Exposure to familiar versus novel conspecifics is associated with differential activity of oxytocin circuits

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Ron Christian Blaza

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Abstract

Oxytocin (OT) signalling has been shown to be significantly involved in the regulation of social behaviour, particularly in regards to pro-sociality. Familiarity with another conspecific is a major modifier of social behaviour. Here we investigated whether exposure to a familiar versus novel conspecific was associated with differential activity in OT neuronal circuits. We placed male mice in a chamber with a restrainer containing either a familiar or novel conspecific, with an empty restrainer as a control. Immunhistochemical analysis following this exposure showed greater activation of OT neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus in response to exposure to a familiar conspecific, which was not found to occur in response to exposure to a novel or unfamiliar conspecific. Furthermore, there is increased general neuronal activation in the medial posterior amygdala in response to a familiar, but not novel, conspecific, and increased general neuronal activation in the medial anterior amygdala to exposure to both familiar and novel conspecifics, but the magnitude of this activation is significantly greater in response to the familiar conspecific. To substantiate the claim of a relationship between familiarity and OT, we injected male mice with the OT receptor (OT-R) antagonist L-368,899 and placed them in a scenario where they could choose to spend time in a chamber with a trapped conspecific or a chamber with a palatable tastant. OT-R antagonism reduced time spent with a familiar, but not novel conspecific. We conclude that familiarity and social context result in differences in OT neuronal activity and OT signalling.

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AVP – vasopressin

AVPR1a – arginine vasopressin receptor 1a

BST – bed nucleus of the stria terminalis

icv – intracerebroventricular

ip - intraperitoneal

MPOA – medial preoptic area

NAcc – nucleus accumbens

NTS – nucleus of the solitary tract

OT – oxytocin

OT-R – oxytocin receptor

PVN – paraventricular nucleus of the hypothalamus

SON – supraoptic nucleus of the hypothalamus

VTA – ventral tegmental area

1. Introduction and Literature Review

1.1. Neurobiology of oxytocin

Oxytocin (OT) is a cyclic nonapeptide, consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-GlyNH_2, with a sulphur bridge that links the two cysteines (Lee, Macbeth, Pagani, & Young, 2010). It is well-conserved across the animal kingdom. The presence of isoleucine at position 3 in OT and members of the OT family is essential for stimulation of OT receptors. Homologues of OT in vertebrates differ in two amino acids at most: for example, the closely related vasopressin (AVP) only differs as follows: OT contains the neutral amino acid leucine at position 8 and isoleucine at position 3, while AVP has the basic arginine at position 8 and phenylalanine at position 3. (Gimpl & Fahrenholz, 2001).

OT is primarily synthesised by magnocellular neurons located in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus (Calcagnoli et al., 2014). OT-containing vesicles produced in the PVN and SON are then directed via axonal projections to the posterior pituitary (neurohypophysis) where they are stored and then released into the blood stream. Once these neuropeptides circulate into the bloodstream, they can exert their effects in the periphery as neurohormones (Lukas & Neumann, 2013).

OT synthesising neurons also project widely elsewhere throughout the brain: magnocellular neurons project centrally to targets like the amygdala; discrete populations of parvocellular OT found in the PVN innervate hindbrain and limbic areas, such as the medial septum, hippocampus and, again, the amygdala (Debiec, 2007). The central receptor for OT to act on is the OT-receptor (OT-R). The OT-R is, as expected, present in the SON and PVN, but it is also widely distributed throughout the brain: found in the hippocampus, amygdala, bed nucleus of the stria terminalis (BST, septum, ventral pallidum, nucleus accumbens (NAcc) and caudate putamen. These neuropeptides form an intricate neural network (Lee et al., 2010; Lukas & Neumann, 2013).

Neuropeptides, particularly OT, play a major role in the modulation of social behaviours. Historically, however, OT has been best characterised for its role in the facilitation of reproduction in vertebrates. OT has uterotonic activity, inducing labour and uterine contractions to facilitate child birth. It also regulates milk ejection in lactation. In addition, it has regulatory roles in glucose metabolism, adrenal function and sperm transport (Gimpl & Fahrenholz, 2001; Lukas & Neumann, 2013).

Oxytocin also regulates food intake and the anorexigenic effect of OT is well documented. Lesions of the PVN in rats resulted in hyperphagia and increased body weight (Sabatier, Leng, & Menzies, 2013). Mice bred to be unable to produce the OT peptide or lacking the OT receptor developed a late-onset obesity phenotype (Camerino, 2009). Both the central (intracerebroventricular, icv) and systemic administration of OT agonists similarly decreases food intake and meal

duration, even in starved, obese or leptin-resistant animals (Morton et al., 2012; Olszewski et al., 2010). Parvocellular OT neurons in the PVN appear to be involved in the gastrointestinal vago-vagal reflex (Blevins et al., 2003). Here, the activity of OT in this smaller circuit appears to regulate this reflex, acting to inhibit gastric motility (Sabatier et al., 2013). Magnocellular OT neurons in both the PVN and SON are also activated shortly after the initiation of food intake by the same gastrointestinal stimuli that activate vagal afferents to the nucleus of the solitary tract, including satiety peptides and stomach distension. Subsequent dendritic release of OT from these magnocellular neurons in high volumes is suggested to diffuse to sites in the hypothalamus to inhibit food intake (Olszewski et al., 2010; Sabatier et al., 2013).

1.2. OT and the regulation of social behaviour in rodents

1.2.1. Reproductive-related social behaviours

Reproduction-related social behaviours include maternal behaviour and socio-sexual interactions such as mating, for which the role of OT is well-characterised. OT acts on the BST, ventral tegmental area (VTA) and medial preoptic area (MPOA) during nursing to facilitate maternal care (Lukas & Neumann, 2013). Furthermore, the infusion of synthetic OT into the lateral ventricle of steroid-primed virgin female rats induces spontaneous maternal care, whereas the icv injection of OT-receptor antagonist or anti-OT antiserum instead impairs the onset of maternal behaviour and lowers the display of existing pup-directed behaviour.

In rats made insensitive to chemotactic stimuli from pups that promote maternal behaviour (e.g. made anosmic), icv injection of OT restores maternal behaviour. Furthermore, mice either lacking the OT-receptor or with OT knocked out completely display deficient maternal care (Bosch & Neumann, 2012).

The effects of OT on maternal care are most important during parturition, where OT-receptor antagonism impairs or delays the onset of all components of maternal care. Similar treatments during lactation do not result in such comprehensive deficiencies in all aspects of maternal care, nor to the same extent (Bosch & Neumann, 2012).

Pair bonding is another reproductive-related social behaviour that is regulated by OT. Pair bonding is extensively studied in the prairie vole, a species of rodent that establishes enduring monogamous relationships among sexually mature adults. OT immunoreactivity was widely distributed in several regions in the vole brain, including the PVN, SON, BST, and MPOA. Prairie voles also have higher OT-R density in the BST, medial prefrontal cortex and NAcc (Young et al., 2011).

Interestingly, the relative contribution of OT to pair bonding in prairie voles is gender-specific, with the effects of OT more significant in the female, whereas it appears AVP has a more significant role in the male. Icv administration of OT into the NAcc and prefrontal cortex induces pair bonding in female prairie voles in the absence of prior mating, which can be blocked by pre-treatment with the OT-R antagonist in these areas. OT infusion also induced partner preferences in males, although higher doses were required compared to females (Cho et al.,

1999). OT levels in the NAcc are also elevated in response to sociosexual interactions with a male conspecific. Exposure to male chemosensory cues also induces in changes in OT-R density in the olfactory bulb (Lukas & Neumann, 2013; Young et al., 2011).

1.2.2. Social recognition and social memory

Recognition and discrimination of a conspecific is an essential component of appropriate social interactions and responses, including reproductive social behaviours or whether affiliative versus aggressive behaviour should be displayed (Lee et al., 2010). Pair bonding, partner preference and maternal-offspring bonding require the ability to recall and distinguish a specific individual, known as social memory. This is demonstrated in the social discrimination test, where a rat is exposed to a conspecific and then, after an interval between exposures, the rat is simultaneously exposed to this familiar conspecific and an unfamiliar, novel conspecific. Greater social investigation of the novel conspecific during this second exposure suggests the recollection and social recognition of the familiar conspecific (Lukas & Neumann, 2013).

