-deficient Brattleboro rats exhibit reduced territorial aggression in the resident intruder test (Fodor et al., 2014). Veenema et al. (2010) showed that AVP release within the LS had a positive correlation with aggression, whereas AVP release within the BNST had a negative correlation with aggression in rats using the resident intruder test. Importantly, central infusion of AVP into the BNST was able to inhibit aggression in rats showing the highest level of aggression, whereas infusion of a V1aR antagonist into the LS inhibited aggression. In contrast, another study found that less aggressive mice have higher AVP innervation in the LS and density of AVP neurons in the BNST (Compaan et al., 1993), while Everts et al. (1997) found higher levels of AVP in nonaggressive rats compared to aggressive rats. Interestingly, V1aR knockout mice exhibit heightened aggression in the resident intruder test but do not display escalated aggression when tested in a neutral context (Wersinger et al., 2007b). Following 7 weeks of post weaning social isolation, reduced V1aR binding was observed in the lateral hypothalamus and dentate gyrus of both male and female rats (Oliveira et al., 2019). However, V1aR binding in the anterior portion of the BNST was decreased in males and increased in females (Oliveira et al., 2019).

Conversely, there appears to be greater consensus on the role of the vasopressin V1b receptor (V1bR) in aggression. Activation of the V1bR has been strongly associated with increased aggression, while antagonism of the V1bR reduces aggression. For example, V1bR knockout mice exhibit significantly less

aggression compared to their wild type littermates (Pagani et al., 2015; Wersinger et al., 2007a; Wersinger et al., 2002). Moreover, administration of a selective V1bR antagonist SSR149415 produces significant reductions in aggression in hamsters in the resident intruder test (Blanchard et al., 2005).

A range of human studies examining the relationship between AVP and aggression are at least somewhat reflective of the results from animal studies. Human gene studies have shown V1aR polymorphisms to be associated with impulsive aggression in patients with borderline personality disorder (Vogel et al., 2012), and childhood aggression (Pappa et al., 2016), however the functional significance of these polymorphisms is currently unknown. Similarly, polymorphisms in the V1bR are associated with childhood aggression (Zai et al., 2012), which may be specific to a more reactive form of aggression (Luppino et al., 2014). CSF AVP has been shown to be positively correlated with trait level aggression in patients with impulsive aggressive personality disorders (Coccaro et al., 1998). Furthermore, it has been shown that intranasal administration of AVP to healthy subjects impaired their recognition of negative emotions (Uzefovsky et al., 2012), although Guastella et al. (2010b) found that intranasal AVP enhanced the encoding of happy and angry faces. Consistent with AVP reducing reactivity to angry faces, AVP decreased amygdala blood oxygen level dependent (BOLD) responses to angry faces and this was blocked by oral administration of a the V1aR antagonist SRX246 (Lee et al., 2013).Overall, the effect of AVP on aggression appears to differ depending on a range of factors. In rodents, it appears to be region dependent but also strongly dependent on the type of aggression. The studies conducted in hamsters showing AVP promoted aggression examined aggression within the resident intruder test, whereas the majority of studies in rats examined maternal aggression. Importantly

these effects also appear to be mediated by actions at different receptors, with reduced V1aR activity associated with increased aggression in pathological models (Oliveira et al., 2019) and V1bR activity associated with heightened aggression (Blanchard et al., 2005; Terranova et al., 2017; Wersinger et al., 2002). In humans, it appears that AVP may be beneficial in clinical populations (Coccaro et al., 1998; Vogel et al., 2012) but has the opposing or no effect in healthy samples (Lee et al., 2013; Uzefovsky et al., 2012) .

1.4 Conclusions

The literature discussed in this chapter highlights that aggression is highly prevalent across a wide range of psychological disorders, such as ASD and dementia, which place a large burden on the healthcare system. Unfortunately, there are a lack of efficacious treatment options available on the market that selectively treat aggression. As a result, inappropriate techniques must often be employed to manage aggression in these populations. These include the use of restraints and the administration of high doses of antipsychotics, often off label, which do not target aggression directly and can have myriad side effects. As such, aggression is a growing but ultimately unmet clinical need.

Hence there is a great need for safe and efficacious therapeutics. A promising opportunity is to explore targeting the oxytocin and/or vasopressin system which play a critical role in social and affiliative behaviour. The literature in this chapter demonstrate that the neuropeptides oxytocin and vasopressin can reduce aggression. Previous research has primarily examined the role of these neuropeptides in models of adaptive aggression such as territorial or maternal aggression such that it is not established whether they will have efficacy within a

clinically relevant population. In this thesis, the effects of these neuropeptides are examined within a mouse model of maladaptive aggression in which socially isolated mice are tested within a neutral context. The main advantages that this model provides are superior predicative validity in screening anti-aggressive serenics and greater translatability to pathological human aggression. Some of the pro-social effects of oxytocin have been previously shown to be mediated via actions at the V1aR. It is not known whether the anti-aggressive effects of oxytocin are similarly mediated through the V1aR. Hence, a greater understanding of the underlying pharmacology which underpins the behavioural effects of oxytocin and vasopressin will be of value in directing future drug discovery efforts

The serotonergic system is critically involved in the control of aggression and is thought to be involved in the anti-aggressive effects of some atypical antipsychotics. Pro-social effects on 5-HT1a agonism have previously been shown to be at least partly mediated via downstream release of endogenous oxytocin, but it has not been explored whether downstream actions mediated by endogenous oxytocin are also involved in the anti-aggressive effects of 5-HT1aR agonism. Whilst activation of the 5-HT_{1A}R can inhibit aggression, potential adverse side effects may rule out directly targeting it. Hence, a deeper examination of this serotonergic-oxytocin pathway may lead to the identification of either a more precise method of targeting this pathway or the identification of an entirely different pathway that may be relevant in the treatment of impulsive aggression.

1.5 Overview of Experimental Chapters

The work presented in this thesis provides evidence for three main hypotheses derived from the literature review presented in this chapter:

1. Targeting the OXT system will reduce aggressive behaviour in a non-territorial rodent model of pathological impulsive aggression

2. Anti-aggressive effects of OXT will involve actions at the V1aR and/or OXTR.

3. Anti-aggressive and pro-social effects of $5HT_{1A}R$ stimulation will involve downstream activation of the V1aR and/or OXTR.

Chapter 2 (Tan et al., 2019) aims to serve as a proof of concept to demonstrate the utility of the OXT system for treating aggression in a non-territorial rodent model of social isolation induced aggression which more closely resembles human clinical aggression. The involvement of the OXTR and V1aR in mediating the effects of peripheral OXT and AVP are examined.

Chapter 3 (Tan and Bowen., Under review) expands our understanding of the interactions between the serotonergic and oxytocin systems by determining whether the anti-aggressive and pro-social effects of $5\text{-}HT_{1A}R$ stimulation can be explained by downstream release of endogenous OXT and its subsequent actions at the OXTR and/or V1aR. To the best of our knowledge this is the first study to attempt to delineate the roles of OXT and 5-HT in pro-social and aggressive behaviour.

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Chapter 2: Oxytocin and Vasopressin Inhibit Hyper-Aggressive Behaviour in Socially Isolated Mice

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Abstract

Despite the high prevalence of aggression across a wide range of disorders, there is a severe lack of pharmacological treatments. Recent rodent studies have shown both centrally and peripherally administered oxytocin is effective in reducing territorial aggression, an adaptive form of aggression not reflective of pathological hyper-aggression. The current study tested i.p. administered oxytocin and vasopressin in a model of non-territorial hyper-aggression and examined the involvement of oxytocin receptors (OXTR) and vasopressin V1a receptors (V1aR). Male Swiss mice (N=160) were either socially isolated or group housed for 6 weeks prior to the commencement of testing; wherein two unfamiliar weight and condition matched mice were placed into a neutral context for 10 minutes. Socially isolated mice exhibited heightened aggression that was powerfully and dose-dependently inhibited by oxytocin and vasopressin and that was accompanied by dose-dependent increases in close social contact (huddling) and grooming. These anti-aggressive effects of oxytocin were blocked by pretreatment with a higher dose of selective V1aR antagonist SR49059 (20 mg/kg i.p.), but not a lower dose of SR49059 (5 mg/kg i.p.) or selective OXTR antagonist L-368,899 (10 mg/kg i.p.). This is consistent with a growing number of studies linking a range of effects of exogenous oxytocin to actions at the V1a receptor. Interestingly, the highest dose of the OXTR agonist TGOT (10 mg/kg) also reduced isolation-induced aggression. These results suggest that while activation of the V1a receptor appears critical for the anti-aggressive effects of oxytocin, activation of the oxytocin receptor cannot be excluded.

Keywords: Aggression; oxytocin; vasopressin; V1a receptor; oxytocin receptor; social behavior.

Introduction

A high prevalence of aggression has been observed across a range of psychiatric (Barlow et al., 2000), developmental (Kanne & Mazurek, 2011), substance use (Zhao et al., 2016), neurological (Fazel et al., 2009) and neurodegenerative disorders (Voyer et al., 2005); as well as following stroke (Kim, 2016), and traumatic brain injury (Rao et al., 2009). Despite considerable interest in developing a 'serenic' drug, a drug to specifically reduce aggression, to-date no true serenic drug has reached the market (Verhoeven & Tuinier, 2007). Instead, sedating doses of anti-psychotics and benzodiazepines, with their myriad liabilities, are most frequently used to manage aggressive behaviour in the aforementioned patient populations (Corbett et al., 2014). There is thus a clear need to explore novel therapeutic targets for the treatment of aggression.

The neuropeptide oxytocin (OXT) is well-known for its role in a number of important physiological processes and for its regulation of social behaviour and stress and anxiety responses (Neumann & Landgraf, 2012). However, more recently, the brain OXT system has garnered considerable attention as a potential therapeutic target for treating a range of disorders, including: social disorders; substance-use disorders; stress and anxiety disorders; and numerous other psychiatric disorders (Bowen & Neumann, 2017; Bowen & Neumann, 2018; Bradley & Woolley, 2017; McGregor & Bowen, 2012; Neumann & Slattery, 2016; Slattery & Neumann, 2010). There is also mounting interest in the brain OXT system as a novel treatment target for aggression (de Jong & Neumann, 2018).

Numerous lines of evidence point to an involvement of the OXT system in regulating aggressive behaviour in mammals (for a recent review see de Jong & Neumann, 2018). For instance, aggression is negatively correlated with activation of OXT neurons in rodents (Calcagnoli et al., 2014a; Hathaway et al., 2016). Moreover, recent animal studies found that

both acute (Calcagnoli et al., 2013) and chronic (Calcagnoli et al., 2014b) inctracerebroventricular (i.c.v.) infusion of OXT, intranasal administration of OXT (Calcagnoli et al., 2015a), and infusion of OXT directly in the central amygdala (Calcagnoli et al., 2015b) successfully inhibited inter-male territorial aggression in the resident intruder test.

Two of these studies (Calcagnoli et al., 2013; Calcagnoli et al., 2015b) were able to at least partially block OXT effects with an OXTR antagonist. However, these studies were conducted in rats and the peptidergic antagonist used lacks considerable selectively for the rat OXTR over the rat vasopressin 1a receptor (V1aR), and the non-peptidergic antagonist used has *greater* affinity for the rat V1aR over the rat OXTR (Manning et al., 2012). It is thus difficult to determine whether the effects of exogenous OXT on aggression reported in these studies were mediated by the OXTR or the V1aR, especially given the growing body of literature demonstrating effects of exogenous OXT mediated by actions at the V1aR.

For instance, Ramos et al. (2013) showed that peripheral administration of OXT and arginine vasopressin (AVP) resulted in an increase in social interaction, which was subsequently blocked by pre-treatment with SR49059, a V1aR antagonist, but not the OXTR antagonist C25. Similarly, Bowen and McGregor (2014) demonstrated that peripheral OXT increased social huddling behaviour in response to threat, which was also blocked by pre-treatment with SR49059. Unsurprisingly, given its greater affinity for V1aRs than OXTRs, AVP was substantially more potent at augmenting behaviour than OXT in both of those studies. Of particular relevance here, it has been shown that heightened levels of aggression in OXTR KO mice could be significantly reduced by peripheral administration of OXT, the effects of which were subsequently blocked by pre-treatment with SR49059, (Sala et al., 2011).

The role of AVP in aggression, however, appears complex. For example, Veenema et al. (2010) demonstrated that AVP release within the lateral septum (LS) and bed nucleus of the stria terminalis (BNST) correlated positively and negatively, respectively, with inter-male aggression in rats in the resident intruder test. Moreover, they found infusion of AVP into the BNST was able to inhibit aggression in highly aggressive rats, whereas administration of a V1aR antagonist into the LS of the highly aggressive rats inhibited aggression. However, in contrast, several other studies in rodents have found aggression to correlate negatively with AVP in the LS (Compaan et al., 1993; Everts et al., 1997). Overall, it is clear that more work is required to clearly characterise the effects of OXT and AVP on aggression and the receptors through which they act to exert their effects.

Moreover, as it pertains to the study of abnormal aggression (as is most relevant to the expression of aggression in a clinical setting), a limitation of the majority of the aforementioned studies examining OXT and AVP is their utilisation of the resident intruder paradigm. The resident intruder paradigm assesses territorial aggression, which can be considered a normal, adaptive response (Olivier & Young, 2002). It has been well documented within the animal literature that mice socially isolated for extended periods of time exhibit both heightened levels of aggression and numerous other behavioural abnormalities (see Brain (1975) for a detailed review). Thus, examining isolation-induced aggression in a neutral context provides a more appropriate model for examination of maladaptive, impulsive aggression akin to that observed in a wide range of human clinical populations (Olivier & Young, 2002). Moreover, the model provides excellent predictive validity and is a preferred model for serenic drug-screening programs (Olivier & Young, 2002).

The present study aimed to examine the effects of peripherally administered OXT and AVP on social isolation-induced aggression and to examine the involvement of the OXTR and V1aR. Importantly, there is now considerable evidence demonstrating that peripherally administered OXT and AVP lead to elevated cerebrospinal fluid and brain extracellular fluid levels of the neuropeptide being administered (Born et al., 2002; Chang et al., 2012; Dal Monte et al., 2014; Freeman et al., 2016; Modi et al., 2014; Neumann et al., 2013; Striepens et al., 2013). This study comprised of a series of 7 primary experiments using a social isolation-induced model of aggression in two cohorts of male Swiss mice. Experiment 1 compared baseline levels of aggression between single-housed mice and group-housed mice, to demonstrate successful induction of hyper-aggressive behaviour. Experiment 2 examined the effectiveness of different doses of OXT at reducing hyper-aggressive behaviour in isolated mice. Experiment 3 aimed to determine whether the anti-aggressive effects of OXT could be blocked by pre-treatment with the OXTR antagonist L-368,899. Experiments 4 and 5 subsequently aimed to determine whether the anti-aggressive effects of OXT could be blocked by pre-treatment with the selective AVP V1aR antagonist SR49059. Based on the findings of the previous experiments, experiment 6 examined whether AVP, across a range of doses, also had any anti-aggressive effects. Experiment 7 examined whether TGOT, a selective OXTR agonist, would produce any anti aggressive effects. To the best of our knowledge this is the first study to examine exogenous OXT and AVP effects on isolation-induced aggression in a neutral context.

Methods

Subjects

Two cohorts of mice were used over the course of the study. Male Swiss mice (ARC, Perth, Australia) aged 7-9 weeks and with an average weight of 37.3 g upon arrival (N=80 experiments 1 - 4) and aged 6-7 weeks with an average weight of 32.3 g upon arrival (N=80 experiments 5 - 7). Subjects were housed five per cage in a temperature controlled colony room (22˚C ± 1˚C) under standard laboratory conditions (12 – hour light/dark cycle; light phase 0700 – 1900 hours) upon arrival. Mice were housed in standard IVC cages. Mice had *ad libitum* access to food and water in their home cages. All experiments were performed during the light phase, between 11:00 and 18:00. All experimental procedures were approved by University of Sydney Animal Ethics Committee.

Procedure

Social isolation induced aggression paradigm

After allowing 1 week for acclimatisation, n = 64 mice from each cohort were socially isolated by moving them into single housing, whereas n = 16 animals from each cohort were housed in groups of four. During this time, all mice were weighed at least once per week. One week prior to the commencement of testing mice were given a single habituation injection with saline (0.9%, 10 mL/kg) to reduce the potential confounding stress and anxiety from injections during testing. Please note that we determined for successful induction of isolation-induced aggression the airflow from the IVC cages must be passed through the filters and exhausted back into the room. We believe this is due to the important role pheromones play in aggression (Chamero et al., 2007; Corridi et al., 1993; Hammour et al., 1982; Katz, 1976).

Two mouse social interaction test and drug administration

Testing commenced from 6 weeks post-isolation. All experiments were conducted in testing arenas constructed from acrylic, with matte blue internal walls and floor (400 mm x 400 mm x 400 mm). The lighting at the centre of the arenas during testing was <10 lux. Prior to testing, one mouse in each pair was marked on its back with a non-toxic marker, this allowed the automated behavioural tracking software (see below) to distinguish between the two mice. On test days, weight and condition matched pairs of unfamiliar mice were administered their drug treatment(s) as described below and were then placed into the testing arena together for a 10 min test session. Thus, both mice in each pair received the same drug treatment and were from the same housing condition. All drugs were administered via intraperitoneal (i.p.) injection (injection volume 10 mL/kg). The testing arenas were cleaned between sessions with ethanol (80%) and F10 disinfectant to eliminate residual odours. Cohort 1 mice were used for Experiments 1 through 4, and Cohort 2 mice for Experiments 5 through 7. New weight-matched pairs were formed in each experiment with weight differences between mice in each pair being no greater than 5 g. Drug treatments were randomly allocated to pairs and mice were given a one-week washout between tests.

Drugs

OXT (ChinaPeptide, Shanghai, China) was dissolved in saline (0.9%). AVP (ChinaPeptide, Shanghai, China) was dissolved in saline (0.9%). [Thr^4,Gly^7]OT (TGOT) (ChinaPeptide, Shanghai, China) was dissolved in saline (0.9%). The non-peptidergic OXTR antagonist L-368,899 (Santa Cruz Biotechnology, Dallas, Texas) was dissolved in saline (0.9%). The non-peptidergic AVP V1aR antagonist SR49059 (Axon MedChem BV, The Netherlands) was dissolved in a solution of DMSO (10%) Tween 80 (5%) and saline (85%).

OXT has high pM to low nM affinity for the mOXTR and ~20 fold selectivity over the mV1aR (Busnelli et al., 2013). AVP has low nM affinity for both the mOXTR and

mV1aR with little selectivity (Busnelli et al., 2013; Manning et al., 2012). TGOT has mid to high pM affinity for mOXTR and > 1000 fold selectivity over the mV1aR (Busnelli et al., 2013). SR49059 has high pM affinity for the mV1aR with ~15 fold selectivity of the mOXTR (Busnelli et al., 2013). Affinity and selectivity of L-368,899 for the mOXTR and mV1aR has not been determined. However, BRET studies have established that L-368,899 is a full antagonist of OXTR through all OXTR-dependent messenger systems (Duque-Wilckens et al., 2018). Moreover, the compound has been successfully used to alter OXTR mediated behaviours following systemic administration in mouse studies (Duque-Wilckens et al., 2018).

Timing of the drug administration (see below) was chosen based on the literature and pharmacokinetic properties of the drugs. Briefly, testing commenced 20 min after OXT administration so that testing would correspond to the peak brain concentration following i.p. administration in mice (Neumann et al., 2013), and we chose to use the same timing for TGOT. Testing commenced 5 min after administration of AVP as AVP reaches peak brain concentrations after systemic administration much more rapidly than OXT (Born et al., 2002; Neumann et al., 2013) and this timing has previously been used successfully in rodent behavioural studies (Bowen & McGregor, 2014). SR49059 was administered 15 min prior to OXT and L-368,899 10 min prior as these timings have been used previously to successfully block OXT effects in rodents (Hicks et al., 2014; Olszewski et al., 2015).

Experiments

Experiment 1: Initial screen for isolation-induced hyper-aggressive behaviour

This experiment utilised a 2 group between-subjects design. VEH (saline 0.9%) was administered to all mice 20 min prior to being placed in the open field testing arena. The two conditions were the group housed mice and socially isolated mice. The sample size in each

group was as follows: Group housed (n = 14); Single Housed (n = 64). Two mice in the group-housed cohort were not tested in this experiment due to the severity of fight wounds obtained in their home cage. Testing took place between 12:00 – 15:00 and time of testing was randomised across groups.

Experiment 2: Oxytocin dose response

An 8 group between-subjects design was utilised. 20 min prior to testing, group-housed mice received vehicle treatment (saline 0.9%; VEHg) and socially isolated mice received one of seven different doses of OXT (0, 0.03, 0.1, 0.3, 1, 3 and 10 mg/kg). This dose range of OXT was chosen as it covers doses that have been shown to enhance positive social behaviours in rodents (Bowen & McGregor, 2014; Ramos et al., 2013). The sample size in each group was as follows: group housed (n = 10); singled housed, OXT dose 0 (n = 10), 0.03 (n = 10), 0.1 (n = 10), 0.3 (n = 10), 1 (n = 8), 3 (n = 8), 10 (n = 8) mg/kg. An additional four mice from the group housed condition were not tested in this experiment due to fight wounds obtained in their home cage. Testing took place between 12:00 – 16:00 and time of testing was randomised across groups.

Experiment 3: Effect of OXTR antagonist on OXT effects on aggressive behaviour

A 5 group between-subjects design was utilised. Four groups of socially isolated mice received their first injection of VEH (0.9% saline) or L-368,899 (10mg/kg) 5 min prior to their second injection of VEH (0.9% saline) or OXT (3 mg/kg), 20 minutes after which the mice were placed into the testing arena. The group housed mice received VEH for both injections. The dose of L-368,899 was chosen as it has previously been shown to block the effects of an OXTR agonist in mice (Olszewski et al., 2014). The dose of 3 mg/kg OXT was chosen based on the results from experiment 2. N = 40 mice from cohort 1 were used for this experiment (n = 8 per group). Two of the injured group housed mice had recovered

sufficiently to resume testing. Testing took place between 14:00 – 16:00 and time of testing was randomised across groups.

Experiment 4: Effect of V1aR antagonist on OXT effects on aggressive behaviour

A 5 group between-subjects design was utilised. Four groups of socially isolated mice received their first injection of VEH (10% DMSO, 5% Tween 80, 85% physiological saline) or SR49059 (5 mg/kg) 5 min prior to their second injection of VEH (0.9% saline) or OXT (3 mg/kg), 20 minutes after which the mice were placed into the testing arena. The group housed mice received VEH for both injections. The dose of 5 mg/kg SR49059 was chosen as a similar dose had been shown to block OXT effects in rats (Bowen & McGregor, 2014). The mice from cohort 1 that were not tested in Experiment 3 were used in this experiment (n = 4 group housed, n = 8 in each single housed condition). Testing took place between 16:00 – 18:00 and time of testing was randomised across groups.

Experiment 5: Effect of a higher dose of V1aR antagonist on OXT effects on aggressive behaviour

A 5 group between-subjects design was utilised. Four groups of socially isolated mice received their first injection (VEH or SR49059 20 mg/kg) 15 min prior to their second injection (VEH or OXT 3 mg/kg), 20 minutes after which the mice were placed into the testing arena. The group housed mice received VEH for both injections. Given the partial blockade of some OXT effects in experiment 4 with a lower dose of SR49059, we aimed to determine whether a higher dose would lead to a complete blockade. Cohort 2 was used for Experiment 5 onward. The sample size for Experiment 5 was n = 14 in the group housed condition and n = 16 in each of the single housed conditioned. Two mice from the group housed condition were not tested in this experiment due to fight wounds obtained in their home cage. These mice did not recover sufficiently to be included in testing and thus were

not used for any of the experiments with cohort 2. Testing took place between 13:00 – 17:00 and time of testing was randomised across groups.

Experiment 6: AVP dose response

A 5 group between-subjects design was utilised. 5 min prior to testing, group housed mice received vehicle treatment and four groups of single housed mice received one of four different doses of AVP (0, 0.01, 0.03 or 0.1 mg/kg). The doses of AVP were chosen as we predicted AVP to be more potent than OXT at reducing aggression as it has greater affinity at the V1aR than OXT (Busnelli et al., 2013). Moreover, previous studies which have demonstrated V1aR mediated effects of OXT in rodents have found AVP to be substantially more potent (Bowen & McGregor, 2014; Ramos et al., 2013). The sample size for Experiment 6 was n = 14 in the group housed condition and n = 16 in each of the single housed conditions. Testing took place between 11:00 – 15:00 and time of testing was randomised across groups.

Experiment 7: TGOT dose response

The experiment utilised a 5 group between-subjects design. 20 min prior to testing, group housed mice received vehicle treatment and four groups of single housed mice received one of four different doses of TGOT (0, 1, 3 or 10 mg/kg). This dose range was chosen to match the effective dose range for OXT as TGOT and OXT have been shown to have similar affinity for the OXTR in hippocampal membrane preparations (Elands et al., 1988). The sample size for Experiment 7 was n = 14 in the group housed condition and n = 16 in each of the single housed conditioned. Testing took place between 12:00 – 15:00 and time of testing was randomised across groups.

Data Acquisition and Statistical Analysis

Experimental sessions were recorded with an overhead camera using CaptureStar (Version 1.00, CleverSys, Virginia, USA). The behaviours detailed below were automatically quantified from the videos using the behavioural tracking software TopScan, SocialScan and AgressionScan (CleverSys, Virginia, USA). The behaviours of interest were: (1) time (in seconds) spent fighting; (2) time (in seconds) spent huddling (defined as the mice being in close physical contact with each other while stationary); (3) time (in seconds) spent grooming themselves; and (4) distance travelled (in mm) by each mouse during the experimental session. Grooming and huddling behaviours were included as they have been shown to be sensitive to manipulations of the OXT and AVP system (Bowen & McGregor, 2014; Ramos et al., 2013; Schorscher-Petcu et al., 2010).

Data were analysed using SPSS (Version 24.0, IBM, USA). In the OXT, AVP and TGOT dose response experiments, multiple doses were analysed using planned linear and quadratic trend contrasts, excluding the group housed condition. In experiment 1 and the antagonist experiments, data were analysed with a one-way ANOVA and (for the antagonist experiments) planned contrasts. The planned contrasts are presented in Table 1. Significance was set at $p < 0.05$. Effect sizes are reported as eta-squared (η^2; small = 0.01, medium = 0.06, large = 0.14) for the linear and quadratic trends for the dose response experiments and Cohen's *d* (small = 0.2, medium = 0.5, large = 0.8) for the contrasts in all other experiments (Cohen, 1988).

Table 1. Planned contrasts for antagonist experiments

Contrast	What the contrast examines
1. Vehicle treated single housed mice (VEHs) vs vehicle treated group housed mice (VEHg)	The presence of a behavioural change (fighting, grooming, huddling, and/or distance travelled) in the socially isolated mice that received no antagonist or treatment compared to the group housed mice
2. single housed mice that received drug treatment only vs VEHs mice	Whether the drug treatment altered behaviour relative to vehicle treated single housed mice.
3. single housed mice that received drug treatment only vs VEHg mice	Whether the drug treatment altered behaviour relative to group housed mice.
4. single housed mice that received antagonist and drug treatment vs single housed mice that received drug treatment only	Whether pre-treatment with an antagonist inhibited the effects of drug treatment.
5. single housed mice that received antagonist and drug treatment vs VEHs mice	Whether pre-treatment with an antagonist was able to completely block the effects of drug treatment
6. single housed mice that received only antagonist treatment vs VEHs mice	Whether the antagonist alone was having any effects.

Results

Experiment 1: Initial screen for isolation-induced hyper-aggressive behaviour

The primary aim of this experiment was to establish the presence of isolation-induced aggression. Data are presented in Figure 1.

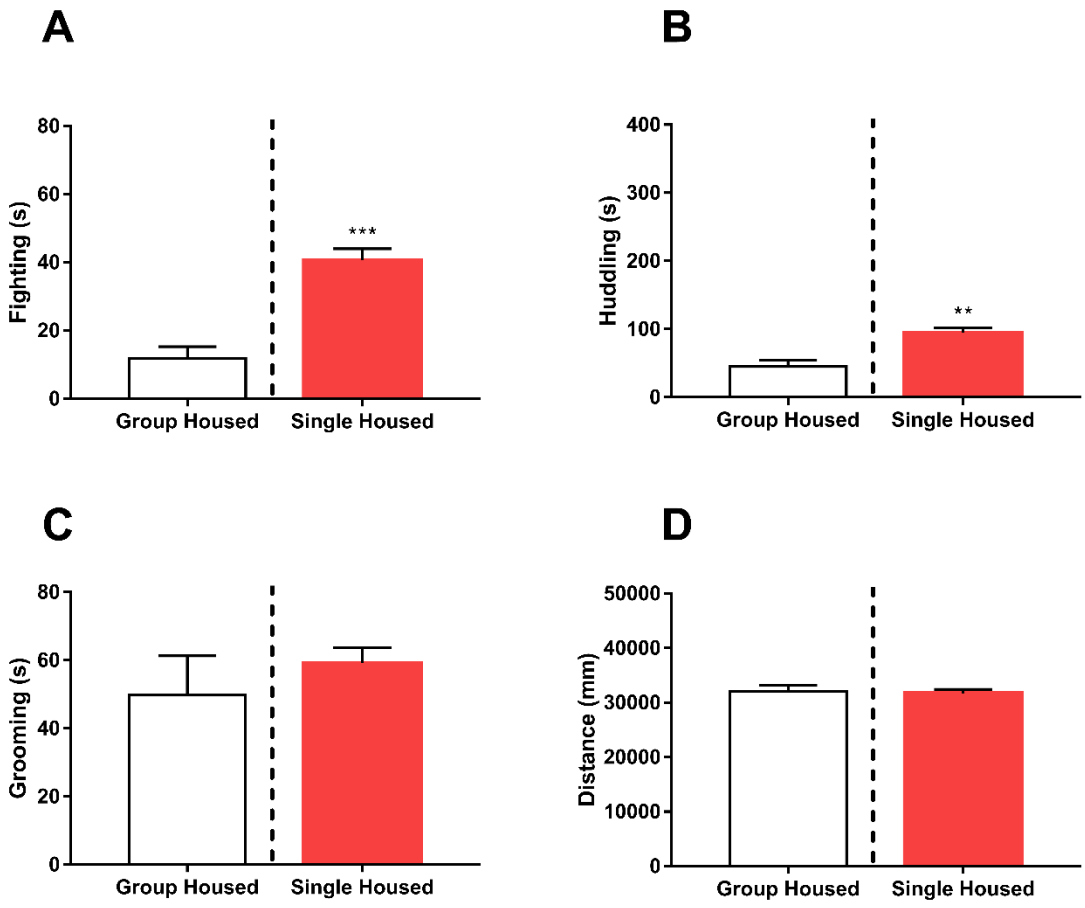

Figure 1. Experiment 1. The effect of social isolation on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. Socially isolated mice spent more time fighting and huddling and fighting but did not differ in grooming or distance travelled. Columns represent means ± SEM. **p<0.01; ***p<0.001

Single housed mice engaged in significantly more fighting [F(1,76) = 15.38, p = 0.0002, *d*=1.38] and huddling compared to group housed mice [*F*(1,76) = 11.51, p = 0.0011, *d*=1.12]. The groups did not differ significantly in time spent grooming [*F*(1,76)= 0.367, p=0.547, *d*= 0.23] or distance travelled [*F* (1, 76) = 0.288, p = 0.593, *d*=0.06].

Experiment 2: Oxytocin dose response

The primary aim of this experiment was to assess the impact of a range of OXT doses on aggression. Data are presented in Figure 2.

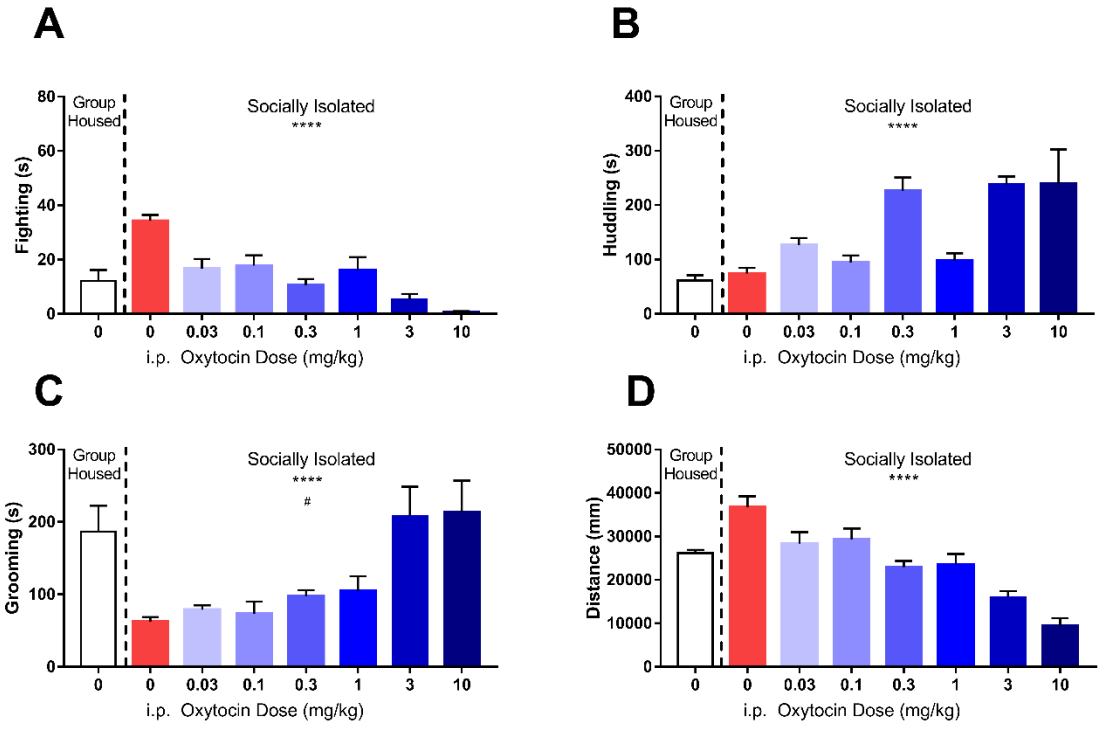

Figure 2. Experiment 2. The effect of peripheral OXT (0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg, 3.0 mg/kg or 10 mg/kg) on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. Oxytocin dose dependently inhibited aggressive behaviour, increased huddling and grooming and reduced distance travelled. Columns represent means ± SEM.