OT has been shown to modulate this social memory in rats. There is a male-specific extension of the duration of social memory following the icv administration of OT, which is prevented by OT-R blockade. In addition, OT and OT-R knockout mice show attenuated social memory of conspecifics in both males and females. Again, there was male-specific reversibility of these deficits in response to icv OT administration prior to the first exposure of the animal,

suggesting that OT activity is essential for the initial formation of social memory (Lukas & Neumann, 2013). In contrast, OT knockout mice did not perform differently in tests of social discrimination compared to wild-type mice, with no difference between the two groups in time spent with a novel stranger conspecific when introduced simultaneously with a familiar conspecific (Crawley et al., 2007).

OT-mediated prolonging of social memory in juvenile male rats has been localised to the lateral septum, olfactory bulb, ventral hippocampus, MPOA and medial amygdala. Synthetic OT administration to the medial amygdala is sufficient to re-establish the capacity for social recognition in male OT knockout mice. In contrast, the direct administration of OT-R antagonist to the lateral septum and olfactory bulb in male rats weakens social recognition. In both male and female rats, the medial amygdala appears to be particularly important for the recognition of female conspecifics. Blockade of the OT-R directed to the medial amygdala appears to improve social recognition of females, while the recognition of juvenile males is confined to OT activity in the lateral septum (Lukas & Neumann, 2013; Lukas et al., 2013).

1.2.3. Social approach

For social interactions between conspecifics to occur, particularly when the conspecific is novel, the balance must be tipped from avoidance or reserved behaviour towards a motivation to approach and interact. Social approach and exploration of a novel conspecific by male rats and mice can be impaired through

the central administration of synthetic OT-R antagonist, which can be later reversed by central infusion of synthetic OT. This suggests that endogenous OT facilitates approach and, in turn, blocking its effects attenuates it. Even avoidance behaviour in animals following a social defeat can be reversed by synthetic OT administration. Normally, following a social defeat, re-exposure of a rat to the conspecific that defeated it (the "defeater" rat) with simultaneous exposure to an object stimulus results in decreased preference and avoidance of the defeater rat and increased preference for the object. However, icv OT administration 20 minutes prior to re-exposure was sufficient to reinstate preference for the defeater (Lukas & Neumann, 2013; Lukas et al., 2011). Together, this might suggest that the absence of OT signalling should impair social approach; however, in OT knockout mice, tendencies to approach, explore and spend time with a novel conspecific remained intact, suggesting OT is not required for spontaneous social approach behaviour (Crawley et al., 2007).

Voles also provide a useful comparative model system for neural mechanisms that underpin social approach. Prairie voles and montane voles are genetically similar yet behave differently in a social context. As previously mentioned, prairie voles are monogamous animals, seeking lifelong pair bonding. They proactively seek social contact and, when observed in large enclosures that mimic their natural habitat, spend greater than half their time interacting or huddling with another prairie vole. Montane voles are instead promiscuous and more solitary, only seeking out others for the purpose of mating. The neurological bases for these social differences has been attributed to differential distribution of OT and AVP receptors, particularly in regions related to reward. Prairie voles have higher OT-R

density in the nucleus accumbens and higher AVP receptor density in the ventral pallidum – both regions part of the dopaminergic reward system. Therefore, activation of reward regions, facilitated by OT, during social interaction may be rewarding and reinforcing, promoting the pursuit of social contact (Young, 2002).

Social fear conditioning, such as through the delivery of foot shocks during exposure to and investigation of a conspecific, can result in significant reduction and fear of subsequent social investigation – similar to a social a defeat. This can be reversed by the central administration of synthetic OT, showing that it has a pivotal role in overcoming learned social avoidance. However, these effects are limited to conditions where sociality is already low at baseline; synthetic OT administration was not able to enhance sociality to supranormal levels. (Lukas & Neumann, 2013).

1.2.4. Aggression

Intermale aggression is a common example of a non-affiliative social behaviour in rodents. In terms of a dominant, aggressive resident male rodent defeating a subordinate male intruder, central OT and AVP release was found in both animals. Social defeat appears to triggers OT release from the SON and septum, but not PVN, while AVP is released from the PVN but not the SON or septum (Lukas & Neumann, 2013).

Furthermore, manipulation of OT appears to modify the development and magnitude of aggression altogether. The injection of OT, both acutely and

chronically, via the icv route in a feral strain of male rats (wild-type Groningen) resulted in dose-dependent suppression of aggressive behaviour and also increased social exploration following the introduction of an intruder into their home territory. This responsivity to this OT manipulation was positively correlated to the level of baseline aggression, with the anti-aggressive response greatest in animals displaying the highest baseline aggression level. In keeping with these results, OT-receptor blockade instead potentiated aggressive displays, particularly in those animals with the lowest baseline aggression levels (Calcagnoli et al., 2014). This is supported by analysis of oxytocin expression and receptor binding in the male rat brain. Rats were repeatedly contested against intruder conspecifics and categorised into phenotypes as having low, high or excessive levels of aggressive. Greater magnitudes of aggression correlated with decreased OT mRNA expression in the PVN, but highest OT-R binding in the central amygdala and BST (Calcagnoli et al., 2014).

1.3. Empathy and emotional contagion

At its essence, empathy is a complex social behaviour that involves understanding and experiencing another's feelings and emotions for one's self – often emerging when that other individual is in need or in distress. However, the formal definition in an academic sense remains imprecise. Some definitions additionally require awareness of the distinction between the affective state of the other individual and one's own (Panksepp & Lahvis, 2011). Researchers have also pointed to a separation between cognitive empathy – the ability to recognise or identify

another's emotional state, e.g. seeing an upset individual and realising they are upset, and emotional empathy – actual sharing and experiencing another's emotions, e.g. seeing an upset individual and feeling upset or sad for them (Uzefovsky et al., 2015).

Empathetic concern can then drive pro-social behaviour to alleviate and address the perceived distress or needs of the target (Bartal, Decety, & Mason, 2011). Human children as young as one-year-old have been shown to act pro-socially in response to the recognition of sadness in another (Sato et al., 2015). People clearly do not act solely in terms of their own self-interest; instead, they recognise and respond to unfairness and inequities, provide care for others, and share valued resources without receiving extrinsic benefits (Hernandez-Lallement et al., 2015).

One of the most basic and primitive forms of empathy is 'emotional contagion' – a form of emotional empathy, in which the emotional state of another elicits a similar, shared emotional response in the individual. The observer accesses the subjective state of another from their own perspective, triggering a similar emotional state. For example, in emotional contagion, an individual may observe another experiencing pain and, as a result, experience this pain for themselves. Emotional contagion involves mere mimicry of another's emotional state, whereas more stringent definitions of empathy may refer to understanding another's affective state for one's self and recognising this can be separate from one's own state but is occurring in another – which is difficult to ascertain in non-verbal animals. Empathy may be viewed as an evolution from emotional contagion – beyond mimicry and towards concern, compassion, pro-social responses or

helping, and altruism (Mogil, 2012; Nakahashi & Ohtsuki, 2015; Panksepp & Lahvis, 2011). In humans, emotional contagion is supported by neurobiological evidence, as neuroimaging studies suggest that the neural networks activated in an individual while observing another's pain overlap with networks involved in directly experiencing physical pain for oneself (Gonzalez-Liencres et al., 2014).