****indicates a significant linear trend for dose, p<0.0001. # indicates a significant quadratic trend for dose, p<0.05.

Results of the trend analysis examining dose response relationships are presented below. For the linear trends, with increasing doses of OXT there was a significant decrease in total fight time [$F(1,66)= 50.465$, $p<0.0001$, $\eta^2= 0.38$], increase in time spent huddling [$F(1,66)= 30.736$, $p<0.0001$, $\eta^2=0.23$], increase in time spent grooming [$F(1,66)= 38.924$, $p<0.0001$, $\eta^2=0.34$], and reduction in distance travelled [$F(1,66)= 100.711$, $p<0.0001$, $\eta^2=0.58$]. However, the quadratic trend was also found to be significant for grooming [$F(1,66)= 4.987$, $p=0.0290$, $\eta^2=0.04$], indicating that the rate of increase in grooming increased as dose increased. No other quadratic trends were significant (all $p > 0.29$). Due to the dip in the dose response curve at 1 mg/kg for huddling and grooming, doses 0.3 mg/kg and up were repeated in two experiments (this experiment and supplementary S1). As the same pattern was not observed in both experiments, the dip at this dose was deemed to likely be spurious.

Experiment 3: Effect of OXTR antagonist on OXT effects on aggressive behaviour

This experiment aimed to determine whether the anti-aggressive effects of OXT (3 mg/kg) could be blocked by pre-treatment with the selective OXTR antagonist L-368,899 (10 mg/kg). The results are presented in Figure 3.

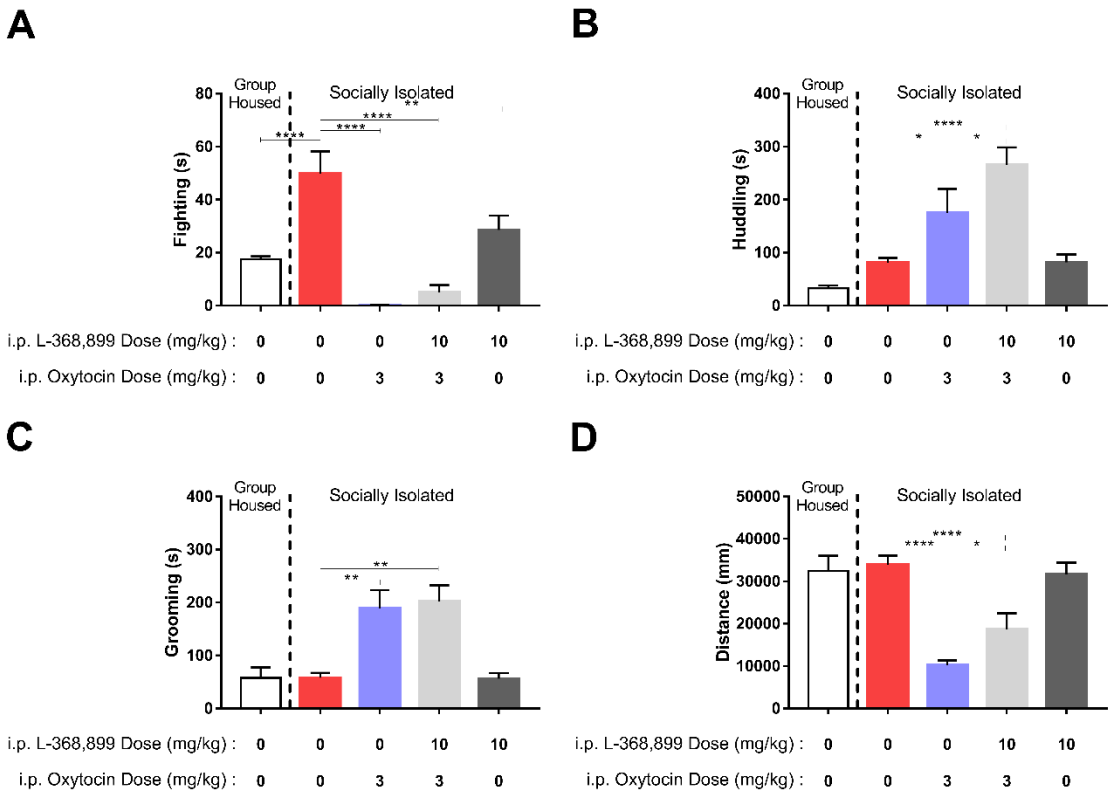

Figure 3. Experiment 3. The effect of pre-treatment with either the OXTR antagonist L-368,899 (10 mg/kg) or vehicle on OXT effects on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. OXT (3 mg/kg) reduced time spent fighting, increased time spent huddling and grooming and reduced distance travelled. Pre-treatment with L-368,899 (10 mg/kg) did not block the anti-aggressive effects of OXT, but did appear to partially block the locomotor suppressive effects of OXT. Columns represent means ± SEM. * $p<0.05$ ** $p<0.01$, **** $p<0.0001$.

Fight time, huddling, grooming and distance travelled all differed significantly as a function of group [$F(4,35)=17.784$, $p<0.0001$; $F(4,35)= 12.223$, $p<0.0001$; $F(4,35)=10.564$, $p< 0.0001$; $F(4,35)= 13.298$, $p<0.0001$ respectively] as such the planned contrasts were performed for all of these variables.

Isolation increased fighting (VEHs vs VEHg, p<0.0001, *d*=1.86) and OXT not only completely blocked isolation induced aggression (OXT vs VEHs, p<0.0001, *d*=2.90), but reduced it below the levels seen in the group housed mice (OXT vs VEHg p= 0.0132, *d*=8.03). L-368,899 did not inhibit the effect of OXT on fighting (L-368,899 + OXT vs OXT p= 0.478, *d*=0.85; L-368,899 + OXT vs VEHs p<0.0001, *d*=1.26). The antagonist on its own significantly reduced isolation induced aggression (L-368,899 vs VEHs p = 0.0032, *d*=1.05).

Isolation did not significantly affect huddling (VEHs vs VEHg, p = 0.204, *d*=2.35). OXT significantly increased huddling in the isolated mice (OXT vs VEHs p = 0.0180, *d*=1.00), and L-368,899 enhanced the effect of OXT on huddling (L-368,899 + OXT vs OXT p=0.0207, *d*=0.80; L-368,899 + OXT vs VEHs p<0.0001, *d*=2.69). The antagonist on its own did not have a significant effect on time spent huddling (L-368,899 vs VEHs p = 0.819, *d*=0.016).

There was no effect of isolation on grooming (VEHs vs VEHg, p = 0.995, *d*<0.01). OXT increased grooming in the isolated mice (OXT vs VEHs p = 0.0003, *d*=1.85), and L-368,899 did not inhibit the effect of OXT on grooming (L-368,899 + OXT vs OXT p=0.702, *d*=1.38; L-368,899 + OXT vs VEHs p=0.0001, *d*=2.23). The antagonist on its own did not have a significant effect on grooming (L-368,899 vs VEHs p = 0.953, *d*=0.07).

There was no effect of isolation on distance travelled (VEHs vs VEHg p = 0.713, *d*=0.18). OXT significantly reduced distance travelled (OXT vs VEHs p< 0.0001, *d*=4.94), and L-368,899 inhibited but did not fully block the effect of OXT on distance travelled (L-368,899 + OXT vs OXT p = 0.0445, *d*=1.06; L-368,899 + OXT vs VEHs p = 0.0006, *d*=1.76). The antagonist on its own did not have a significant effect on distance travelled (L-368,899 vs VEHs p = 0.567, *d*=0.33).

Experiment 4: Effect of V1aR antagonist on OXT effects on aggressive behaviour

This experiment examined whether the anti-aggressive effects of OXT (3 mg/kg) could be blocked by pre-treatment with selective V1aR antagonist SR49059 (5 mg/kg). The results are presented in Figure 4.

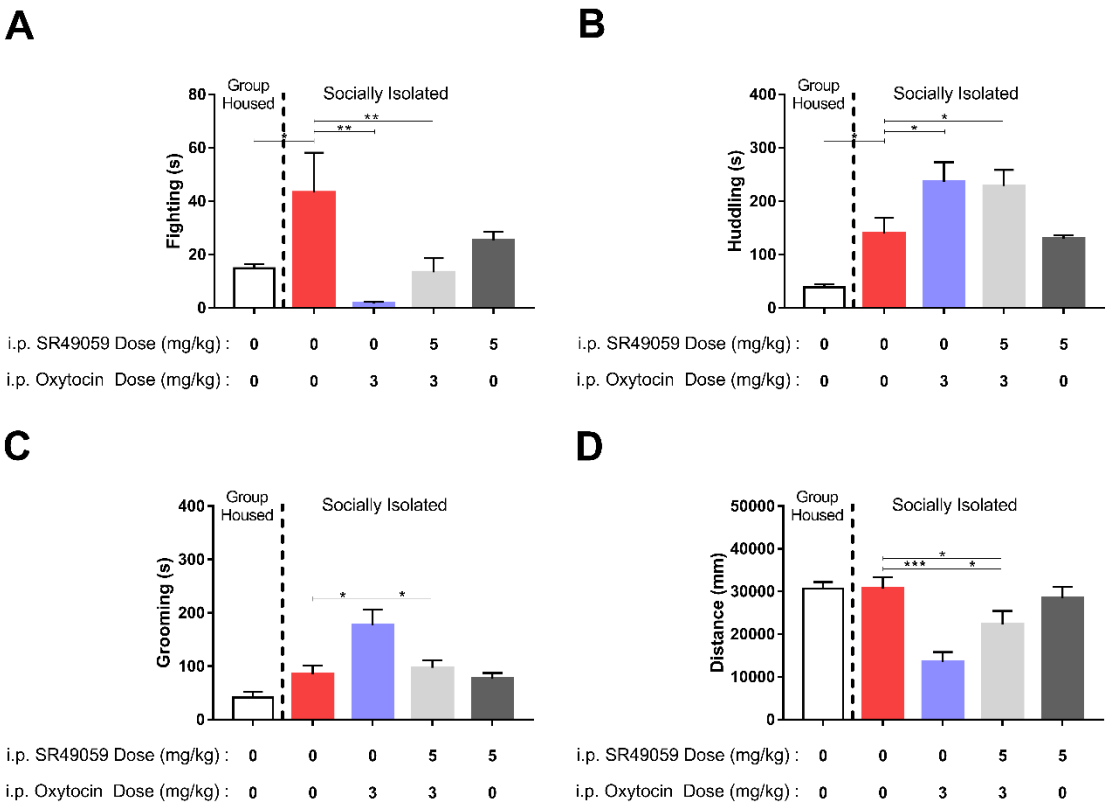

Figure 4. Experiment 4. The effect of pre-treatment with either the V1aR antagonist SR49059 (5 mg/kg) or vehicle on OXT effects on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. OXT (3 mg/kg) decreased time spent fighting, increased huddling and grooming, and reduced distance travelled. Pre-treatment with SR49059 partially blocked the locomotor suppressive effects of OXT and completely blocked OXT effects on grooming, but did not inhibit OXT effects on fighting or huddling. Columns represent means ± SEM. * $p<0.05$ **$p<0.01$. ***$p<0.001$.

Fight time, time spent huddling, grooming and distance travelled, and all differed significantly as a function of group [$F(4,31)= 4.072$, $p= 0.0091$; $F(4,31)= 6.581$, $p= 0.0005$; $F(4,31)= 6.452$, $p<0.0001$; $F(4,31)= 7.450$,$p=0.0002$ respectively].

Isolation again significantly increased fighting (VEHs vs VEHg, p = 0.0410, *d*=0.95). OXT completely blocked isolation induced aggression (OXT vs VEHs p = 0.0005, *d*=1.39; OXT vs VEHg p = 0.330, *d*=5.09). SR did not significantly inhibit the effect of OXT on fighting (SR + OXT vs OXT p = 0.291, *d*=1.08; SR + OXT vs VEHs p = 0.0099, *d*=0.94). The antagonist on its own did not have a significant effect on fighting (SR vs VEHs p = 0.112, *d*=0.58).

Isolation significantly increased huddling (VEHs vs VEHg, p = 0.0369, *d*=1.72). OXT significantly increased huddling in the isolated mice (OXT vs VEHs p = 0.0165, *d*=1.02), and SR did not inhibit the effect of OXT on huddling (SR + OXT vs OXT p = 0.848, *d*=0.08; SR + OXT vs VEHs p = 0.0258, *d*=1.06). The antagonist on its own did not have a significant effect on huddling (SR vs VEHs p =. 0.7951, *d*=0.17).

There was no effect of isolation on grooming (VEHs vs VEHg, p = 0.1675, *d*=1.26). OXT increased time spent grooming in the isolated mice (OXT vs VEHs p = 0.0011, *d*=1.38), and SR completely blocked the effect of OXT on grooming (SR + OXT vs OXT p = 0.0038, *d*=1.23; SR + OXT vs VEHs p = 0.6466, *d*=0.28). The antagonist on its own did not have a significant effect on grooming (SR vs VEHs p =. 0.7420, *d*=0.22).

There was no effect of isolation on distance travelled (VEHs vs VEHg, p = 0.991, *d*=0.01). OXT significantly reduced distance travelled (OXT vs VEHs p<0.0001, *d*=2.42), and SR inhibited but did not completely block the effect of OXT on distance travelled (SR + OXT vs OXT p = 0.0218, *d*=1.13; SR + OXT vs VEHs p = 0.0289, *d*=1.02). The antagonist

on its own did not have a significant effect on distance travelled (SR vs VEHs p = 0.551, *d*=0.30).

Experiment 5: Effect of a higher dose of V1aR antagonist on OXT effects on aggressive behaviour

This experiment examined whether the anti-aggressive effects of OXT (3 mg/kg) could be blocked by pre-treatment with a higher (20 mg/kg) dose of SR49059. The data are presented in Figure 5.

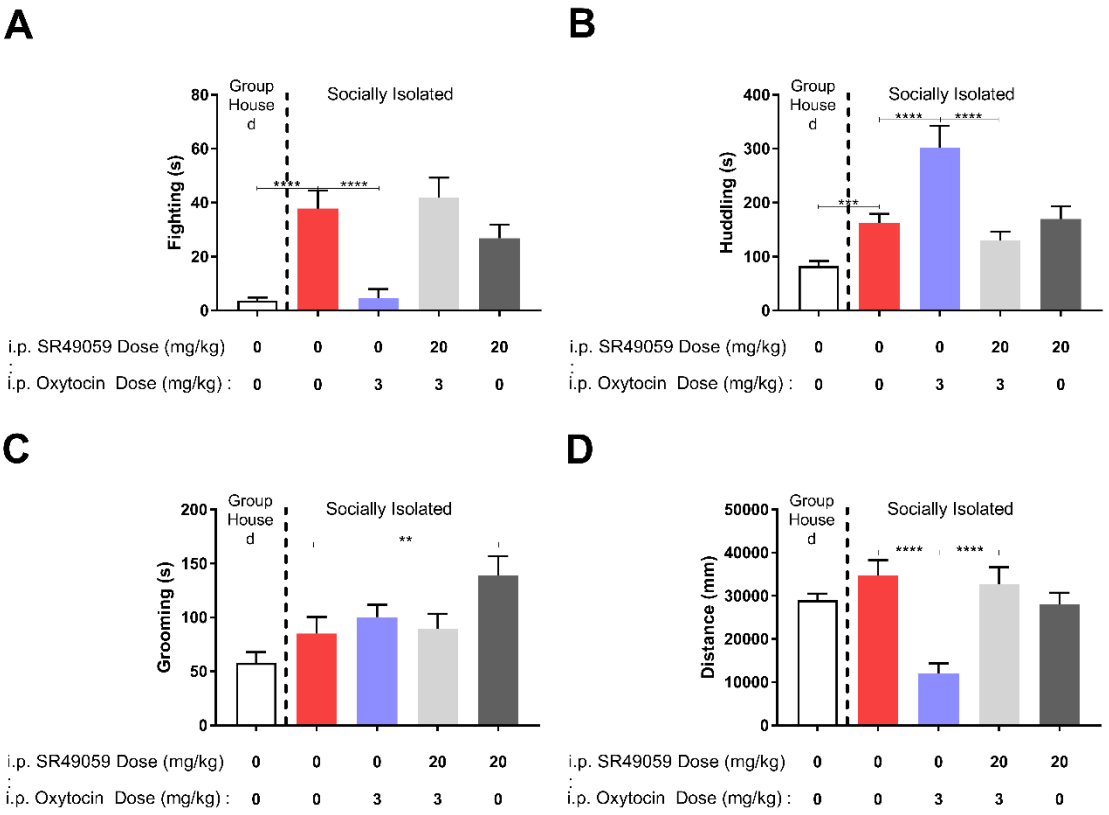

Figure 5. Experiment 5. The effect of pre-treatment with either the V1aR antagonist SR49059 (20 mg/kg) or vehicle on OXT effects on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. OXT (3 mg/kg) decreased time spent fighting, increased huddling, and reduced distance travelled. Pre-treatment with SR49059 (20 mg/kg)

fully blocked the effects of OXT on fighting, huddling, and distance travelled. Columns represent means ± SEM. **p<0.01. ***p<0.001, ****p<0.0001.

Fight time, huddling, grooming and distance travelled all differed significantly as a function of group [$F(4,73)= 11.333$, $p<0.0001$; $F(4,73)= 11.387$, $p<0.0001$; $F(4,73)= 4.243$, $p= 0.0038$; $F(4,73)= 9.184$,p $p<0.0001$ respectively].

Isolation significantly increased fighting (VEHs vs VEHg, $p < 0.0001$, d=1.80). OXT completely blocked isolation induced aggression (OXT vs VEHs $p < 0.0001$, d=1.59; OXT vs VEHg $p = 0.890$, d=0.11), and SR completely blocked the effect of OXT on fighting (SR + OXT vs OXT $p < 0.0001$, d=1.65; SR + OXT vs VEHs $p = 0.580$, d=0.15). The antagonist on its own did not have a significant effect on fighting (SR vs VEHs $p = 0.143$, d=0.47).

Isolation significantly increased huddling (VEHs vs VEHg, $p = 0.0260$, d=1.47). OXT significantly increased huddling in the isolated mice (OXT vs VEHs $p<0.0001$, d=1.14), and SR completely blocked the effect of OXT on huddling (SR + OXT vs OXT $p<0.0001$, d=1.41; SR + OXT vs VEHs $p = 0.336$, d=0.48). The antagonist on its own did not have a significant effect on huddling (SR vs VEHs $p = 0.819$, d=0.10).

There was no effect of isolation on grooming (VEHs vs VEHg, $p = 0.188$, d=0.53). OXT did not have an effect on grooming in the isolated mice. (OXT vs VEHs $p = 0.442$, d=0.28) However, the antagonist on its own appeared to increase grooming (SR vs VEHs $p = 0.008$, d=0.82).

There was no effect of isolation on distance travelled (VEHs vs VEHg, $p = 0.185$, d=0.53). OXT significantly reduced distance travelled (OXT vs VEHs $p<0.0001$, d=1.91), and SR completely blocked the effect of OXT on distance travelled (SR + OXT vs OXT

p<0.0001, *d*=1.58; SR + OXT vs VEHs p = 0.611, *d*=0.14). The antagonist on its own did not have a significant effect on distance travelled (SR vs VEHs p = 0.114, *d*=0.54).

Experiment 6: AVP dose response

This primary aim of this experiment was to assess the impact of a range of doses of AVP on aggression. The results are presented in Figure 6.

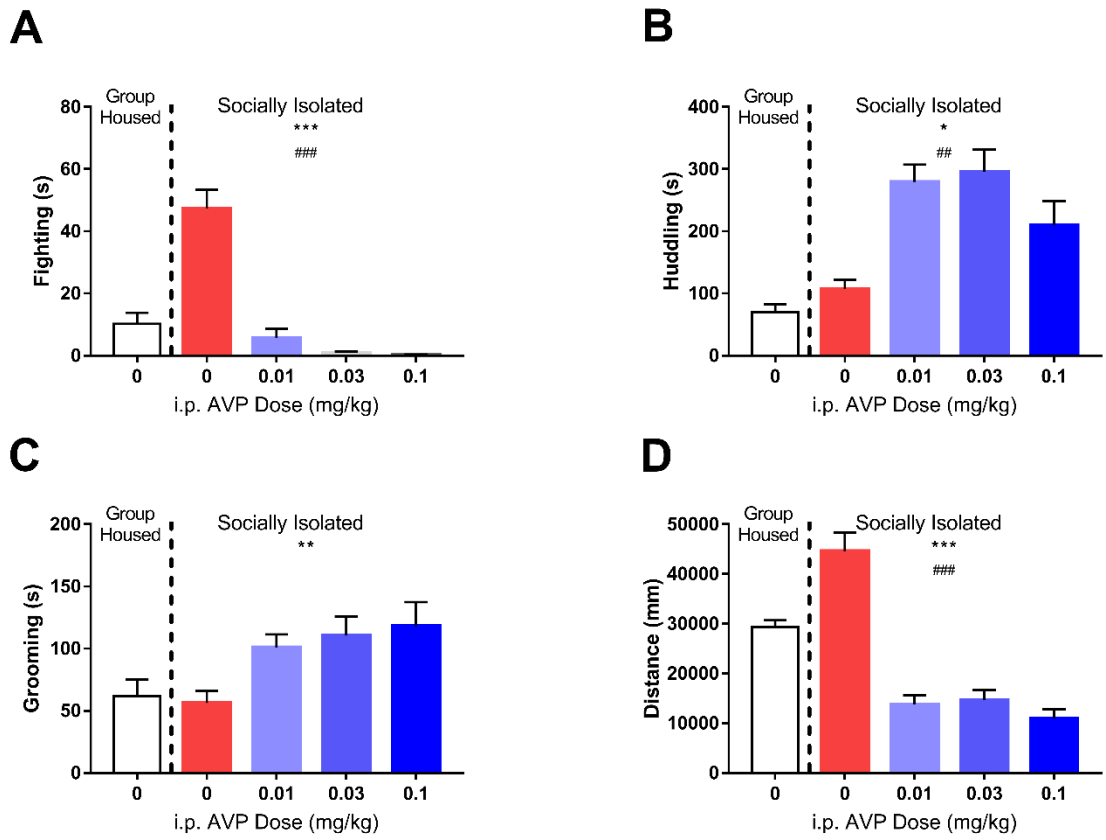

Figure 6. Experiment 6. The effect of AVP (0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg) on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. AVP dose dependently inhibited aggressive behaviour, increased huddling and grooming, and decreased distance travelled. Columns represent means ± SEM. ***indicates a significant linear trend, p<0.001.

# indicates a significant quadratic trend $p<0.05$; ## indicates a significant quadratic trend $p<0.01$; ### indicates a significant quadratic trend $p<0.001$.

Results of the trend analysis examining dose response relationships are presented below. With increasing doses of AVP there was a significant decrease in time spent fighting [$F(1,73)= 73.258$, $p<0.0001$, $\eta^2=0.41$], increase in time spent huddling [$F(1,73)= 4.919979$, $p=0.0297$, $\eta^2=0.04$], increase in time spent grooming [$F(1,73)= 7.584$, $p=0.0074$, $\eta^2=0.09$], and reduction in distance travelled [$F(1,73)= 57.319$, $p<0.0001$, $\eta^2=0.35$]. However, the quadratic trend was also found to be significant for fight time [$F(1,73)= 26.755$, $p<0.0001$, $\eta^2=0.15$], and for distance travelled [$F(1,73)= 17.537$, $p<0.0001$, $\eta^2=0.11$], such that the rate of decrease began to decrease at higher doses. The quadratic trend was also significant for huddling [$F(1,73)= 16.542$, $p= 0.0001$, $\eta^2=0.14$], indicative of the slight dip in efficacy at the highest dose.

Experiment 7: TGOT dose response

This experiment aimed to assess whether TGOT, a selective OXTR agonist, would have any impact on aggression. The data are presented in Figure 7.

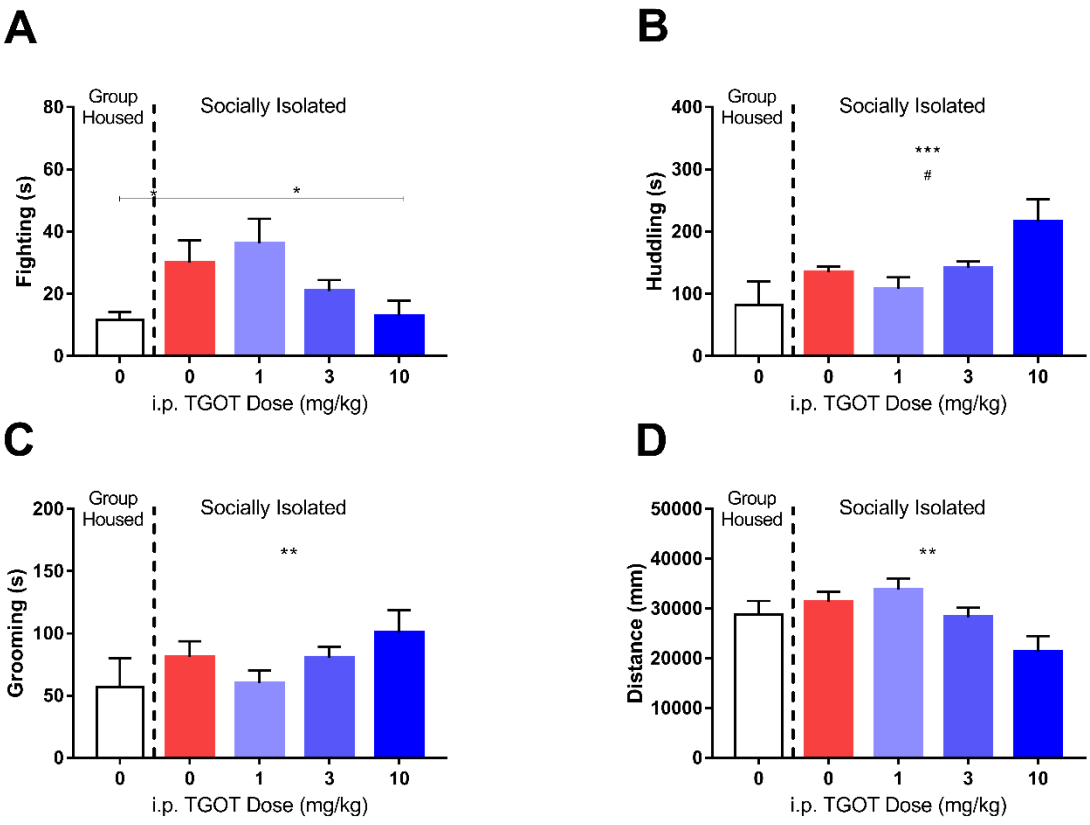

Figure 7. Experiment 7. The effect of TGOT (1 mg/kg, 3 mg/kg, 10 mg/kg) on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. TGOT dose dependently increased huddling and grooming and decreased locomotor activity. Only the highest dose of TGOT (10 mg/kg) significantly reduced aggression. Columns represent means ± SEM. For A: *$p<0.05$. For B – D: **indicates a significant linear trend at $p<0.01$; ***indicates a significant linear trend $p<0.001$; # indicates a significant quadratic trend $p<0.05$.

Results of the trend analysis examining dose response relationships are presented below. For total fight time, neither the linear [$F(1,73)= 0.0439$, $p= 0.835$, $\eta^2=0.08$] nor quadratic [$F(1,73)= 1.622$, $p= 0.207$, $\eta^2=0.02$] trends were statistically significant. However, examination of the data (Figure 7A) revealed that the two higher doses did indeed appear to be reducing aggression. Thus, a post-hoc one-way ANOVA with follow-up comparisons was

conducted to determine if there were any significant group differences. This revealed that total fight time differed significantly as a function of condition [F(4, 73)= 3.568, p= 0.0103], isolation significantly increased fighting (VEHs vs VEHg p = 0.0276, *d*=0.88), but only the highest dose of TGOT (10 mg/kg) significantly decreased fighting (TGOT 10 mg/kg vs VEHs p = 0.0317, *d*=0.71).

For other trend analyses, with increasing doses of TGOT there was a significant increase in time spent grooming, [F(1,73)= 9.185, p= 0.0034, η^2=0.02], increase in time spent huddling [F(1,73)= 20.813, p< 0.0001, η^2=0.07], and decrease in distance travelled [F(1,73)= 10.85, p= 0.0015, η^2=0.12]. The quadratic trend was also significant for time spent huddling [F(1,73)= 4.471, p= 0.03789, η^2=0.05], indicating the rate of increase began to increase as the doses increased. No other quadratic trends were significant, all p>.05.

Discussion

The current study examined the effects of OXT and AVP on isolation-induced aggression in male Swiss mice and explored the involvement of the OXTR and V1aR in the observed effects. Most previous studies exploring the involvement of OXT and AVP in aggression have used models of territorial or maternal aggression. We thus sought to use the isolation-induced aggression model with testing in a neutral context. It has been argued that this paradigm more closely models the pathological aggression observed in numerous clinical populations (Olivier & Young, 2002) and, as such, this model is more relevant for examining the therapeutic potential of targeting the brain OXT system to treat pathological aggression, an area of growing interest (de Jong & Neumann, 2018).

We found that hyper-aggressive behaviour in socially isolated mice was inhibited by both peripheral administration of OXT and peripheral administration of AVP across a range of doses. With regards to OXT, this is consistent with recent studies which demonstrated that OXT administered either centrally or peripherally inhibited territorial aggression in rats in the resident intruder test (Calcagnoli et al., 2013; Calcagnoli et al., 2015a; Calcagnoli et al., 2014b; Calcagnoli et al., 2015b). We demonstrated that peripheral OXT was also able to inhibit non-territorial hyper-aggressive behaviour in male mice.

Our findings also contribute to the literature regarding the complex effects of AVP on aggressive behaviour. For instance, whilst Veenema et al. (2010) found that AVP in the LS was associated with increased aggression in male rats in the resident intruder test, other studies have found the opposite association (Compaan et al., 1993; Everts et al., 1997). Veenema et al. (2010) also found that infusion of AVP into the BNST inhibited aggression in rats. In contrast, another study found that infusion of AVP into the anterior hypothalamus decreased latency to attack in male Syrian golden hamsters in the resident intruder test,

although it appears this effect is only present in hamsters previously trained to fight (Ferris et al., 1997; Terranova et al., 2017). Our findings indicate that, at least in the context of non-territorial isolation-induced hyper-aggressive behaviour in male mice, AVP has an inhibitory effect on aggression. However, there are clearly complexities regarding context of testing, type of aggression being examined, regional-specificity of actions, and species and sex differences that remain to be comprehensively understood as pertaining to AVP effects on aggression.

Inhibition of aggression by OXT and AVP was accompanied by increases in huddling and grooming and a decrease in distance travelled. OXT and AVP have previously been reported to cause dose-dependent increases in close social contact (aka huddling) and grooming in rodents (Bowen & McGregor, 2014; Drago et al., 1986; Meisenberg, 1988; Ramos et al., 2013). Whilst increased huddling following OXT and AVP treatment have previously been reported in rats, this is the first demonstration of this behaviour in response to OXT in mice. Moreover, whilst grooming has previously been reported in response to central OXT or AVP administration, to the best of our knowledge, this study provides one of, if not the first demonstrations of increased grooming following peripheral administration of these neuropeptides.

The dose dependent effects of AVP and OXT on distance travelled, can, at least in part, be attributed to the substantial increases in huddling and grooming behaviours. To elaborate, the huddling and grooming both involve the mice staying in the same position within the arena; as the increase in time spent engaged in these behaviours was of substantial magnitude, it is thus not surprising that the measure of distance travelled around the arena during the session was reduced. To assess this more objectively, we conducted a post-hoc analysis (see supplementary S2), which revealed that once the increased time spent engaged in huddling and grooming following OXT treatment was controlled for, the average distance

travelled between the vehicle treated isolated mice (57.96 mm/s) and the mice treated with 3 mg/kg OXT (61.36 mm/s) did not differ significantly. Finally, we conducted a control experiment in which mice were treated with VEH, OXT (3 mg/kg, i.p.), AVP (0.1 mg/kg, i.p.) or TGOT (10 mg/kg, i.p.) and then tested for locomotor activity in the testing arenas in the absence of a conspecific. There was no significant effect of any of the treatments on locomotor activity, further supporting our conclusion that the suppression observed in the main experiments was due to the increased huddling and grooming. Detailed methods and results for this control experiment are presented in supplementary S3.

Interestingly, we found that pre-treatment with a higher (but not lower) dose of the V1aR antagonist SR49059 (20 mg/kg i.p.), but not the OXTR antagonist L-368,899 (10 mg/kg i.p.), completely blocked the anti-aggressive effects of OXT as well as OXT-induced increases in huddling and reductions in distance travelled. This aligns with a growing body of research demonstrating a range of behavioural effects of exogenously administered OXT, including increases in close social contact and grooming, are mediated via actions at the V1aR (Bowen & McGregor, 2014; Schorscher-Petcu et al., 2010). Importantly, this is consistent with a previous study which found that peripheral administration of OXT could reduce aggression in OXTR KO mice and this effect was blocked by SR49059 (Sala et al., 2011).

Previous studies in rodents have also shown a clear pattern of neuronal activation induced by peripherally administered OXT that shares overlap with neural substrates underlying intermale aggression in rodents. Moreover, this pattern of OXT-induced neuronal activation is inhibited by SR49059. Specifically, OXT administered i.p. caused a significant increase in c-fos expression in the supraoptic nucleus, paraventricular nucleus, central amygdala, lateral parabrachial nucleus, locus coeruleus, nucleus of the solitary tract, BNST;

and decreased expression the lateral habenula (Carson et al., 2010; Hicks et al., 2012; Hicks et al., 2016). Importantly, SR49059 was able to inhibit the OXT-induced c-fos in the supraoptic nucleus, paraventricular nucleus, locus coeruleus, nucleus of the solitary tract, central amygdala and lateral parabrachial nucleus (Hicks et al., 2016). Interestingly, the paraventricular nucleus, BNST, amygdala and locus coeruleus are all regions which have been identified to play a role in intermale aggression in rodents (Takahashi & Miczek, 2014).