Emotional contagion is also well-characterised in animals. One of the earliest examinations of emotional contagion in rodents involved rats performing a lever-pressing task while simultaneously being exposed to a conspecific receiving an electric shock. Observation of the shock-induced distress resulted in decreased lever pressing, demonstrating an ability to perceive the distress of another rat and to modulate their behaviour accordingly (Church, 1959). Another example is the common experimental paradigm in rodents which involves an observer mouse watching a conspecific receive some form of noxious stimuli, such as an electric foot shock. The observing rodent indicates a shared experience of distress through behaviour such as freezing or quantity of faecal droppings (Gonzalez-Liencres et al., 2014). It is assumed that the observer mouse has copied or perceived the emotional state of the distressed target and the resultant behavioural responses are driven by those emotions. This provides a distinction from behavioural mimicry, when the behaviour itself is merely copied; in emotional contagion, behaviour can differ so long as the underlying emotions appear to be shared. This allows these behaviours to be used as a surrogate measure for emotional mimicry (Nakahashi & Ohtsuki, 2015).

Langford *et al.* (2006) examined mice receiving a painful stimuli and the effects of observing another mouse simultaneously receiving that same stimuli. In this instance, the stimuli was the intraperitoneal (ip) injection of dilute acetic which induces writhing behaviour. Mice displayed more writhing behaviour when observing a mouse receiving the injection versus a non-injected mouse. Therefore, observation of another mouse in pain appears to potentiate one's own experience of pain.

Interestingly, emotional contagion in rodents is also modulated by the social context. The freezing behaviour observed in response to watching a conspecific receive electric foot shocks is significantly increased when this conspecific is a familiar cagemate, as opposed to an unfamiliar rodent from a non-cagemate condition. This indicates the importance of familiarity in the development of empathic-like processes (Gonzalez-Liencres et al., 2014). This is supported by the study of mice simultaneously receiving acetic acid injections: the increased writhing of the observer mouse also occurs only when exposed to a cagemate also receiving the injection, but not a novel conspecific (Langford et al., 2006).

1.4. Pro-social behaviour

1.4.1. Pro-social behaviour in animals

While empathy and pro-social behaviour are linked, with empathy often thought to drive pro-social behaviour, emotional contagion and pro-social action are still distinct. Pro-social behaviour describes actions or activities performed with the

intention of alleviating need or improving the wellbeing of others (Bartal et al., 2011; Cronin, 2012). It involves directed action to benefit another; a response beyond mere emotional mimicry. For example, the resultant freezing behaviour and inaction described in the aforementioned emotional contagion experiments does little for the observed rodent undergoing foot shocks or writhing (Bartal et al., 2011).

The potential adaptive benefits of pro-social behaviour can include promoting survival and reproduction within a group. For example, kin selection theory may drive pro-social behaviour, as an individual invests or contributes to the reproductive success or genetic fitness of relatives, even to the detriment of their own survival (Porter, Moore, & White, 2014). Kin selection may be the reason pro-social behaviour extends to conspecifics in general, as it is likely that conspecifics co-existing in a group context are genetically related (Vasconcelos, Hollis, Nowbahari, & Kacelnik, 2012). However, it can also come at a cost to the individual, such as when sharing finite and valuable resources (Ben-Ami Bartal, Rodgers, Bernardez Sarria, Decety, & Mason, 2014).

An example of pro-social behaviour that encompasses both costs and benefits to an individual is food sharing. Food sharing is defined as a type of pro-social behaviour when a food-motivated individual, with the capacity to defend a food source that could be monopolised, lets another consume a portion of that food. This definition allows for both passive sharing, where theft of the food by another individual is tolerated, to active sharing, where the transfer of food to another individual is facilitated (Stevens & Gilby, 2004). Food sharing, even between

non-kin, has been observed in non-human and non-primate animals. Vampire bats share food through regurgitation of blood with reciprocating non-kin group members (Wilkinson, 1984); groups of largely non-related Taiga voles nest together during winter and co-operatively accumulate and utilise food stores (Wolff & Jr, 1981); and non-spiny pairs of mice share a central cup of food, with no difference in food sharing members between unfamiliar sibling pairs (i.e. born in successive litters) and unfamiliar non-sibling pairs (Lukas et al., 2011).

Such food sharing is a paradoxical phenomenon. As animals share or donate food, which is often scarce, it comes at a personal cost. However, food sharing, in addition to assisting the survival of kin, can occur to prevent a contest, to recruit others to help defend a kill against potential predators, or in the hope that the recipient may reciprocate with a favour in the future (Stevens & Gilby, 2004). Alternatively, food sharing – and perhaps pro-social behaviour in general – may be motivated by empathy and altruism.

Whether or not pro-social behaviour in non-primate mammals is driven by empathy or altruism is not entirely clear (Bartal et al., 2011; Vasconcelos et al., 2012). Regardless of whether or not empathy is the driving motivator, pro-social behaviour has been demonstrated in rodents. Bartal et al. (2011) produced an arena where a free rat was allowed to roam without restriction. A restrainer was placed in the centre of the arena, which was filled with either a trapped cagemate, plush toy, or left empty. A door to the restrainer could be opened by the free rat applying pressure to tip it over, liberating the trapped rat. Over 12 days of the experiment, increasing proportions of free rats opened the door to liberate the

trapped rat with decreasing latency; however, no such increase was observed in the control conditions where the restrainer was filled with a toy or left empty. In a modified set-up, free rats still continued to liberate trapped rats even when the trapped rats were only able to exit into a separate area, indicating that the anticipation of social interaction was not required for the motivation of door opening. It was also shown that the rats in the trapped cagemate condition showed more distress themselves compared to the control conditions, with significantly more alarm calls detected through sampling of ultrasonic vocalisations. However, the researchers were unable to distinguish whether the alarm calls originated from the free or trapped rat in this condition. Therefore, these alarm calls do not extend as conclusive evidence that isolate that the free rat was empathically experiencing distress in response to the observing trapped rat.

Bartal et al. (2011) extended this experiment by placing free rats in a similar arena with two restrainers: one containing palatable chocolate chips and the other containing either a trapped cagemate or left empty as a control. In the trapped cagemate vs. chocolate condition, doors were opened in no specific order and there was no difference in latency for door opening, whereas in the empty restrainer vs. chocolate condition, the door to the chocolate-containing restrainer was opened significantly quicker than the empty container. This showed that the relative value of liberating a trapped cagemate was equivalent to a highly palatable tastant. The free rats even shared these chocolate chips with the now-liberated rats, despite previous tests showing that the free rat was capable of eating all of the chips in a single meal.

It was later shown that this experimental paradigm for pro-social behaviour was, like emotional contagion, modified by social experience and familiarity (Ben-Ami Bartal et al., 2014). Rats were shown to liberate trapped rats regardless of whether they were familiar cagemates or strangers, under the condition that they belonged to the same strain (Sprague-Dawley) and not a different strain (Long-Evans). This was consistent with preferential pro-social behaviour towards kin or members of one's own group. In humans, pro-social behaviour is also modulated by the degree of affiliation, where it is usually preferentially directed towards those members belonging to the group.

Next, the liberating behaviour of free Sprague-Dawley rats under three further conditions was tested: 1) initial pair-housing with Long-Evans rat, with that same cagemate used as the trapped rat (familiar with rat condition), 2) pair-housing with a Sprague-Dawley rat, with a Long-Evans rat used as trapped rat (unfamiliar with both strain and individual rat condition), and 3) pair-housing with a Long-Evans rat, with a stranger Long-Evans rat used as trapped rat (familiar with strain but not individual rat condition). Free Sprague-Dawley rats that were familiar with the Long-Evans strain liberated both cagemate and stranger Long-Evans rats (i.e. conditions 1 and 3); in contrast, Sprague-Dawley rats unfamiliar with the Long-Evans strain did not open the restrainer door upon exposure to trapped Long-Evans rats (i.e. condition 2). Therefore, familiarity with a rat strain, not limited to familiarity with the specific individual rat to which they were exposed, was determined to be necessary for pro-social liberation.

Finally, to investigate whether rats possessed an innate motivation to assist other rats of their own strain, Bartal et al. (2014) fostered Sprague-Dawley rats from birth with Long-Evans rats, without exposure to rats belonging to the Sprague-Dawley strain. These fostered rats helped to liberate trapped Long-Evans rats but did not free Sprague-Dawley rats, demonstrating that it is the social experience of strain familiarity, as opposed to an innate motivation to act pro-socially towards rats belonging their own genetic strain, which is the essential determinant for pro-social behaviour.