In the present study we did not observe increased aggression following administration of L-368,899 on its own, as has previously been reported (Calcagnoli et al., 2013). In fact, a significant *decrease* in fighting was observed in the present study. Of note, Calcagnoli et al (2013) found that L-368,899 increases territorial aggression, whereas the present study examined aggression in a neutral context. Also of note, Calcagnoli et al (2013) observed an inverted-U shaped dose response whereby the middle dose of centrally administered L-368,899 increased aggression but the highest dose had no effect. It is therefore possible that similar effects with peripherally administered L-368,899 may have been observed in our mouse model at a different dose to that used.

In the present study, V1aR antagonism alone did not lead to a heightened level of aggression. This is consistent with the findings of Wersinger et al. (2007) who demonstrated that socially isolated V1aR KO mice exhibit enhanced territorial aggression in the resident-intruder test compared to WT mice, but show no differences in aggression when tested in a neutral context. This again highlights the importance of being cognisant of the different types of aggression modelled by different paradigms.

We also tested the effect of a selective, peptidergic OXTR agonist TGOT. We expected TGOT to be ineffective in inhibiting aggressive behaviour in our model due to its low level of activity at the mouse V1aR (Busnelli et al., 2013). Contrary to our expectations,

however, we found that the highest dose tested (10 mg/kg) led to both a significant reduction in aggression (albeit only when unplanned post-hoc comparisons were made), and an increase in huddling. Given both the lower dose of SR49059 (5 mg/kg) and L-368,899 (10 mg/kg, i.p.) caused a similar partial blockade of OXT effects on distance travelled and that TGOT at the highest dose appeared to be having some impact on aggression, it is possible that in this model there is also a lower efficacy (relative to V1aR activation) inhibitory effect of OXTR activation on aggression. Future studies should therefore further explore the possibility of both V1aR activation and OXTR activation being involved in OXT inhibition of hyper-aggressive behaviour. Finally, as TGOT only had an impact on aggression at the highest dose, it is also possible that it is acting at the V1aR to exert these effects as it does have some affinity for the mouse V1aR and a previous study found that a high dose of TGOT augmented social behaviour in OXTR KO mice and this was blocked by SR49059 (Sala et al., 2013).

Another point that warrants further discussion is that L-368,899 on its own appeared to reduce aggression (Fig. 3A) and when co-administered with OXT appeared to amplify the effects of OXT on huddling (Fig. 3B). Whilst these effects may be mediated via OXTR antagonism, it is also possible L-368,899 may be having these effects through activity at another receptor. Off-target effects are common with small molecules (Schenone et al., 2013) and, as is common with tool/probe compounds, the off-target effects of L-368,899 have not been systematically explored. Indeed, there has been no examination of activity outside of OXTR and AVP receptors. It is therefore possible that L-368,899 may be having these effects through actions at another receptor target.

A limitation of the current study is that a higher dose of L-368,899 was not tested. The dose of L-368,899 was chosen as it has been shown to be the maximally effective dose in mice (Olszewski et al. 2015). Moreover, other studies in mice have shown clear effects with doses as low as 1 and 3 mg/kg i.p. (Olszewski et al. 2010). Given that the antagonist on its

own appeared to decrease aggression and that OXT induced huddling was amplified by pre-treatment with L-368,899 (perhaps due to off-target effects), we felt that testing a higher dose was unlikely to provide more clarity. Nonetheless this limitation should be noted and some involvement of the OXTR cannot be conclusively ruled out.

A limitation of this model that warrants highlighting is that across repeated testing it does not always lead to the same phenotype in the vehicle treated isolated mice relative to the vehicle treated group housed mice. For instance, whereas aggression was consistently increased, huddling was either increased (Fig. 1B, 3B, 4B, 5B, 6B, S1B) or unchanged (Fig. 2B, 7B). Similarly, grooming was either increased (Fig. 4C, 5C), not changed (Fig. 1C, 3C, 6C, 7C) or decreased (Fig. 2C, S1C), and locomotion was either increased (Fig. 2D, 5D, 6D, S1D) or unchanged (Fig. 1D, 3D, 4D, 7D). However, whilst there was some variability in these behavioural measures with regards to the effect of isolation, the impact of OXT on these behaviours was consistent. Moreover, the level of aggression observed in the group housed mice and the single-housed mice treated with vehicle was consistent across experiments (see Figures 1 to 7). We therefore do not believe that repeated testing had an impact on our results with regards to aggression.

Conclusions

Overall, the present study adds to a growing body of evidence showing a number of pronounced effects of exogenously administered OXT are mediated by actions at the V1aR. This has important implications for therapeutic development, suggesting that ligands selectively targeting the OXTR might lack some important therapeutic effects mediated by the V1aR. However, regarding aggression, whilst we saw striking V1aR mediated inhibition of isolation-induced hyper-aggressive behaviour in the present study, this must be considered in the context of previous studies, which highlight the complex array of factors mediating the impact of the brain OXT and AVP systems on aggression. Unravelling these factors will play

an important role in more fully understanding the potential of targeting brain OXT systems to treat pathological aggression.

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Chapter 3: Divergent pathways mediate the effects of a 5-HT_{1A} receptor agonist on close social interaction, grooming and aggressive behaviour: exploring involvement of the oxytocin system

This is a word document version of a manuscript under review: Tan, O., Bowen, M. (under review). Divergent pathways mediate the effects of a 5-HT1A receptor agonist on close social interaction, grooming and aggressive behaviour: exploring involvement of the oxytocin system. *Journal of Psychopharmacology*.

Abstract

Background: 5-HT_{1A} receptor (5-HT_{1A}R) abnormalities are implicated in aggression and there has been considerable interest in developing 5-HT_{1A}R agonists for treating aggression. Endogenous oxytocin released upon stimulation of 5-HT_{1A}Rs in the hypothalamus mediates at least some of the effects of 5-HT_{1A}R agonists on social behaviour.

Aims: Given 5-HT_{1A}R, oxytocin receptor (OXTR) and vasopressin V1a receptor (V1aR) agonists can all reduce aggression, the current study aimed to determine whether the anti-aggressive effects of 5-HT_{1A}R stimulation can also be explained by downstream actions at OXTRs and/or V1aRs in a mouse model of non-territorial, hyper-aggressive behaviour.

Methods: Male Swiss mice (N=80) were socially isolated or group housed for 6 weeks prior to the start of testing. Testing involved placing two unfamiliar weight and condition matched mice together in a neutral context for 10 min.

Results: Social isolation led to a pronounced increase in aggressive behaviour, which was dose-dependently inhibited by the 5-HT_{1A}R agonist 8-OH-DPAT (0.1, 0.3, 1 mg/kg i.p.) with accompanying increases in close social contact (huddling) and grooming. The effects of 8-OH-DPAT on aggression, huddling and grooming were blocked by pre-treatment with a selective 5-HT_{1A}R antagonist (WAY-100635, 0.1 mg/kg i.p.). The anti-aggressive effects of 8-OH-DPAT were unaffected by an OXTR antagonist (L-368,899, 10 mg/kg i.p.), whereas the effects on huddling and grooming were inhibited. Pre-treatment with a V1aR antagonist (SR49059, 20 mg/kg i.p.) had no effect.

Conclusions: Our study suggests that stimulation of endogenous oxytocin is involved in the effects of 5-HT_{1A}R activation on close social contact and grooming but not aggression.

Introduction

Disruption to the serotonergic system is strongly implicated in the underlying pathophysiology of heightened aggression (Coccaro et al., 2015; Olivier, 2004; Rosell & Siever, 2015; Seo et al., 2008; Siever, 2008; Takahashi et al., 2012) and numerous attempts to create a serenic drug (Olivier & Mos, 1986) have targeted the serotonergic system, in particular 5-HT_{1A}Rs (Verhoeven & Tuinier, 2007). Whilst 5-HT_{1A}R agonists such as 8-OH-DPAT, S-15535 and Buspirone reduce aggression in rodents (Centenaro et al., 2008; de Boer & Koolhaas, 2005; de Boer et al., 2000; Olivier & Mos, 1992; Sanchez & Hyttel, 1994) and show potential for this application in humans (Santa Cruz et al., 2017), they can lead to numerous dangerous side effects (Bartlett, 2017; Buckley et al., 2014; Ener et al., 2003; Volpi-Abadie et al., 2013). Identifying the pathways downstream of 5-HT_{1A}R activation that mediate the anti-aggressive effects of 5-HT_{1A}R agonists might therefore uncover a more selective therapeutic target with fewer liabilities.

Interestingly, stimulation of postsynaptic 5-HT_{1A}Rs on oxytocin neurons in the paraventricular nucleus of the hypothalamus leads to downstream release of endogenous oxytocin (Bagdy & Kalogeras, 1993; Hunt et al., 2011; Jørgensen et al., 2003; Petrunich-Rutherford et al., 2018). As administration of a 5-HT_{1A}R agonist or oxytocin can reduce aggression in rodents (Centenaro et al., 2008; De Almeida & Lucion, 1994; De Almeida & Lucion, 1997; Miczek et al., 1998; Sanchez & Hyttel, 1994; Tan et al., 2019; White et al., 1991) one possibility is that that the anti-aggressive effects of 5-HT_{1A}R agonism involves this downstream release of oxytocin.

Numerous rodent studies have shown exogenous oxytocin administration to be effective in reducing territorial aggression (Calcagnoli et al., 2013; Calcagnoli et al., 2015a; Calcagnoli et al., 2014; Calcagnoli et al., 2015b). In rodents, prolonged social isolation leads

to heightened aggression (Brain, 1975; Tan et al., 2019). Importantly, social isolation is accompanied by disruptions to the serotonergic system and oxytocin systems.

The majority of studies report a concurrent reduction in 5-HT_{1A}Rs (Bibancos et al., 2007; Preece et al., 2004; Schiller et al., 2003) and socially isolated rodents show decreased OXTR mRNA in the central amygdala, reduced OXTR binding in the nucleus accumbens, and reduced V1aR binding in the lateral hypothalamus and dentate gyrus (Han et al., 2018; Oliveira et al., 2019). Consistent with this, we recently demonstrated that peripherally administered oxytocin powerfully and dose-dependently inhibits maladaptive aggression in socially isolated mice within a neutral context (Tan et al., 2019) and does so via actions at the V1aR, rather than the OXTR. This is consistent with OXT having relatively high affinity for the mouse V1aR (Busnelli et al., 2013) and numerous studies demonstrating some of exogenous OXT's effects are mediated via actions at V1aRs (Bowen & McGregor, 2014; Everett et al., 2018; Ramos et al., 2013; Sala et al., 2011; Song et al., 2014; Tan et al., 2019).

Further evidence for interaction between oxytocin and serotonergic systems comes from an examination of effects of 5-HT_{1A} stimulation on social behaviour. The pro-social effects of both the 5-HT circuit stimulator MDMA and the 5-HT_{1A}R agonist 8-OH-DPAT in rats were blocked by pre-treatment with a non-selective OXTR antagonist (Thompson et al., 2007). MDMA pro-social effects were also blocked by pre-treatment with the V1aR antagonist SR49059 (Ramos et al., 2013). MDMA-induced c-Fos expression in oxytocin containing neurons was blocked by pre-treatment with the 5-HT_{1A}R antagonist WAY-100635 (Hunt et al., 2011). In addition, chronic administration of 8-OH-DPAT during the neonatal period in a genetic mouse model of autism restored normal sociability when the mice had reached adulthood, an effect which was blocked when the OXTR antagonist L-368,899 was

co-administered with 8-OH-DPAT (Nagano et al., 2018). The pro-ejaculatory effect of 8-OH-DPAT could also be blocked with an OXTR antagonist (de Jong & Neumann, 2015).

Considering the aforementioned research, we proposed that the beneficial therapeutic outcomes of 5-HT_{1A}R activation in the context of aggression might also be mediated by downstream stimulation of oxytocin release and subsequent activation of V1aRs or OXTRs. Given our recent demonstration (Tan et al., 2019) that OXT reduced hyper-aggressive behaviour, circumventing 5-HT_{1A}Rs and directly targeting the OXT system might thus provide an opportunity to harness the serenic potential of 5-HT_{1A} receptor agonism while avoiding many of the side effects.

In a series of 4 experiments we thus aimed to examine whether that the anti-aggressive effects of 5-HT_{1A}R activation by 8-OH-DPAT involve downstream release of endogenous oxytocin and subsequent actions at the OXTR and/or V1aR. In keeping with our previous work (Tan et al., 2019) we utilised the social isolation-induced mouse model of aggression with testing in a neutral context. This more closely models the maladaptive, impulsive aggression observed in human clinical populations (Olivier & Young, 2002). In addition to aggression, we also sought to explore the involvement of downstream actions at the OXTR and/or V1aR in the effects of 8-OH-DPAT on close social interaction and grooming.

Methods

Subjects

Male Swiss mice (ARC, Perth, Australia) aged 6-7 weeks with an average weight of 33.2 g upon arrival (N=80). Subjects were housed four per cage in standard IVC cages in a temperature-controlled colony room (22˚C ± 1˚C) under standard laboratory conditions (12 – hour light/dark cycle; light phase 0700 – 1900 hours) upon arrival. All experiments were performed during the light phase, between 1100 and 1600 hours. All experimental procedures were approved by University of Sydney Animal Ethics Committee.

Procedure

Social isolation and two mouse social interaction test

The experimental procedure is described in detail in (Tan et al., 2019). Briefly, n = 64 mice were socially isolated and n = 16 remained group housed. Testing commenced following 6 weeks of isolation involved placing weight and condition matched pairs of unfamiliar mice, following administration of their drug treatment(s) as described below, into the testing arena (400 mm x 400 mm x 400 mm) for a 10 min test session. Experiments were conducted one week apart to allow for washout. New weight-matched pairs were formed for each experiment. The sample sizes per group for all experiments were between n = 14 and n = 16.

Drugs

The 5-HT_{1A}R agonist 8-OH-DPAT (Sigma Aldrich, St. Louis, Missouri) and 5-HT_{1A}R antagonist WAY-100635 (Acme Bioscience, Inc. Palo Alto, California) were dissolved in saline (0.9%). The non-peptidergic OXTR antagonist L-368,899 (Acme Bioscience, Inc. Palo Alto, California) and non-peptidergic AVP V1aR antagonist SR49059 (Acme Bioscience, Inc. Palo Alto, California) were dissolved in a solution of Tween 80 (5% v/v), DMSO (10%

v/v), and saline (85% v/v). All drugs were administered via intraperitoneal (i.p.) injection (injection volume 10 mL/kg).

Experiment 1: 8-OH-DPAT Dose Response

A 5 group between-subjects design was utilised. 15 minutes prior to testing, group housed mice received vehicle treatment whereas socially isolated mice received either vehicle treatment or one of three different doses of 8-OH-DPAT (0.1, 0.3 or 1.0 mg/kg). This range of doses was chosen as it has been shown previously to reduce aggressive behaviour across a range of rodent models of aggression (Centenaro et al., 2008; De Almeida & Lucion, 1994; De Almeida & Lucion, 1997; de Boer & Koolhaas, 2005; Miczek et al., 1998; Sanchez et al., 1993; Sanchez & Hyttel, 1994).

Experiment 2: Effect of 5-HT_{1A}R antagonist on 8-OH-DPAT effects

A 5 group between-subjects design was utilised. Four groups of socially isolated mice received their first injection (VEH or WAY-100635 0.1 mg/kg) 5 min prior to their second injection (VEH or 8-OH-DPAT 0.3 mg/kg), 15 minutes after which the mice were placed into the testing arena. Group housed mice received vehicle treatment for both injections. The dose of WAY-100635 was chosen as it has previously been shown to block the effects of 8-OH-DPAT across a range of rodent models of aggression (Centenaro et al., 2008; de Boer & Koolhaas, 2005; Miczek et al., 1998). The dose of 0.3 mg/kg 8-OH-DPAT was chosen based on the results from Experiment 1.

Experiment 3: Effect of OXTR antagonist on 8-OH-DPAT effects

A 5 group between-subjects design was utilised. Four groups of socially isolated mice received their first injection (VEH or L-368,899 10 mg/kg) 5 min prior to their second injection (VEH or 8-OH-DPAT 0.3 mg/kg), 15 minutes after which the mice were placed into

the testing arena. Group housed mice received vehicle treatment for both injections. The dose of L-368,899 was chosen as it has been shown to be the highest dose previously used in mice (Olszewski et al., 2015) with studies in mice showing clear effects with doses as low as 1 and 3 mg/kg i.p. (Olszewski et al., 2010).

Experiment 4: Effect of V1aR antagonist on OXT effects

A 5 group between-subjects design was utilised. Four groups of socially isolated mice received their first injection (VEH or SR49059 20 mg/kg) 15 min prior to their second injection (VEH or 8-OH-DPAT 0.3 mg/kg), 15 minutes after which the mice were placed into the testing arena. Group housed mice received VEH for both injections. The dose of SR49059 was chosen as we had previously shown it to block the effects of oxytocin on hyper-aggressive behaviour in this model (Tan et al., 2019).

Data Acquisition and Statistical Analysis

Experimental sessions were recorded with an overhead camera using CaptureStar (Version 1.00, CleverSys, Virginia, USA). The behaviours of interest were: (1) time (in seconds) spent fighting; (2) time (in seconds) spent huddling (defined as the mice being in close physical contact with each other while stationary); (3) time (in seconds) spent grooming themselves; and (4) distance travelled (in mm) by each mouse during the experimental session. The behaviours described were automatically quantified from the videos using the behavioural tracking software TopScan, SocialScan and AgressionScan (CleverSys, Virginia, USA). Grooming and huddling behaviours were included as, in addition to aggression, they have also been shown to be sensitive to manipulations of the OXT and AVP system in both this model and other rodent models (Bowen & McGregor, 2014; Ramos et al., 2013; Schorscher-Petcu et al., 2010; Tan et al., 2019). Moreover, previous studies have shown that 5-HT_{1A}R agonism induces close physical contact, which, in those studies, was at least partly

mediated by downstream stimulation of the OXTR and/or V1AR (Ramos et al., 2013; Thompson et al., 2007). Data were analysed using SPSS (Version 24.0, IBM, USA). Data were analysed with a one-way ANOVA and planned contrasts. Significance was set at $p < 0.05$.

Results

Experiment 1. 8-OH-DPAT Dose Response

The aims of this experiment were to assess the effects of a range of doses of 8-OH-DPAT on aggressive behaviour, huddling, grooming and distance travelled. The results are presented in Figure 1.

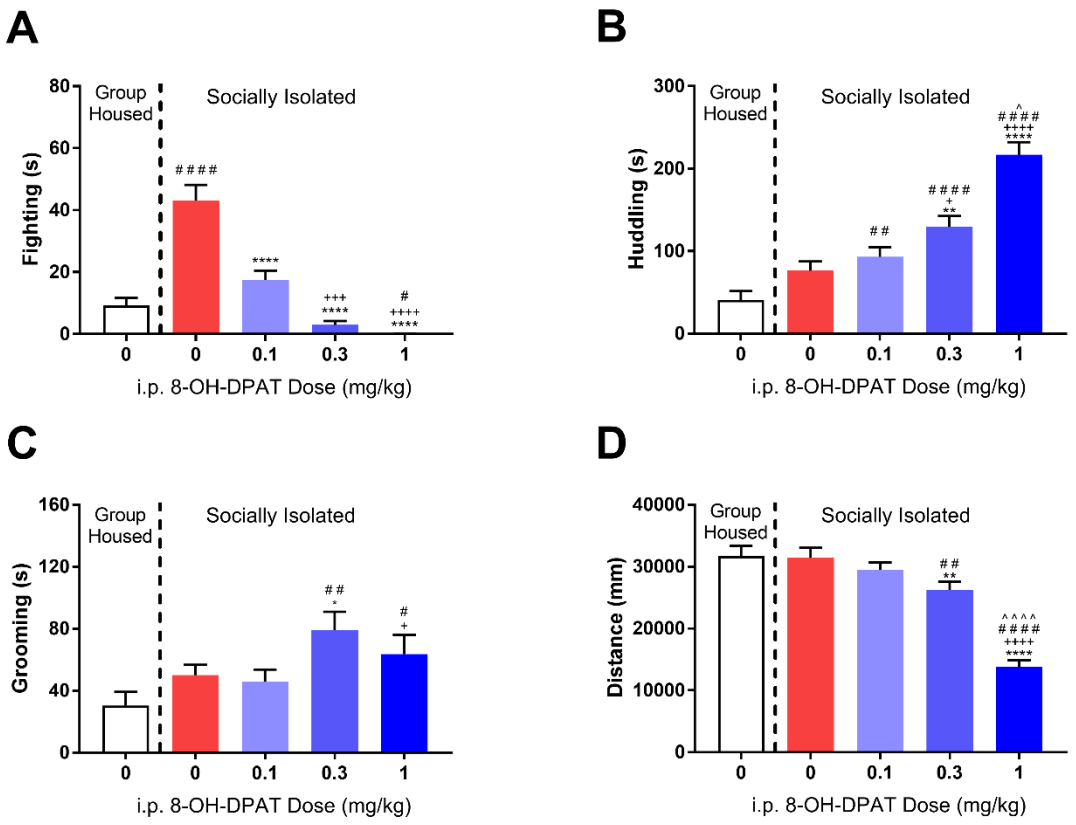

Figure 1. Experiment 1. The effect of 8-OH-DPAT (0.1 mg/kg, 0.3 mg/kg or 1.0 mg/kg) on time spent fighting (A), huddling (B), grooming (C), and locomotor activity (D) during a 10-minute social interaction test with a novel conspecific. 8-OH-DPAT dose dependently inhibited aggressive behaviour, increased huddling, increased grooming, and decreased locomotor activity and. Columns represent means ± SEM. * p<0.05 vs VEHs; ** p<0.01 vs VEHs; *** p<0.001 vs VEHs; **** p<0.0001 vs VEHs; # p<0.05 vs VEHg; ## p<0.01 vs VEHg; ### p<0.001 vs VEHg; #### p<0.0001 vs VEHg; + p<0.05 vs 0.1 mg/kg; ++ p<0.01 vs 0.1 mg/kg; +++ p<0.001 vs 0.1 mg/kg; ++++ p<0.0001 vs 0.1 mg/kg; ^ p<0.05 vs 0.3 mg/kg; ^^^^ p<0.0001 vs 0.3 mg/kg

Fight time, huddling, grooming and distance travelled all differed significantly as a function of group $F(4,73)=34.80$, $p<0.0001$; $F(4,73)=28.08$, $p<0.0001$; $F(4,73)=3.418$, $p<0.01283$; $F(4,73)=30.24$, $p<0.0001$ respectively.

Isolation significantly increased fighting (VEHs vs VEHg, $p<0.0001$). All doses of 8-OH-DAP significantly inhibited isolation-induced fighting (all $p<0.0001$). Mice who received the 1.0 mg/kg dose spent significantly less time fighting than VEHg mice ($p=0.03572$). The 0.3 mg/kg and 1.0 mg/kg doses were significantly more effective at inhibiting fighting than the 0.1 mg/kg dose ($p=0.000772$ and $p=0.000072$, respectively). No other pairwise comparisons were significant (all $p>0.05$)

Isolation had no on effect huddling ($p=0.05620$). All doses of 8-OH-DPAT significantly increased huddling compared to vehicle (all $p<0.01$) The 0.3 mg/kg and 1.0 mg/kg doses were significantly more effective than the 0.1 mg/kg dose at increasing huddling ($p=0.04179$ and $p<0.0001$, respectively) and the 1 mg/kg dose was the most effective (vs 0.3 mg/kg $p<0.0001$). No other pairwise comparisons were significant (all $p>0.05$)

Isolation had no effect on grooming ($p=0.1723$). The 0.3 and 1 mg/kg doses of 8-OH-DPAT significantly increased time spent grooming compared to vehicle (both $p<0.05$). No other pairwise comparisons were significant (all $p>.05$).

Isolation had no effect on distance travelled ($p=0.8803$). The 0.3 mg/kg and 1.0 mg/kg doses of 8-OH-DPAT significantly reduced distance travelled relative to vehicle (all $p<.01$). The effect of the 1.0 mg/kg dose on distance travelled was significantly more pronounced than the 0.3 mg/kg dose ($p<0.0001$). No other pairwise comparisons were significant (all $p>0.05$).

Experiment 2: Effect of 5-HT_{1A}R antagonist on 8-OH-DPAT effects

This experiment aimed to determine whether the effects of 8-OH-DPAT (0.3 mg/kg) on aggression, huddling, grooming and distance travelled could be blocked by pre-treatment with the 5-HT$_{1A}$R antagonist WAY-100635 (0.1 mg/kg). Results are presented in Figure 2.

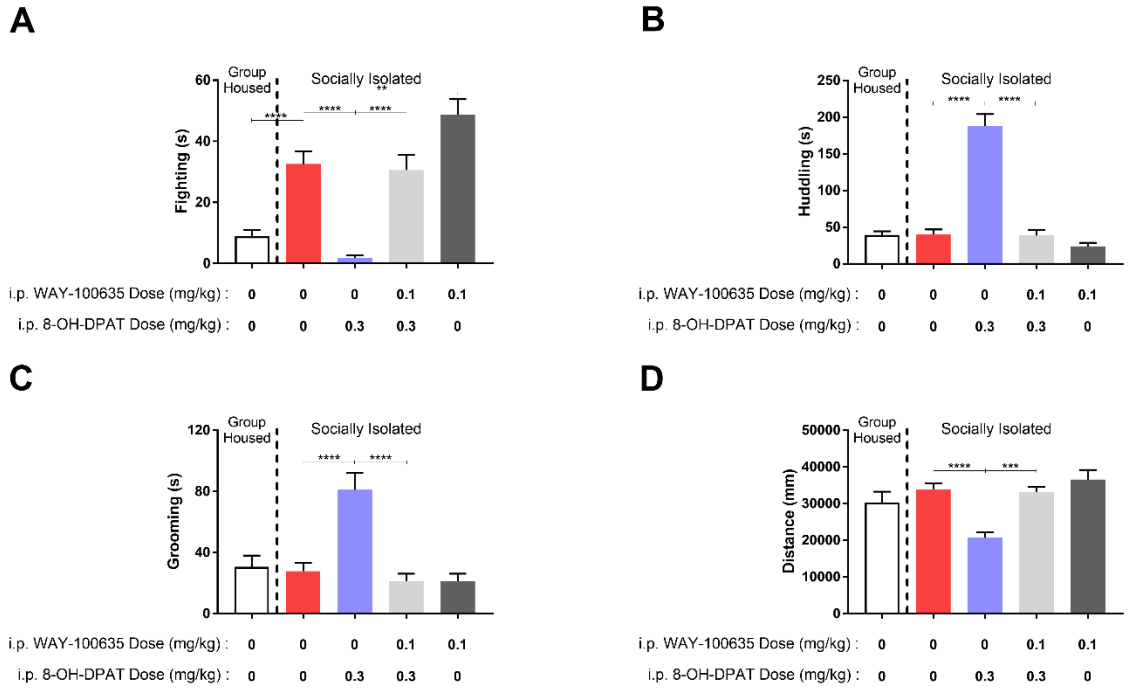

Figure 2. Experiment 2. The effect of pre-treatment with either the 5HT$_{1A}$R antagonist WAY-100635 (0.1 mg/kg) or vehicle on 8-OH-DPAT effects on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. 8-OH-DPAT (0.3 mg/kg) reduced time spent fighting, increased time spent huddling and grooming and reduced distance travelled. Pre-treatment with WAY-100635 (0.1 mg/kg) blocked the effects of 8-OH-DPAT on aggression, huddling grooming and locomotor suppression. Columns represent means ± SEM. ** p<0.01, ***p<0.001 **** p<0.0001.

Fight time, huddling, grooming and distance travelled all differed significantly as a function of group [F(4,69)=23.52, p<0.0001; F(4,69)=58.33, p<0.0001; F(4,69)=12.98, p<0.0001; F(4,69)=8.110, p<0.0001 respectively] hence the planned contrasts were performed for all of these variables.

Isolation increased fighting (VEHs vs VEHg, p=0.0001) and 8-OH-DPAT completely blocked this isolation induced increase in aggression (8-OH-DPAT vs VEHs, p<0.0001; 8-OH-DPAT vs VEHg, p= 0.2253). WAY-100635 prevented the effect of 8-OH-DPAT on fighting (WAY-100635 + 8-OH-DPAT vs 8-OH-DPAT p<0.0001; WAY-100635 + 8-OH-DPAT vs VEHs p=0.7275). The antagonist on its own significantly increased isolation induced aggression (WAY-100635 vs VEHs p=0.003623).

Isolation did not affect huddling (VEHs vs VEHg, p=0.9682). 8-OH-DPAT significantly increased huddling in the isolated mice (8-OH-DPAT vs VEHs p<0.0001) and WAY-100635 blocked the effect of 8-OH-DPAT on huddling (WAY-100635 + 8-OH-DPAT vs 8-OH-DPAT p<0.0001; WAY-100635 + 8-OH-DPAT vs VEHs p=0.9187). The antagonist on its own did not have a significant effect on time spent huddling (WAY-100635 vs VEHs p=0.1887).

There was no effect of isolation on grooming (VEHs vs VEHg, p=0.7720). 8-OH-DPAT increased grooming in the isolated mice (8-OH-DPAT vs VEHs p<0.0001), and WAY-100635 prevented the effect of 8-OH-DPAT on grooming (WAY-100635 + 8-OH-DPAT vs 8-OH-DPAT p<0.0001; WAY-100635 + 8-OH-DPAT vs VEHs p=0.4012). The antagonist on its own did not have a significant effect on grooming (WAY-100635 vs VEHs p=0.4846).

There was no effect of isolation on distance travelled (VEHs vs VEHg p=0.2219). 8-OH-DPAT significantly reduced distance travelled (8-OH-DPAT vs VEHs p<0.0001), and WAY-100635 completely blocked the effect of 8-OH-DPAT on distance travelled (WAY-100635 + 8-OH-DPAT vs 8-OH-DPAT p=0.000150; WAY-100635 + 8-OH-DPAT vs VEHs p=0.7853). The antagonist on its own did not have a significant effect on distance travelled (WAY-100635 vs VEHs p=0.3825).

Experiment 3: Effect of OXTR antagonist on 8-OH-DPAT effects

This experiment aimed to determine whether the effects of 8-OH-DPAT (0.3 mg/kg) on aggression, huddling, grooming and distance travelled could be blocked by pre-treatment with the OXTR antagonist L-368,899 (10.0 mg/kg). Results are presented in Figure 3.

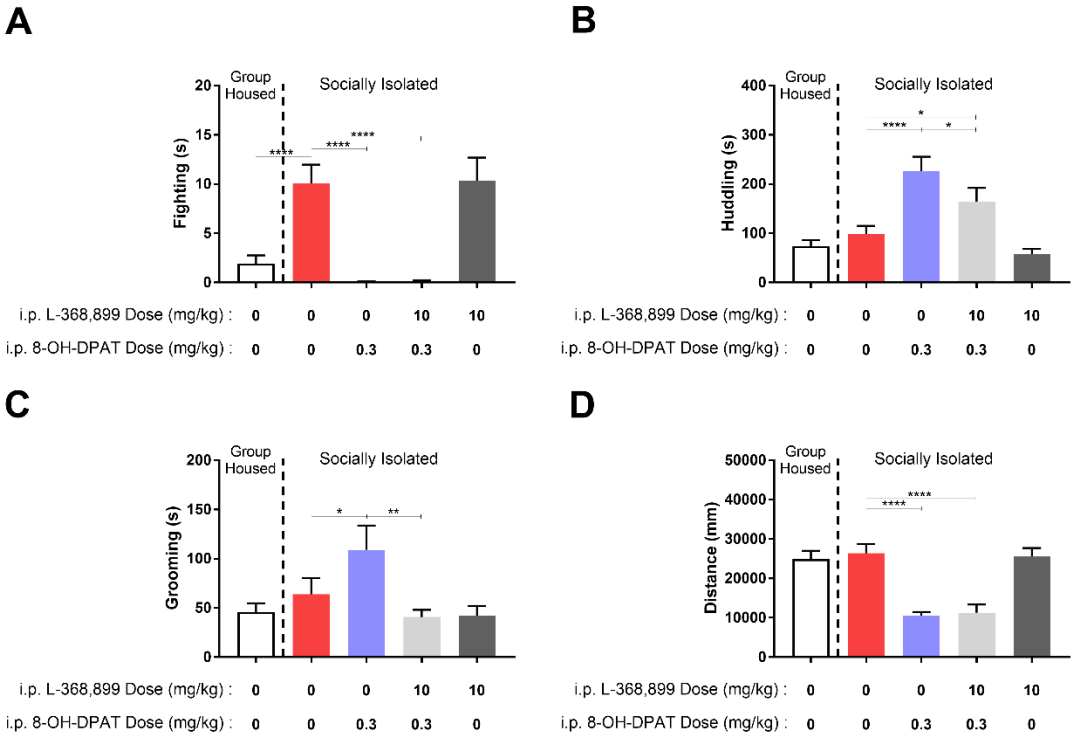

Figure 3. Experiment 3. The effect of pre-treatment with either the OXTR antagonist L-368,899 (10 mg/kg) or vehicle on 8-OH-DPAT effects on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. 8-OH-DPAT (0.3 mg/kg) reduced time spent fighting, increased time spent huddling and grooming and reduced distance travelled. Pre-treatment with L-368,899 (10 mg/kg) did not block the anti-aggressive or locomotor suppressive effects of 8-OH-DPAT, but did appear to partially block the effects of 8-OH-DPAT on huddling and grooming. Columns represent means ± SEM. * $p<0.05$, ** $p<0.01$, **** $p<0.0001$.