Sato et al. (2015) also used an experimental paradigm involving liberation of a distressed rat to study pro-social behaviour. The trapped rat was soaked in a compartment filled with water, while the free rat was placed in an adjacent dry compartment. These two compartments were separated by transparent partition, with a door that could be opened by the free rat in the dry compartment. During the experiment, the free rats quickly liberated the trapped rats with decreasing latency. These rats opened the door significantly quicker in this soaked rat condition compared to the control conditions when the compartment contained either a soaked toy, was filled with only water or was completely empty. Furthermore, the free rats did not show door-opening behaviour when the compartment of the trapped rat was not filled with water and distress was not apparent, suggesting the liberation only occurs when the free rat perceives the trapped rat to be in distress.

A third compartment, filled with a food reward (palatable chocolate cereal), was added to this set-up, and connected to the dry compartment with a transparent

partition and a door. Like the door to the water-filled trapped rat compartment, it could only be opened from within the dry compartment by the free rat. When given the choice to open either door, the free rats chose to open the door liberating the trapped rat before accessing the food reward in the majority of trials. This is additional support that this helping, pro-social behaviour is as valued as a palatable food reward.

Another pro-social behavioural experimental paradigm has also been developed for rodents, where an actor rat was able to enter one of two compartments (Hernandez-Lallement et al., 2015). After entering one of the two compartments, a partner rat was released into an adjacent compartment, separated by only a transparent, perforated wall allowing olfactory, auditory and visual communication. Depending on which compartment the actor rat entered, either the actor rat alone received sucrose pellets ("own reward" condition), or both the actor and partner rats received sucrose pellets ("both reward" condition). As a control, the partner rat was replaced with a toy rat. Actor rats showed a significant preference for entering the "both reward" compartment when paired with a partner rat; this preference was not observed when paired with a toy rat. This showed that, when given the choice, rats preferred a mutual reward and derived some value from enabling another rat's access to food.

These experimental paradigms demonstrate evidence pro-social behaviour in rodents, often times in response to a distressed conspecific. Pro-social behaviour appears to be modulated by social experience, requiring familiarity with the strain of the target animal. The value of pro-social behaviour seems to be on par with

palatable, rewarding tastants, based on situations where rats are given the choice between both. The liberation of trapped rats was also shown to be dependent on whether or not these trapped rats were observably distressed. Rats also prefer mutual rewards over rewards only for one's self, even when this appears to provide no additional benefit for the rat making this choice. While it is clear that the actions of these rats result in the benefit of others, it is difficult to delineate the psychological motivations that drive these actions. Control experiments show that these rats do not direct these same behaviours towards toys or in the absence of the other animals; these are certainly social actions. Nevertheless, in non-verbal animals like rodents, it cannot be stated from these experiments that these animals are, for example, driven by empathy or show understanding of another's emotional state that drives them to act or help. Instead, only assumptions can be made about the perceived, unobservable mental state of the animal, based on the actions we can observe and rudimentary indicators, such as emotional contagion.

It has been noted that while it is uncontroversial that an animal may respond to another animal's affective state, it is different to claim that an animal has an understanding of the affective state and that this is a causal factor of that response. Pro-social behaviour driven by empathy implies that these actions that benefit another are primarily motivated by the goal of contributing positively to another's wellbeing. However, pro-social behaviour may instead be adaptive, such as those discussed earlier, like contributing to the reproductive fitness of probable relatives (Vasconcelos et al., 2012). Conversely, empathic pro-social behaviour cannot be ruled out, either. Paradigms where rats more readily free trapped rats in distress

rather than those who do not show distress begin to suggest that the target animal's wellbeing may be a major motivator.

1.4.2. Neurobiology and OT in empathy and pro-social behaviour

In humans, neuroimaging and lesion studies have identified neurobiological substrates associated with empathy. Different – but interconnected – circuitry have been characterised for cognitive versus emotional empathy. Multiple questionnaires have also been developed to measure these different dimensions of empathy. Neurological patients with lesions localised to the ventromedial prefrontal cortex assessed with one of these questionnaires scored less on measures of cognitive empathy, while lesions of the inferior frontal gyrus indicated deficits in emotional empathy (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009).

The mirror neuron system, associated with state-matching of another's behaviour, has been discovered in in the inferior frontal gyrus. Activation of the mirror neuron system in the inferior frontal gyrus has been shown to occur in both motor action imitation and emotional recognition, and also during imitation and passive viewing of emotional faces. However, the more complex cognitive processing involved in "mentalising", has been connected with the ventromedial prefrontal cortex, among other regions (Shamay-Tsoory et al., 2009). Mentalising is a central part of cognitive empathy, and involves rationalising and interpreting another's emotional or mental state (Feeser et al., 2015).

Based on this, a “mentalising network”, including the ventromedial prefrontal cortex, temporo-parietal junction and temporal poles”, has been defined for cognitive empathy; meanwhile, in emotional empathy, the human mirror neuron system, which in addition to the inferior prefrontal gyrus includes the inferior parietal lobule and the amygdala, is involved. In a normal empathic experience, these areas are simultaneously activated (Uzefovsky et al., 2015).

Considering the role of OT in a wide range of social and affiliative behaviours, as elaborated in section 1.4, this involvement may also extend to empathy. Social behaviours like acting pro-socially, reducing distress of another or altruism may potentially be driven by an empathetic-like recognition of the emotional state of the other individual (Shirtcliff et al., 2009).

It is plausible that neuropeptides like OT play an important role in the neural pathways, such as the mentalising network or mirror neuron system, which underlie empathy. Analysis of the rs53576 single polymorphic region in the OT-R gene shows that the rs537576-GG genotype is associated with increased social cognition. In the arginine vasopressin receptor 1a (*AVPR1a*) gene, a 327 repeat allele in the RS3 polymorphic repeat region located in the promoter was associated with autism, elevated amygdala activation, lower partner bonding in men, and lower altruistic giving. Consistent with this, individuals were given three questionnaires that provide self-reported measures of empathy and result in a combined “complete empathy score”. The presence of either a G to A change in rs53576 (rs537676-A) in the *OT-R* gene or a 327-repeat allele in the *AVPR1a* gene independently predicted lower complete empathy scores. Further regressions

analyses to delineate the effect of these genotypes on specific types of empathy showed that the presence of the rs53576-A allele predicted lower emotional empathy but not cognitive empathy. The opposite was true for the 327-repeat allele, which predicted lower cognitive empathy but had no predictive power for emotional empathy. Therefore, not only do the *OT-R* and *AVPR1a* genes have a role in empathy, but they appear to have distinct involvement in the two different types of empathy (Uzefovsky et al., 2015). Similarly, when *OT-R* variant rs53576-GG individuals were shown a social interaction that displayed an individual in distress and physical pain, reported greater levels of empathic concern and also increased electrodermal activity, indicative of increased sympathetic nervous system arousal (Smit et al., 2015).

Polymorphisms in the *OT-R gene,* including rs53576 and rs2268498, have also been correlated with other objective metrics for empathic performance. Individuals were shown videos of a target subject subjected to variable degrees of physical pain via electrical stimulation to the arm and were required to report the magnitude of pain experienced by the target. This empathic accuracy in correctly judging the pain experience of another was greater in *OT-R* rs2268498-CC and rs53576-AA carriers. This finding in regards to the rs-53576-A allele is in contrast to the aforementioned findings that links the A allele to reduced empathy measures compared to the G allele (Laurse et al., 2014).

In contrast, the intranasal administration of OT did have an effect on the cognitive empathy-associated ability of mentalising. These individuals were administered the Reading the Mind in the Eye Test (RMET) to measure their mentalising

ability, where they were shown images of the eye region expressing a complex mental state and were required to identify this state from a multiple-choice selection. Compared to the placebo condition, OT improved mentalising accuracy. When adjusted for individual's questionnaire-based empathy scores, the effect of OT specifically enhanced mentalising ability in individuals with low baseline empathy scores, suggesting the effect of OT only corrects for attenuated empathic abilities (Feeser et al., 2015). OT given intranasally was also found to reduce interpersonal distance (i.e. promote proximity between two people), which is indicative of responsiveness and comfort in a social interaction. This effect was only found in highly empathic individuals; in fact, in patients with low empathic traits, intranasal OT increased interpersonal distance (Perry, Mankuta, & Shamay-Tsoory, 2014).

1.5. Specific Aims

The role of OT in the regulation of social behaviours is well-characterised. In particular, OT has been associated with social context or experience of behaviours, such as social memory or recognition, and the approach of novel and non-novel conspecifics. Despite its apparent role in the development of familiarity and subsequent behavioural responses, less is known about whether the activity of OT neurons and OT-related circuits is differentially modulated during social exposure to a familiar versus and novel (unfamiliar) conspecific. Therefore, the current set of studies will determine (a) the PVN/SON OT neuronal activity changes in response to exposure to novel/familiar conspecifics; (b) concurrent changes in amygdala c-Fos expression in response to novel/familiar conspecifics,

and (c) behavioural responses to OT-R blockade in terms of spending time in the vicinity of the novel/familiar conspecific when the conflict exists between the conspecific and a familiar palatable tastant.

2. Exposure to a familiar versus novel conspecific is associated with changes in PVN and SON OT neuronal activity and concurrent changes in amygdala neuronal activity

2.1. Methods

2.1.1. Animals

Males on a C57BL/CJ background (AgResearch, Hamilton, New Zealand) were housed in individual cages in a temperature- (23^{o}C) and humidity-controlled room, with a 12h-light:12h-dark cycle, with lights on from 0700 to 1900 h. The animals weighed on average 31.5 g (± 0.5 g) Mice were provided with *ad libitum* access to standard rodent chow (Teklad) and tap water until the beginning of experimental manipulation, where any changes are specified. The University of Waikato animal ethics committee provided approval for the procedures described in this study.

2.1.2. Experimental arena

The experimental arena consisted of a single transparent plexiglass compartment. In order to ensure that mice cannot see outside of the arena, white paper was used to externally cover the sides and the bottom of the container.

A small cylindrical restrainer was placed in one corner. These cylindrical restrainers could either be secured with a top piece to ‘lock’ or ‘secure’ the restrainer, or a drinking bottle could be placed on top of the prison top-less prison.

2.1.3. Experiment 1: Activation of OT neurons in the PVN and SON following exposure to a familiar or novel conspecific in a familiar environment

Mice were pair housed in 18 diyads, with each mouse labelled at the base of the tail to designate the “free mouse” and the “trapped mouse”. Each day for the three days prior to the experiment, the free mouse from each diyad was placed in the experimental arena for 30 minutes, with the restrainer containing either the familiar conspecific from its diyad, a novel conspecific, or an empty restrainer. The order of these pre-experimental exposures was randomly generated.

The diyads were allocated into three groups (n = 6), designated to spend 30 minutes in the familiar experimental arena on the experimental day with one of the three exposures: 1) a restrainer containing the familiar conspecific from its diyad, 2) a restrainer containing a novel conspecific, or 3) an empty restrainer. Immediately after the 30 experimental session was completed, the animals were deeply anaesthetised with an overdose of urethane and then perfused with 10 mL of saline followed by 50 mL of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4). Brains were removed and post-fixed overnight in PFA at 4^{o}C.

60 μm brain coronal sections were cut using the Vibrotome (Leica). Sections were incubated in 3% H_2O_2 in 10% methanol (in Tris-buffered saline (TBS), pH 7.4-7.6), and incubated overnight at 4^{o}C in goat anti-c-Fos antibody (1:1500; Santa Cruz) diluted in 'Supermix' (0.25% gelatin and 0.5% Triton X-100 in TBS). Sections were then incubated with rabbit-anti-goat antibody diluted in Supermix (1:400; Vector Laboratories) for 1 h and then in avidin-biotin complex diluted in Supermix (1:800; Vector Laboratories) for 1 h, both at room tempreature. Visualisation of peroxidase was achieved using 0.05% diaminobenzidine tetrahydrochloride, 0.01% H_2O_2 and 0.2% nickel sulphate. Sections were rinsed between incubations with TBS.

For double staining of these coronal sections for OT, these sections were processed for a second time, following c-Fos staining but prior to mounting. Sections were incubated in 3% H_2O_2 in 10% methanol (TBS), and incubated overnight at 4^{o}C in rabbit anti-OT antibody (Santa Cruz) diluted in Supermix. Sections were then incubated with goat-anti-rabbit antibody diluted in Supermix (1:400; Vector Laboratories) for 1 h and then in avidin-biotin complex diluted in Supermix (1:800) for 1 h. Visualisation was achieved using 0.05% diaminobenzidine tetrahydrochloride and 0.01% H_2O_2, but without nickel sulphate (to obtain a contrasting brown stain). Sections were rinsed between incubations with TBS. Sections were mounted on gelatinised slides, dried in ascending concentrations of alcohol, soaked in xylene, and embedded in Entellan.

In the analysis for activated OT neurons in the PVN and SON in each of the three groups, the following estimates were assessed per section and then per PVN and

SON: the total number of OT neurons and the number of OT neurons positive for c-Fos. Cells were counted bilaterally, and the percentage of neurons containing Fos-positive nuclei was tabulated. Data were analyzed with two-way ANOVA with sociality of testing environment and novelty of conspecific as variables. Bonferroni's post-hoc test was used. Significance was set as $p<0.05$.

2.1.4. Experiment 2: Activation of neurons in the medial anterior and posterior amygdala following exposure to familiar or novel conspecifics in a familiar social environment

Coronal sections from Experiment 1, single-stained for c-Fos and then mounted without double-staining for OT, were also analysed for activation of biochemically undefined neurons in the medial anterior and posterior amygdala in each of the three groups. The number of c-Fos positive nuclei in each region was determined bilaterally on four sections containing a given area per animal using Scion Image software. Densities of c-Fos positive nuclei (per mm^2) were averaged per mouse and per group. Data were analyzed with two-way ANOVA with sociality of testing environment and novelty of conspecific as variables. Bonferroni's post-hoc test was used. Significance was set as $p<0.05$.

2.2. Results

2.2.1. Experiment 1: Activation of OT neurons in the PVN and SON following exposure to familiar or novel conspecifics in a familiar environment

Exposure to the familiar conspecific in the familiar social environment significantly increased the percentage of activated OT neurons in the PVN compared to exposure to a novel conspecific in the same familiar social environment, or the familiar environment alone in a non-social context (Figure 2.1). Exposure to the familiar conspecific in the familiar social environment also significantly increased the percentage of activated OT neurons in the SON compared to exposure to a novel conspecific in the same familiar environment, but no significance difference was found when compared to the familiar environment alone in a non-social context (Figure 2.3). An example of c-Fos-OT colocalisation in the PVN is provided in Figure 2.2.