Fight time, huddling, grooming and distance travelled all differed significantly as a function of group [$F(4,73)=13.957$, $p<0.0001$; $F(4,73)=10.908$, $p<0.0001$; $F(4,73)=3.690$,

p=0.008607; F(4,73)=16.677, p<0.0001 respectively] as such the planned contrasts were performed for all of these variables.

Isolation increased fighting (VEHs vs VEHg, p=0.000167) and 8-OH-DPAT completely blocked isolation induced aggression (8-OH-DPAT vs VEHs, p<0.0001; 8-OH-DPAT vs VEHg p=0.3693). L-368,899 did not inhibit the effect of 8-OH-DPAT on fighting (L-368,899 + 8-OH-DPAT vs 8-OH-DPAT p=0.9622; L-368,899 + 8-OH-DPAT vs VEHs p<0.0001). The antagonist on its own had no effect on isolation induced aggression (L-368,899 vs VEHs p=0.8769).

Isolation did not significantly affect huddling (VEHs vs VEHg, p=0.4219). 8-OH-DPAT significantly increased huddling in the isolated mice (8-OH-DPAT vs VEHs p <0.0001), and L-368,899 partially blocked the effect of 8-OH-DPAT on huddling (L-368,899 + 8-OH-DPAT vs 8-OH-DPAT p=0.03951; L-368,899 + 8-OH-DPAT vs VEHs p=0.03085). The antagonist on its own did not have a significant effect on time spent huddling (L-368,899 vs VEHs p=0.1766).

There was no effect of isolation on grooming (VEHs vs VEHg, p=0.4113). 8-OH-DPAT increased grooming in the isolated mice (8-OH-DPAT vs VEHs p=0.03558), and L-368,899 blocked the effect of 8-OH-DPAT on grooming (L-368,899 + 8-OH-DPAT vs 8-OH-DPAT p=0.001820; L-368,899 + 8-OH-DPAT vs VEHs p=0.2770). The antagonist on its own did not have a significant effect on grooming (L-368,899 vs VEHs p=0.3055).

There was no effect of isolation on distance travelled (VEHs vs VEHg p=0.6095). 8-OH-DPAT significantly reduced distance travelled (8-OH-DPAT vs VEHs p<0.0001), and L-368,899 did not block the effect of 8-OH-DPAT on distance travelled (L-368,899 + 8-OH-DPAT vs 8-OH-DPAT p = 0.7602; L-368,899 + 8-OH-DPAT vs VEHs p<0.0001). The

antagonist on its own did not have a significant effect on distance travelled (L-368,899 vs VEHs p=0.7671).

Experiment 4: Effect of V1aR antagonist on 8-OH-DPAT effects

This experiment aimed to determine whether the effects of 8-OH-DPAT (0.3 mg/kg) on aggression, huddling, grooming and distance travelled could be blocked by pre-treatment with the V1aR antagonist SR49059 (20.0 mg/kg). Results are presented in Figure 4.

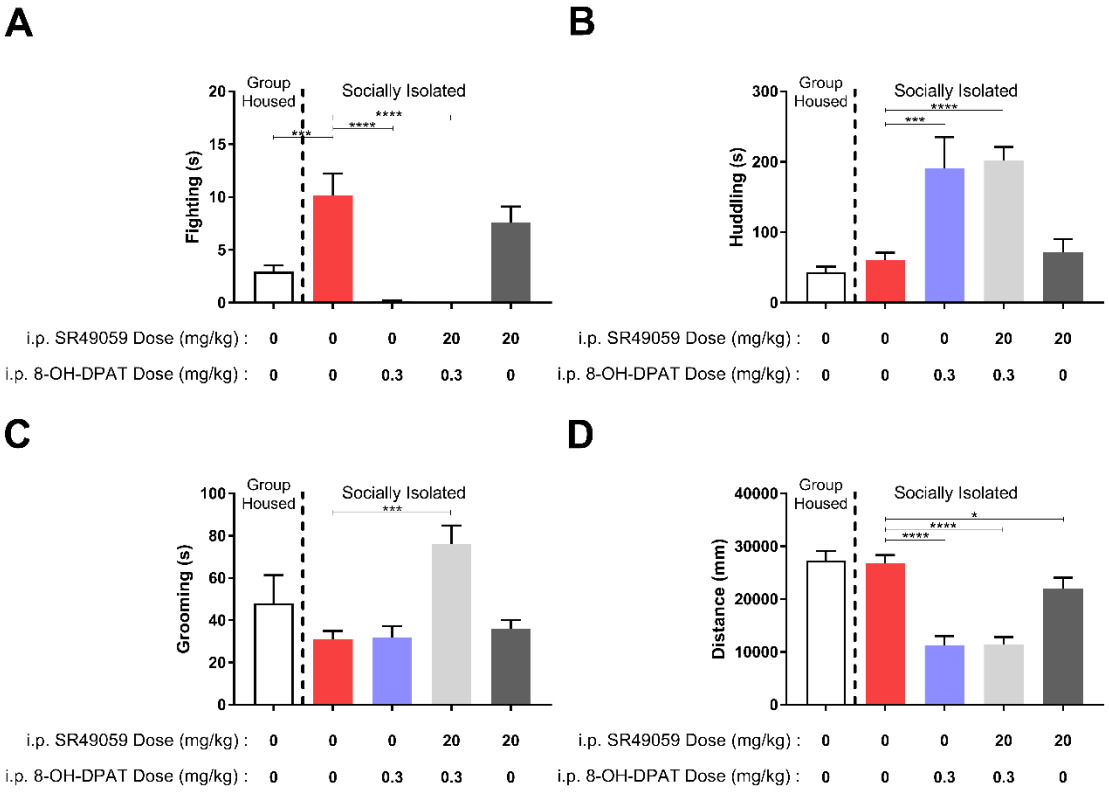

Figure 4. Experiment 4. The effect of pre-treatment with either the V1aR antagonist SR49059 (20 mg/kg) or vehicle on 8-OH-DPAT effects on time spent fighting (A), huddling (B), grooming (C), and distance travelled (D) during a 10-minute social interaction test with a novel conspecific in a neutral context. 8-OH-DPAT (0.3 mg/kg) decreased time spent fighting, increased huddling, and reduced distance travelled. Pre-treatment with SR49059 (20 mg/kg) did not block the effects of 8-OH-DPAT on fighting, huddling or distance travelled, but significantly enhanced the effects on grooming. Columns represent means ± SEM. * $p<0.05$, *** $p<0.001$, ****$p<0.0001$.

Fight time, huddling, grooming and distance travelled all differed significantly as a function of group [$F(4,69)=13.822$, $p<0.0001$; $F(4,69)=12.791$, $p<0.0001$; $F(4,69)=6.114$, $p=0.0003$; $F(4,69)= 22.653$, $p<0.0001$ respectively].

Isolation significantly increased fighting (VEHs vs VEHg, $p=0.0001$). 8-OH-DPAT completely blocked isolation induced aggression (8-OH-DPAT vs VEHs $p<0.0001$; 8-OH-DPAT vs VEHg $p=0.141$), and SR49059 did not block the effect of 8-OH-DPAT on fighting (SR + 8-OH-DPAT vs 8-OH-DPAT $p = 0.9427$; SR + 8-OH-DPAT vs VEHs $p<0.0001$). The antagonist on its own did not have a significant effect on fighting (SR vs VEHs $p=0.146$).

Isolation did not significantly increase huddling (VEHs vs VEHg, $p=0.566$). 8-OH-DPAT significantly increased huddling in the isolated mice (8-OH-DPAT vs VEHs $p=0.0001$), and SR49059 did not block the effect of 8-OH-DPAT on huddling (SR + 8-OH-DPAT vs 8-OH-DPAT $p=0.701$; SR + 8-OH-DPAT vs VEHs $p<0.0001$). The antagonist on its own did not have a significant effect on huddling (SR vs VEHs $p=0.720$).

There was no effect of isolation on grooming (VEHs vs VEHg, $p=0.141$). 8-OH-DPAT did not have an effect on grooming in the isolated mice (8-OH-DPAT vs VEHs $p=0.952$). However, the group pre-treated with the antagonist had a significantly higher time spent grooming relative to VEHs (SR + 8-OH-DPAT vs VEHs $p<0.0001$). The antagonist on its own did not have a significant effect on grooming (SR vs VEHs $p=0.665$).

There was no effect of isolation on distance travelled (VEHs vs VEHg, $p=0.8435$). 8-OH-DPAT significantly reduced distance travelled (8-OH-DPAT vs VEHs $p<0.0001$), and SR did not block the effect of 8-OH-DPAT on distance travelled (SR + 8-OH-DPAT vs 8-OH-DPAT $p=0.9208$; SR + 8-OH-DPAT vs VEHs $p<0.0001$). The antagonist on its own significantly reduced distance travelled (SR vs VEHs $p=0.0458$).

Discussion

The current study examined the effects of $5HT_{1A}R$ stimulation on isolation-induced aggression, positive social interaction, and grooming in male Swiss mice and whether these effects could be explained by downstream release of OXT binding to OXTRs or V1ARs. Consistent with numerous previous studies (Centenaro et al., 2008; De Almeida & Lucion, 1994; De Almeida & Lucion, 1997; de Boer & Koolhaas, 2005; Miczek et al., 1998; Sanchez & Hyttel, 1994; White et al., 1991) we showed that hyper-aggressive behaviour in socially isolated mice was inhibited by the peripheral administration of a 5-$HT_{1A}R$ agonist 8-OH-DPAT, however this appears to be the first study to demonstrate this effect in a non-territorial model of aggression when tested in a neutral context. This inhibition of aggression by 8-OH-DPAT was accompanied by increases in huddling and grooming. Interestingly, it has been previously shown that OXT can produce dose-dependent increases in huddling and grooming and a decrease in distance travelled in rodents (Bowen & McGregor, 2014; Drago et al., 1986; Meisenberg, 1988; Ramos et al., 2013; Tan et al., 2019). As expected, pre-treatment with selective 5-$HT_{1A}R$ antagonist WAY-100635 fully blocked 8-OH-DPAT effects on aggression, huddling, grooming and distance travelled.

However, contrary to our expectations, pre-treatment with neither an OXTR antagonist nor V1aR antagonist blocked the anti-aggressive effects of 8-OH-DPAT. However, the OXTR antagonist was able to partly block 8-OH-DPAT-induced increases in huddling, consistent with previous studies demonstrating 5-$HT_{1A}R$ agonism exerts prosocial effects via downstream activation of OXT pathways (Ramos et al., 2013; Thompson et al., 2007). 8-OH-DPAT increased grooming was completely blocked by WAY-100635, consistent with prior studies showing agonism of 5-$HT_{1A}Rs$ can increase, and antagonism can decrease grooming (Andrews et al., 1994; Centenaro et al., 2008; Marco et al., 2004). Moreover, the OXTR antagonist also completely blocked heighted grooming induced by 8-

OH-DPAT suggesting the effects of 5-HT_{1A}R agonism on grooming are driven by subsequent release of OXT and activation of OXTRs. This finding is consistent with previous work demonstrating OXT increases grooming via actions at the OXTR (Drago et al., 1986; Drago et al., 1991).

It thus appears that the endogenous levels of OXT released by stimulation of the 5-HT_{1A}R are sufficient to cause an increase in huddling and grooming, but not OXT-mediated inhibition of aggression. Our previous study showed that exogenously administered OXT inhibited aggression in this model via actions at the V1AR, rather than the OXTR (Tan et al., 2019). Given the very high doses of OXT required to inhibit aggression in our previous study (Tan et al., 2019) and the lower affinity of OXT for the V1aR compared to the OXTR, it is likely that brain concentrations of OXT achieved via endogenous release following $5HT_{1A}$R stimulation are insufficient to stimulate V1ARs (Bowen, 2019; Smith et al., 2019).

Our results are consistent with the findings of Thompson et al. (2007) who showed that the pro-social effects of both MDMA and 8-OH-DPAT could be blocked by pre-treatment with OXTR antagonist tocinoic acid. However, Ramos et al. (2013) showed that the pro-social effects of MDMA could also be blocked with the pre-treatment of V1aR antagonist SR49059, which we found to be ineffective in our model. One possible explanation is that MDMA may cause a much larger release of endogenous OXT than 8-OH-DPAT and concentrations sufficient to stimulate V1aRs as well as OXTRs are achieved.

Across Experiments 1-3, 8-OH-DPAT consistently increased grooming, however this effect was not observed in Experiment 4. One possibility is that there was an effect of novelty induced grooming (Jolles et al., 1979) in Experiments 1-3 which was no longer present in Experiment 4. It is also of note that in Experiment 4 mice treated with 8-OH-DPAT alone did not show heightened grooming whereas in all of the other experiments there was a clear

effect. We thus think the more plausible explanation is that the effects on grooming observed in the group administered both SR49059 and 8-OH-DPAT are being driven by 8-OH-DPAT and the lack of effect of 8-OH-DPAT on its own was an anomaly.

Conclusion

In conclusion, our findings provide evidence for a divergence of pathways whereby the 5-HT_{1A}R mediated effects on enhanced pro-social behaviour appear to be partly explained by the downstream effects of OXT at OXTRs and the 5-HT_{1A}R mediated effects on grooming appear to be completely explained by downstream OXT actions at the OXTR. Whilst our previous research showed that OXT itself is anti-aggressive (Tan et al., 2019) the results of the current study suggest that anti-aggressive effects of 5-HT_{1A}R agonism cannot be explained by downstream effects on OXT. This indicates that exogenous OXT and 5-HT_{1A}R agonists impact aggression through different pathways. To the best of our knowledge this is the first study to demonstrate this divergence of downstream receptor targets mediating these behavioural effects of 5-HT_{1A}R agonism.

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Chapter 4: General Discussion

4.1 Chapter Overview

The primary aims of this thesis were to: (1) examine the anti-aggressive effects of OXT and AVP in a non-territorial rodent model of hyper aggressive behaviour; (2) delineate the underlying receptor target(s) driving the anti-aggressive effects of exogenously administered OXT and AVP; and (3) explore whether the anti-aggressive and pro-social effects of $5HT_{1A}R$ stimulation can be explained by downstream release of OXT and actions at OXT and/or V1a receptors. These aims were addressed by testing pairs of unfamiliar mice that had either both been group housed or socially isolated (individually housed) for at least six weeks prior to testing. Testing involved a social interaction test in a neutral context with a series of systematic pharmacological manipulations performed during tests to address the aims.

The introductory chapter of this thesis proposed three primary hypotheses regarding the potential of the OXT and AVP systems as targets for treating aggression and the involvement of downstream OXT release in the anti-aggressive effects of 5-$HT_{1A}R$ stimulation. These hypotheses were:

1. Targeting the OXT system will reduce aggressive behaviour in a non-territorial rodent model of pathological impulsive aggression

2. Anti-aggressive effects of OXT will involve actions at the V1aR and/or OXTR.

3. Anti-aggressive and pro-social effects of $5HT_{1A}R$ stimulation will involve downstream activation of the V1aR and/or OXTR.

Overall, the findings presented in this thesis supported hypotheses 1 and 2 and provided partial support for hypothesis 3. This final chapter first summarises and discusses the key findings of the experimental chapters and then relates the findings back to the aforementioned hypotheses. The broader implications of the findings presented in this thesis, including limitations and directions for future research, are then discussed.

4.2 Summary of Findings

4.2.1 Chapter 2. The anti-aggressive effects of OXT appear to be V1aR mediated

Chapter 2 (Tan et al., 2019) demonstrated the powerful, dose-dependent anti-aggressive properties of peripherally administered OXT and AVP in a mouse model of pathological non-territorial aggression. Whilst the anti-aggressive properties of OXT had been demonstrated in previous studies (Calcagnoli et al., 2013; Calcagnoli et al., 2014a; Calcagnoli et al., 2015a; Calcagnoli et al., 2014b; Calcagnoli et al., 2015b), they used the resident intruder paradigm and thus assessed territorial aggression (Olivier & Young, 2002). Therefore, Tan et al. (2019) was the first to study the effects of OXT and AVP in a model of non-territorial, pathological aggression (Olivier & Young, 2002). A potential limitation is whether an increase in aggression can truly be considered pathological as it was not tested if the isolated mice attacked harmless juveniles or females which would otherwise constitute an inappropriate response to a neutral stimulus.

Nonetheless, in this model, OXT dose-dependently inhibited hyper-aggressive behaviour in socially isolated mice. This was accompanied by dose-dependent increases in close social contact (huddling) and grooming behaviour and a dose

dependent decrease in locomotor activity. AVP produced the same dose dependent inhibition of hyper-aggressive behaviour, dose-dependent increases in huddling and grooming, and dose dependent decreases in locomotor activity, but the dose-response curve was shifted considerably to the left relative to OXT

Several key findings uncovered a critical role for the V1aR in the observed effects. Pre-treatment with an OXTR antagonist did not block the anti-aggressive and pro-social effects of OXT. In contrast, these effects were blocked by pre-treatment with a V1aR antagonist. Moreover, AVP exerted the same pro-social and anti-aggressive effects as OXT, but at approximately 30 fold lower doses than OXT, consistent with AVP's approximately 20 fold greater binding affinity at the V1aR than OXT (Busnelli et al., 2013). OXTR activation is clearly involved in several forms of adaptive aggression such as territorial aggression (Calcagnoli et al., 2013; Calcagnoli et al., 2014b; Calcagnoli et al., 2015b) and maternal aggression (Bosch, 2013; Bosch & Neumann, 2012). However, our findings suggest it may have less relevance to maladaptive impulsive aggression and suggest targeting V1aRs may hold more potential for reducing pathological impulsive aggression (Tan et al., 2019).