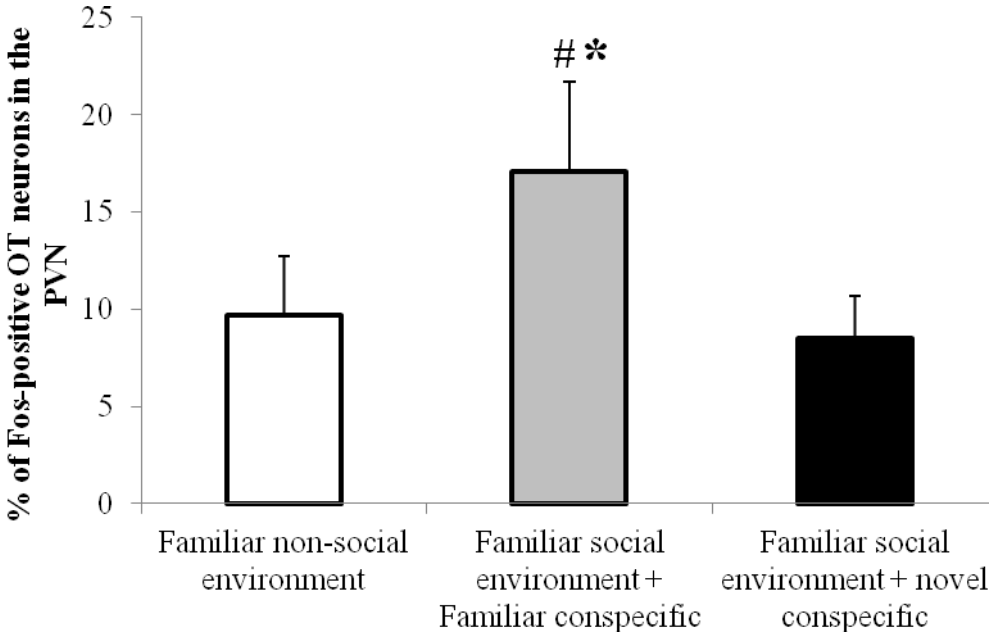

Fig 2.1. The effect of sociality of the environment and familiarity of the conspecific on activity of oxytocin (OT) neurons in the paraventricular nucleus of the hypothalamus (PVN). The percentage of OT neurons in the PVN expressing c-Fos in each group was analysed. Exposure to a familiar conspecific in the familiar social environment resulted in

a significant increase in the percentage of Fos-positive OT neurons in the PVN compared to exposure of a novel conspecific in the familiar social environment, or the familiar non-social environment alone. # - significantly different from "familiar non-social environment" group; * - significantly different from "familiar social environment + novel conspecific group ($P < 0.05$).

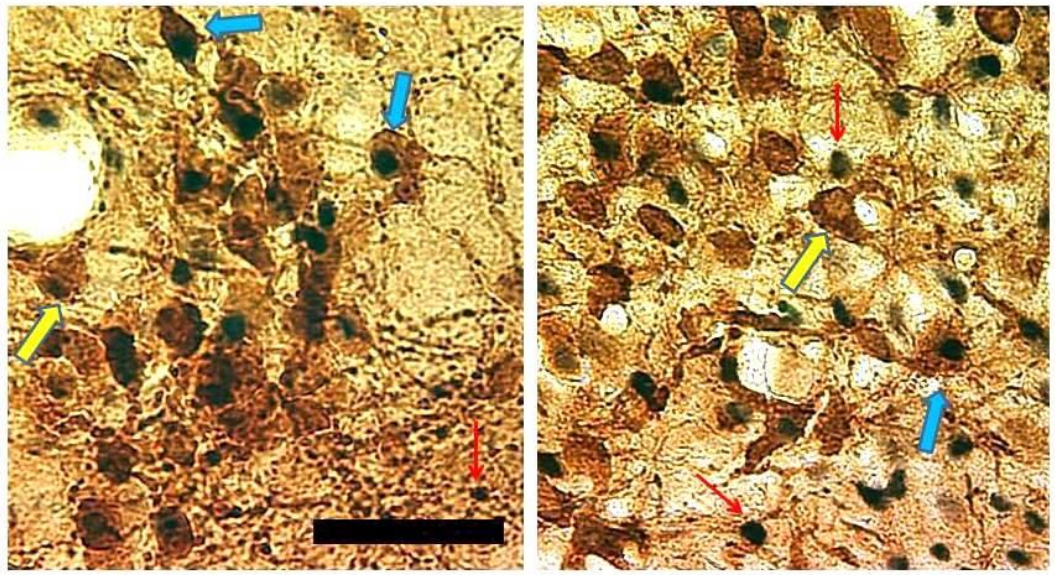

Fig 2.2. Photonmicrograph showing c-Fos-OT colocalisation in the PVN in the animal exposed to the familiar conspecific (L) versus the animal exposed to the novel conspecific (R). Blue arrows indicate Fos-positive OT neurons; yellow arrows indicate OT neurons devoid of Fos; and red arrows indicate Fos-positive nuclei of unidentified neurons (red thin arrows). Scale bar = 0.1 mm.

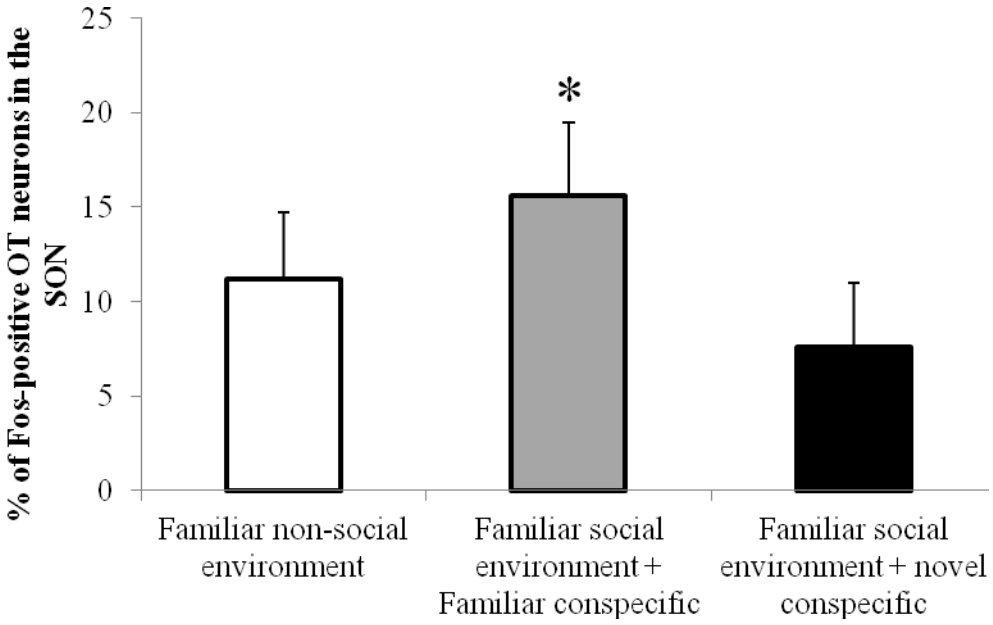

Fig 2.3. The effect of sociality of the environment and familiarity of the conspecific on activity of oxytocin (OT) neurons in the supraoptic nucleus of the hypothalamus (SON). The percentage of OT neurons in the SON expressing c-Fos in each group was analysed. Exposure to a familiar conspecific in the familiar social environment resulted in a significant increase in the percentage of Fos-positive OT neurons in the SON compared to exposure of a novel conspecific in the familiar social environment. * - significantly different from "familiar social environment + novel conspecific group ($P < 0.05$)

2.2.2. Experiment 2: Activation of neurons in the medial anterior and posterior amygdala following exposure to familiar or novel conspecifics in a familiar social environment

Exposure to the familiar conspecific in the familiar social environment significantly increased the density of c-Fos positive neurons in both the medial anterior and posterior amygdala compared to exposure to a novel conspecific in the same familiar social environment, or the familiar environment alone in a non-

social context (Figures 2.4 and 2.5). Exposure to the novel conspecific in the familiar social environment also significantly increased the percentage of activated OT neurons in the medial anterior amygdala compared to the familiar environment alone in a non-social context (Figure 2.4). An example of c-Fos immunoreactivity in the medial amygdala is provided in Figure 2.6.