Whilst mice treated with OXT and AVP travelled less distance in the arenas during the test sessions, we were able to rule out drug-induced sedation as the cause. After accounting for the increased time engaging in the stationary behaviours huddling and grooming there was no difference in distance between treated and untreated mice. Consistent with this, in a control experiment we tested mice individually and observed no effects of OXT or AVP on locomotion. These findings are significant as one of the major issues with the use of antipsychotics for the management of agitation and aggression is that they are only effective at reducing

aggression at sedative doses (Fung et al., 2016; Liu et al., 2016). Moreover, these sedative effects pose a significant risk of injury and death in elderly populations through increasing the likelihood of slips, falls and cardiovascular liabilities (Arai et al., 2016; Corbett et al., 2014; Koponen et al., 2017; Nielsen et al., 2016; Zhai et al., 2016). In contrast to antipsychotics, our findings suggest that exogenously administered OXT and AVP, via actions at the V1aR, change the nature of social interactions from aggressive to pro-social and do so without having sedative effects.

4.2.2 Chapter 3. Divergent pathways mediate effects of a 5-HT$_{1A}$R agonist on pro-social and aggressive behaviours

Chapter 3 (Tan et al., 2019) aimed to determine whether the anti-aggressive and pro-social effects of 5-HT$_{1A}$R stimulation in a mouse model of pathological non-territorial aggression could be explained by stimulation of endogenous OXT release. Consistent with prior research, administration of the 5-HT$_{1A}$R agonist 8-OH DPAT dose-dependently inhibited aggression and increased huddling and grooming with an accompanying dose dependent reduction in locomotor activity. This is consistent with prior studies demonstrating that agonism of 5-HT$_{1A}$Rs can increase grooming whilst 5-HT$_{1A}$R antagonism can decrease grooming (Andrews et al., 1994; Centenaro et al., 2008; Marco et al., 2004). All of the observed effects of 8-OH-DPAT were mediated via actions at post-synaptic 5-HT$_{1A}$Rs as they were completely blocked by pre-treatment with WAY-100635, a selective 5-HT$_{1A}$R antagonist (Chemel et al., 2006; Martel et al., 2007).

In partial opposition to what was initially hypothesised, pre-treatment with a selective OXTR antagonist was unable to block the anti-aggressive effects of 8-OH DPAT. However, the OXTR antagonist did inhibit the pro-social effects of 8-OH

DPAT. 8-OH DPAT-induced grooming was also inhibited by pre-treatment with the OXTR antagonist. These findings suggest that the anti-aggressive effects of 5-HT_{1A}R stimulation do not involve downstream stimulation of endogenous OXT, whereas this pathway is involved in the pro-social effects of 5-HT1aR agonism and effects on grooming.

Given the findings of our previous study (Tan et al., 2019), and the lack of effect of the OXTR antagonist, it remained a possibility that 5-HT1aR stimulated OXT release may be reducing aggression via actions at the V1aR. However, pre-treatment with a V1aR antagonist had no impact on 8-OH DPAT-reduced aggression. The V1aR antagonist also did not inhibit 8-OH-DPAT effects on huddling and grooming. This suggest that the endogenous OXT release stimulated by 8-OH-DPAT is unable to reach concentrations sufficient to act upon V1aRs, and that pharmacologically relevant doses of OXT, such as those used in our previous study (e.g. 3 mg/kg), are required to elicit V1aR-mediated effects on aggression and pro-social behaviours.

Overall, this study shows that the downstream release of OXT is unable to explain the effects of 5-HT_{1A}R stimulation on aggression but may partially explain the pro-social effects and effects on grooming. This suggests that there may be divergent pathways for aggression mediated via the 5-HT_{1A}R whereas social behaviour and grooming may be mediated via actions at the OXTR.

4.3 Implications and Future Directions

Implications for future drug development

Our results (Tan et al., 2019) add to a growing body of literature which suggests that pursuing activation of the V1aR may hold considerably more promise for certain indications than activation of the OXTR. Not only did we demonstrate that OXT acts via the V1aR to inhibit impulsive aggression and to enhance pro-social behaviour, but AVP was considerably more potent than OXT in eliciting these therapeutically-relevant effects. The considerably higher levels of OXT required to elicit these behavioural effects are consistent with OXT's lower affinity for the V1AR (Busnelli et al., 2013; Manning et al., 2012) and this may help to explain why results from clinical studies examining intranasal OXT have been largely underwhelming (Bakermans-Kranenburg & van, 2013).

Specifically, it may be that the levels of OXT required to stimulate V1aRs cannot be reached via intranasal administration at the doses used in humans (Bowen, 2019). Indeed, the results of a recent clinical trial (Parker et al., 2019) found intranasal AVP to be beneficial for the treatment of social deficits in ASD in children on the primary outcome measure of social communication. Despite being a small trial, these results were arguably more striking than any that have been reported with intranasal OXT in clinical studies of ASD populations and are consistent with the hypothesis that attempts to more effectively target the V1aR warrant greater attention.

Neural Circuitry

With regard to maladaptive impulsive aggression, the findings of Tan et al. (2019) and Tan and Bowen (2019) suggest that OXT is not merely serving as a more precise way of targeting the same pathway stimulated by 5-HT_{1A}R agonists. Rather, oxytocin, at pharmacological doses, appears to be reducing this aggression via an entirely different circuit that involves stimulation of V1aRs. Of considerable interest to the model utilised in this thesis, following 7 weeks of post-weaning social isolation V1aR binding was found to be downregulated in the lateral hypothalamus and increased V1aR in the anterior portion of the BNST in male rats (Oliveira et al., 2019). In light of the findings in the current study (Tan et al., 2019), this may suggest that peripheral administration of AVP may be preferentially acting on V1aRs expressed on AVP neurons in the BNST which synapse onto GABAergic neurons or directly onto V1aRs expressed on GABAergic neurons of isolated mice which would then in turn lead to a reduction in aggression through GABAergic signalling to glutamatergic neurons within the HAA (refer back to Figure 1 in the General Introduction and Literature Review).

Whilst it is possible that AVP may be directly activating V1aRs expressed on GABAergic neurons in the LS that send inhibitory inputs to the HAA to decrease aggression, Allaman-Exertier et al. (2007) instead suggest that in the LS V1aRs in are expressed primarily on GABAergic interneurons that synapse onto and subsequently reduce the activity of these GABAergic projection neurons. Increased AVP activity in this region would thus be predicted to promote aggression through disinhibition of the HAA through reduced LS-HAA GABA signalling. This notion is consistent with prior research showing that AVP release within the LS was positively

correlated with aggression whereas AVP release within the BNST was negatively correlated with aggression (Veenema et al., 2010). To investigate this pathway further, one possibility may be to examine whether administration of OXT or AVP directly into the BNST can inhibit aggression in our model, and whether this can subsequently be blocked with the pre-treatment of a V1aR antagonist. Another approach would be to infuse an siRNA or shRNA that downregulates expression of the V1aR into the BNST and assess the impact on aggressive behaviour. These approaches would provide more direct evidence of the V1aR BNST to HAA pathway being involved in the OXT and AVP suppression of aggression.

Supporting potential translatability of our findings, there is some evidence showing that this pathway may be conserved across species. V1aRs were also found to be expressed in the hypothalamus, amygdala, BNST, LS and brainstem of Rhesus monkeys (Young et al., 1999). Importantly, the BNST may be an important region of interest across a range of psychiatric disorders that has been overlooked (Lebow & Chen, 2016). Future research may seek to examine associations between activity in these pathways and aggression in humans. Since activation of V1bRs promote aggressive behaviour (Blanchard et al., 2005; Wersinger et al., 2002), and given the high affinity of AVP at both the V1aR and V1bR, it is likely that the discrepancies in early rodent studies showing AVP to increase aggression can be explained by actions of AVP at differing regions and different receptors. For example, if AVP is administered to a region with a high population of V1bRs this may promote aggression whereas if AVP is administered to a region consisting primarily of V1aRs we would expect this to reduce aggression.

AVP may have aggression enhancing or inhibiting effects via actions at the V1aR which depends on the particular circuit being targeted. Our findings and those

of previous studies, taken together, suggest that whether or not peripherally administered AVP will primarily be pro- or anti-aggressive depends on the context (i.e. type of aggression) and state of the animal (whether a state of pathological aggression been induced). Importantly, our findings suggest that when assessing impulsive aggression in animals with pathological-like hyper aggression, AVP inhibits aggression. The ability of AVP to be anti-aggressive in this context may be driven by social isolation resulting in the upregulation of V1aRs in circuits in which activation suppresses aggressive behaviour (BNST) and downregulation of V1aRs in circuits in which activation enhances aggression (LS) (Oliveira et al., 2019). In this light, perhaps the V1aR plays somewhat of a homeostatic role with regards to aggression – with expression changing to enhance aggression in adaptive contexts and in an attempt to inhibit aggression when it has become maladaptive. This possibility should be explored in greater detail in future research.

4.3.1 Sex differences

One limitation of this current thesis is that all the studies were conducted using male mice. Indeed, female mice do not readily exhibit abnormal heightened aggression. Rodent studies on aggression have largely focused on males, with studies on female aggression primarily focusing on maternal aggression which is an adaptive form of aggression (Olivier & Young, 2002) with very few studies examining inter-female aggression (Been et al., 2018).

However, a new rodent model of female aggression has been recently developed which may provide future avenues to investigate the underlying neurophysiology of inter-female aggression (Newman et al., 2019). In this model, female Swiss mice are co-housed with a castrated male mouse after which they begin to display spontaneous aggression toward unfamiliar female intruders.

It is important to consider possible sex differences as there has been some evidence to suggest that OXT and AVP may have different effects on aggressive behaviour in females compared to males. There is some evidence demonstrating that OXT can inhibit aggression in females outside of maternal aggression models. Within the resident intruder test, Injection of OXT into the AH of female hamsters reduces aggression, whereas administration of an OXTR antagonist increased aggression (Harmon et al., 2002). .Earlier research demonstrated that AVP injected into the AH of male hamsters was pro-aggressive within the resident intruder test (Ferris et al., 1997). Conversely, in female hamsters which display greater levels of aggression in the resident intruder test than males, injection of a AVP into the AH decreased aggression whereas administration of a V1aR antagonist into the AH of increased aggression (Gutzler et al., 2010). Moreover, in a primate study by (Jiang & Platt, 2018), both intranasal administration of AVP and OXT in female macaque monkeys increased aggression towards males but was coupled with increased affiliative behaviours toward other females such as vocalisations which may serve to recruit support of other females to protect against males. The role of AVP in regulating aggression in female rats and mice has not been well characterised (Been et al., 2018).

There has also been preliminary evidence to suggest that the effects of serotonergic drugs may also be moderated by sex. Terranova et al. (2016) demonstrated that in Syrian hamsters within the resident intruder model with sex-matched intruders, injection of 8-OH-DPAT into the AH reduced aggression in males but increased aggression in females. Similarly, systemic fluoxetine administration promoted aggression in females and but inhibited aggression in males.

Thus, given that OXT and AVP appear to affect aggression differently depending on sex, future drug development efforts should ensure adequate testing across both sexes in pre-clinical models before testing in human samples and very recently developed paradigms (Newman et al., 2019) have improved the feasibility of achieving this goal in future research.

4.3.2 Limitations of Oxytocin and Vasopressin as Therapeutics

Despite the strong body of preclinical evidence supporting potential therapeutic effects of oxytocin across a range of different disorders (Bradley & Woolley, 2017; Cochran et al., 2013; de Jong & Neumann, 2018; Lukas et al., 2011; Matsuzaki et al., 2012; Neumann & Slattery, 2016; Peters et al., 2014; Slattery & Neumann, 2010a; Slattery & Neumann, 2010b), the full potential of oxytocin appears to be held back by its poor pharmacokinetic properties posing a strong challenge to its therapeutic application (Bowen, 2019; Bowen & Neumann, 2017; Leng & Ludwig, 2016).

Unfortunately, recent clinical trials examining the efficacy of intranasal oxytocin have, overall, been underwhelming. A recent meta-analysis of clinical trials examining the efficacy of intranasal oxytocin for treating a wide range of psychiatric disorders revealed a small overall non-significant effect size of d=0.32 (Bakermans-Kranenburg & van, 2013). This poor outcome may be due to the pharmacokinetic properties of OXT as a molecule. As oxytocin is a cyclic peptide, it cannot be delivered orally as it does not survive primary metabolism, being broken down by peptidases in the gut. In addition, oxytocin has a very short biological half-life such that it is unable to pass through the blood-brain barrier effectively.

In light of these pharmacokinetic limitations, the intranasal route of administration emerged as a hypothesised non-invasive means of delivering oxytocin directly to the brain through absorption through the olfactory or trigeminal neurons, However, it has been shown that only a very small proportion of intranasally administered oxytocin reaches the brain directly due to its difficulty in diffusing through the blood-brain barrier (see Leng & Ludwig, 2016 for a review). As aforementioned, Smith et al. (2019) demonstrated that OXT could be detected in extracellular fluid from the amygdala and hippocampus of the oxytocin null mice following intranasal OXT administration. Given the EC50 for oxytocin at the mouse oxytocin receptor to be close to 4.5 nM (Busnelli et al., 2013), Bowen (2019) estimated that the brain OXT concentrations arising from the high peripheral doses used in rodent studies are likely to lead to the pharmacologically relevant brain concentrations required to produce behavioural effects. Conversely, studies of intranasal administration of OXT in humans show much lower peak CSF concentrations (Striepens et al., 2013) than in rodents. Given that the EC50 for OXT at the human OXTR is ~ 10 nM (Passoni et al., 2016). Intranasal OXT in humans is thus likely to only provide marginal activation of the OXTR and unlikely to provide therapeutically relevant activation of the V1aR (Bowen, 2019).

One hypothesis was that a small amount of OXT when delivered intranasally would be able to first pass through the blood brain barrier then subsequently act as an OXT circuit stimulator to stimulate the further release of endogenous OXT. This was investigated by (Lee et al., 2018) who demonstrated using deuterated OXT, in which the deuterated OXT was detected in CSF, however this was not accompanied with a corresponding increase in non-deuterated OXT concentrations in the CSF. Thus while the oxytocin system holds promise for the treatment of psychological

disorders, the pharmacokinetic properties of the oxytocin molecule prevent the full realisation of the therapeutic potential of the oxytocin system (Martinetz & Neumann, 2016).

Whilst it is likely that AVP may be prone to the same pharmacokinetic limitations as OXT as it is also a neuropeptide, given that there is greater affinity for AVP at the V1aR than OXT (Busnelli et al., 2013; Manning et al., 2012), this may mean that despite a similarly low concentration of AVP reaching the brain via peripheral administration, this may be enough to sufficiently activate V1aRs for those applications where OXT effects at V1aRs are more relevant. Hence, the findings of the current thesis (Tan et al., 2019) which demonstrate that a much lower dose of AVP was required to produce both pro-social and anti-aggressive effects are consistent with the findings of Parker et al. (2019) suggesting that activation of the V1aR may produce more potent therapeutic effects in children with ASD. An important consideration is that AVP also has a high affinity for the V1bR, which has consistently been shown to promote aggressive behaviour (Blanchard et al., 2005; Koshimizu et al., 2012; Wersinger et al., 2002), such that future drug discovery efforts should strive for agonists that are selective for the V1aR over the V1bR as well as the V2R to avoid any potential unwanted effects on the periphery.

Hence, there is great potential for the development of a selective V1aR agonist to produce more robust therapeutic effects by overcoming the pharmacokinetic challenges presented by the neuropeptides OXT and AVP. This thesis, thus hopes to serve as a proof of concept to demonstrate the potential for the further development novel therapeutics targeting the OXT and AVP system to be exploited for the treatment of pathological aggression.

4.4 Conclusions

Overall, the findings presented within this thesis show that targeting the OXT and AVP systems shift the social phenotype from aggressive to pro-social. The studies presented add to a growing body of evidence showing a number of pronounced effects of exogenously administered OXT are mediated by actions at the V1aR. This has important implications for therapeutic development, suggesting that ligands selectively targeting the OXTR might lack some important therapeutic effects mediated by the V1aR. However, regarding aggression, whilst we saw striking V1aR mediated inhibition of isolation-induced hyper-aggressive behaviour, this must be considered in the context of previous studies, which highlight the complex array of factors mediating the impact of the brain OXT and AVP systems on aggression. Unravelling these factors will play an important role in more fully understanding the potential of targeting brain OXT systems to treat pathological aggression.

Whilst these pre-clinical results demonstrate a high level of efficacy, given the overall poor pharmacokinetic properties, and lack of selectivity, of OXT and AVP it is unlikely that OXT or AVP will be optimal treatment options where activation of central OXTRs or V1aRs is required. Thus, to achieve the greatest clinical impact in fully realising the therapeutic potential of the OXTR and V1aR systems, the development of small molecule novel therapeutics with superior pharmacokinetic properties should be a core goal of future research efforts.

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