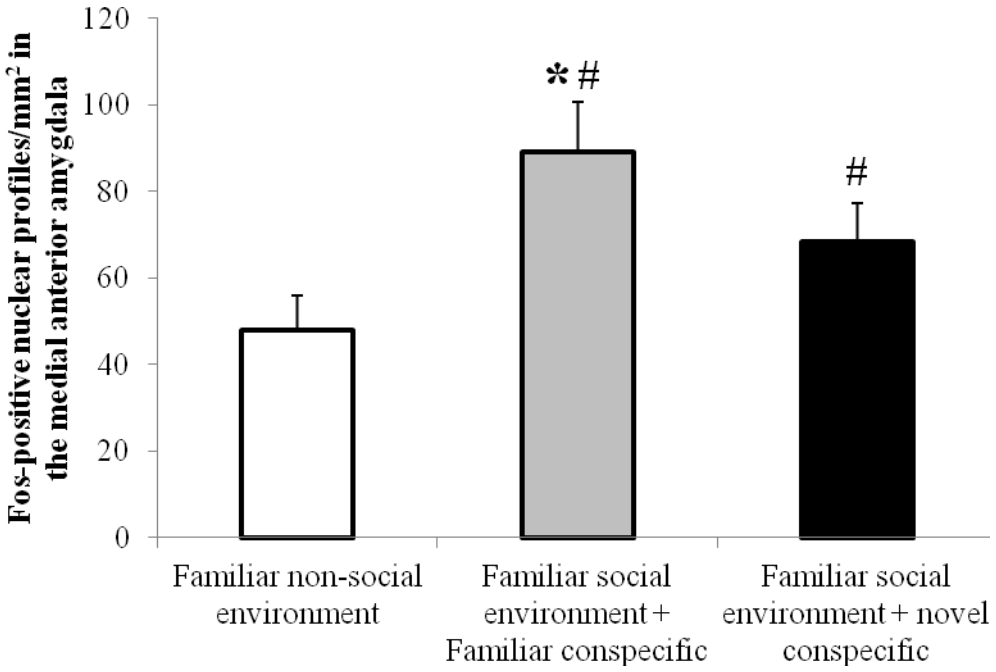

Fig 2.4. The effect of sociality of the environment and familiarity of the conspecific on the activity of biochemically undefined neurons in the medial anterior amygdala. The density of c-Fos positive nuclear profiles in the medial anterior amygdala in each group was analysed. Exposure to a familiar conspecific in the familiar social environment resulted in a significant increase in the density of c-Fos-positive neurons in the medial anterior amygdala compared to exposure of a novel conspecific in the familiar social environment, or the familiar social environment alone. Exposure to a novel conspecific in the familiar social environment also resulted in a significant increase in the density of c-Fos positive neurons in the medial anterior amygdala compared to the familiar social environment alone. # - significantly different from "familiar non-social environment"

group; * - significantly different from “familiar social environment + novel conspecific group ($P < 0.05$).

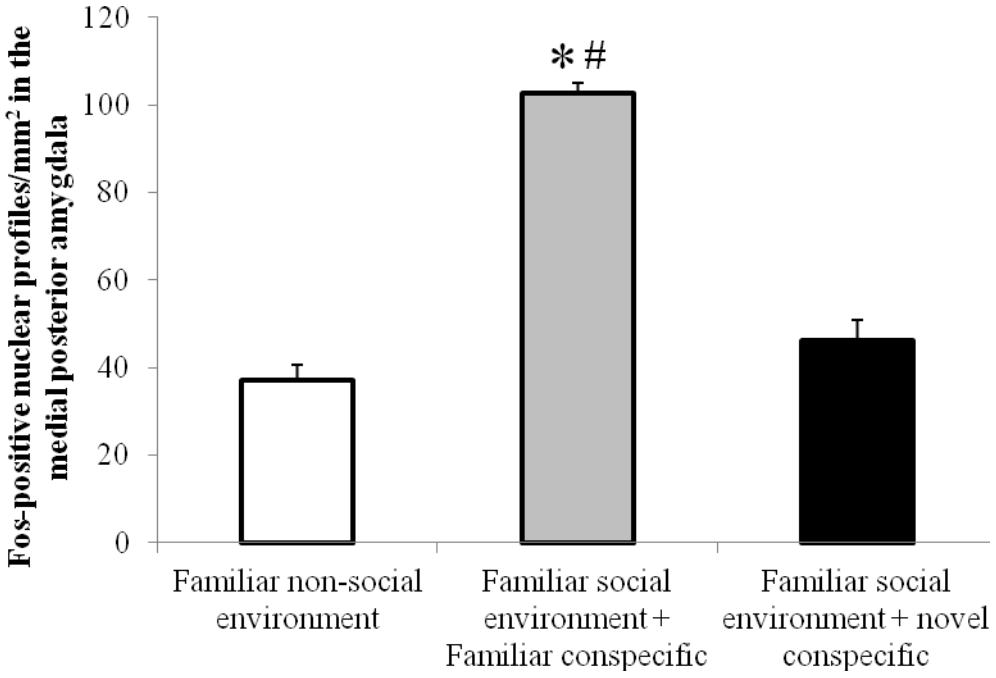

Fig 2.5. The effect of sociality of the environment and familiarity of the conspecific on the activity of biochemically undefined neurons in the medial posterior amygdala. The density of c-Fos positive nuclear profiles in the medial posterior amygdala in each group was analysed. Exposure to a familiar conspecific in the familiar social environment resulted in a significant increase in the density of c-Fos-positive neurons in the medial anterior amygdala compared to exposure of a novel conspecific in the familiar social environment, or the familiar social environment alone. # - significantly different from “familiar non-social environment” group; * - significantly different from “familiar social environment + novel conspecific group ($P < 0.05$).

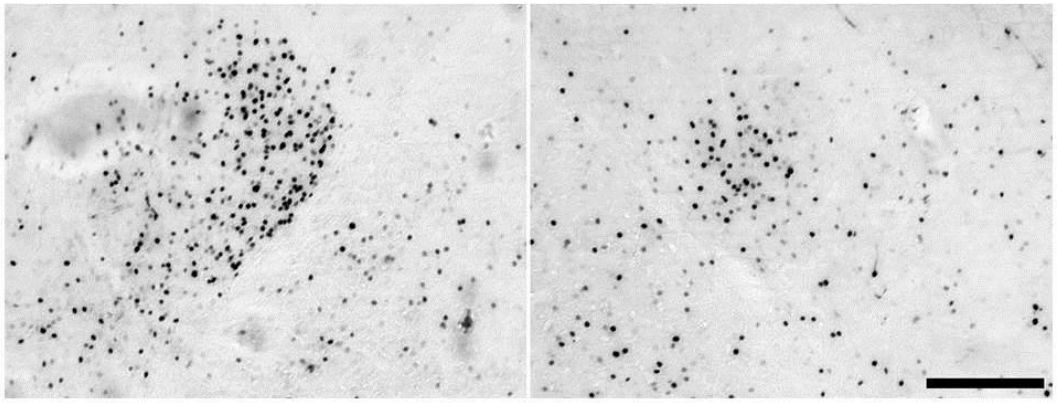

Fig 2.6. Photonmicrograph depicting c-Fos immunoreactivity in the medial amygdala in the animal exposed to a familiar conspecific (L) versus animals exposed to a novel conspecific (R). Scale bar = 0.1 mm.

3. Oxytocin receptor antagonism by L-368,899 reduces time spent with a familiar, but not novel, trapped conspecific, in the presence of a palatable food alternative

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3.1. Materials and Methods

3.1.1. Animals

Male on a C57BL/CJ background (AgResearch, Hamilton, New Zealand) were housed in individual cages in a temperature- (23^{o}C) and humidity-controlled room, with a 12h-light:12h-dark cycle, with lights on from 0700 to 1900 h. The animals weighed on average 31.5 g (± 0.5 g) Mice were provided with *ad libitum* access to standard rodent chow (Teklad) and tap water until the beginning of experimental manipulation, where any changes are specified. The University of Waikato animal ethics committee provided approval for the procedures described in this study.

3.1.2. Experimental arena

The experimental arena consisted of a large, transparent plexiglass container, separated into three equally sized compartments by two plexiglass walls. A small

opening/door in the centre of the plexiglass walls allows movement of mice between the compartments. In order to ensure that mice cannot see outside of the arena, white paper was used to externally cover the sides and the bottom of the container.

In the both left and right compartments, a small cylindrical restrainer was placed in one corner. These cylindrical restrainers could either be secured with a top piece to ‘lock’ or ‘secure’ the restrainer, or a drinking bottle could be placed on top of the prison top-less prison. An example of the arena set-up is in Figure 3.1.

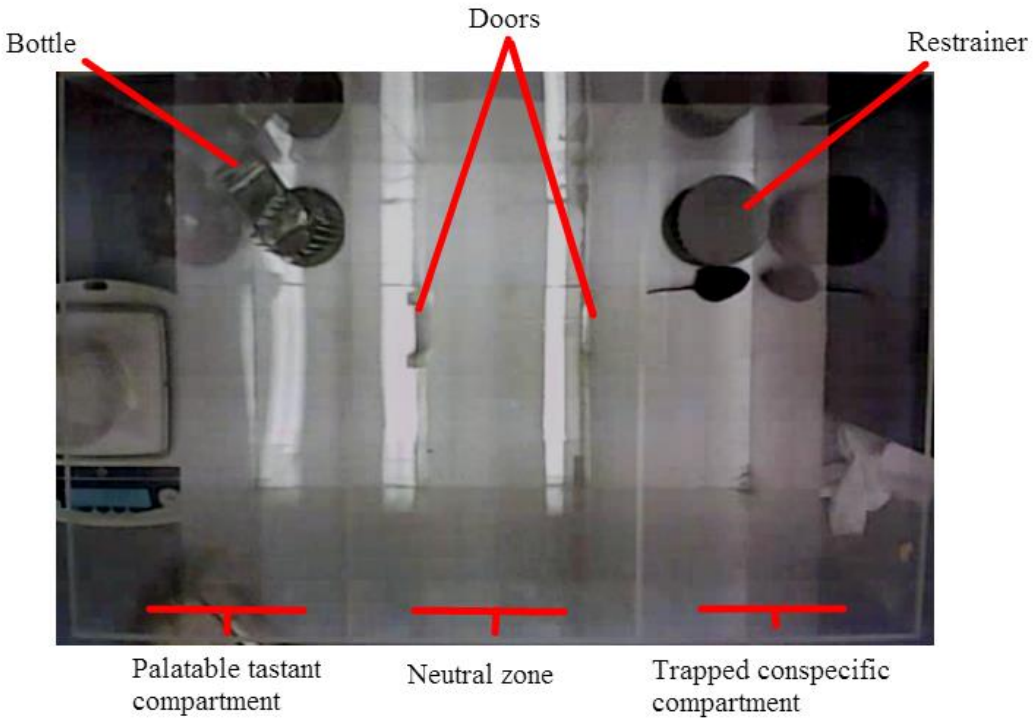

Fig 3.1. Experimental set-up of the arena for the trapped conspecific compartment in a trapped conspecific vs palatable tastant paradigm. Consists of three discrete plexiglass compartments, connected by small openings/doors. An intermediate neutral compartment connects the left and the right compartment, one containing a bottle of 10% sucrose

secured onto the restrainer, the other containing a conspecific trapped in a restrainer. The perimeter walls are covered with paper to obscure vision out of the arena.

3.1.3. Experiment 3: Effect of OT-R antagonism on time spent with a trapped familiar mouse versus a palatable tastant

Mice were pair housed in ten diyads, with each mouse labelled at the base of the tail to designate the “free mouse” and the “trapped mouse”. Prior to the experiment, the free mouse from each diyad was placed in the experimental arena with one compartment set-up with a drink bottle filled with 10% sucrose solution and the other compartment set-up with an empty, locked restrainer. The free mouse was given 10 minutes each day around 1000 h to familiarise themselves with the set-up and the availability of the sucrose solution.

On the night preceding each actual experimental day, the mice were deprived overnight of access to food at 1730 h which was returned the each following morning after each testing session.

The experiment consisted of five consecutive days of testing, beginning at 1000 h. At the beginning of each test session, the free mouse received a single ip injection of either saline, or 0.3, 1 or 3 mg/kg body weight of L-368,899; each free mouse receiving a single dose of all five treatments by the end of the five days. The order of treatments was randomly generated.

Ten minutes after receiving the allocated injection for the day, the mouse was placed in the centre compartment of the arena. Inside the arena, one side compartment contained a bottle of 10% sucrose solution placed on top of an empty restrainer, while the other side compartment housed the restrainer which contained the free mouse's diyad cagemate – the designated "trapped mouse". The free mouse was left in the test arena for 10 minutes and its movements recorded on video by an overhead camera. These recordings were later scored to measure the time the free mouse spent in each compartment. Consumption of the 10% sucrose solution was also measured after each session.

One-way ANOVA followed by Tukey's post-hoc test was used to establish whether L-368,899 at doses ranging from 0 to 3 mg/kg affected the time spent in the each compartment. *P* values <0.05 were considered significantly different.

3.1.4. Experiment 4: Effect of OT-R antagonism on time spent with a trapped novel mouse versus a palatable tastant

The experimental protocol from Experiment 3 was applied. However, during each test session, the restrainer contained a trapped novel (unfamiliar) mouse from a different diyad, distinct from the diyad the free mouse belonged to. During each test session, a different unfamiliar mouse was introduced into the restrainer to ensure that the trapped unfamiliar mouse was always completely novel. Data was analysed with one-way ANOVA followed Tukey's post-hoc test ($P < 0.05$ was considered significant).

3.1.5. Experiment 5: Effect of OT-R antagonism on time spent with a palatable tastant versus an empty restrainer

The experimental protocol from Experiment 3 was applied. However, during each test session, both side compartments housed a restrainer each. One compartment contained a bottle of 10% sucrose solution placed on top of an empty restrainer, while the other compartment contained an empty restrainer. Data was analysed with one-way ANOVA followed Tukey's post-hoc test ($P < 0.05$ was considered significant).

3.2. Results

3.2.1. Experiment 3: Effect of OT-R antagonism on time spent with a trapped familiar mouse versus a palatable tastant

In a two-choice paradigm between a compartment containing a trapped familiar conspecific and a compartment containing a palatable 10% sucrose solution, OT-R blockade by injection of L-368,899 at the highest dose of 3 mg/kg resulted in a significant decrease in time spent by the free mouse in the compartment containing a familiar trapped mouse (mean difference = -163 s) and a corresponding significant increase in time spent in the compartment containing a 10% sucrose solution (mean difference = +161 s), compared to saline controls (Figures 3.2 and 3.3).

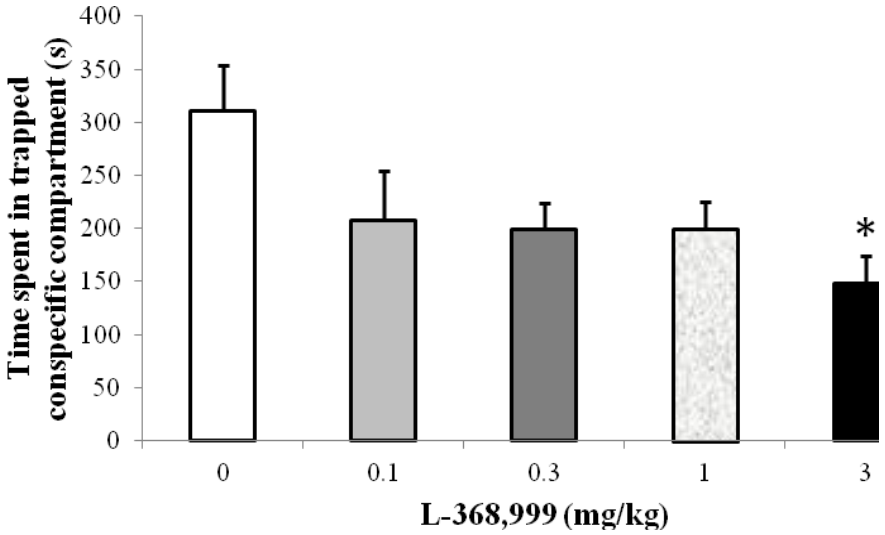

Fig 3.2. Time spent in the trapped familiar conspecific compartment in a trapped conspecific vs palatable tastant paradigm. Mice were injected with either saline (0), or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a trapped familiar conspecific and another compartment with a bottle of 10% sucrose solution for 10 minutes. The time in each compartment was measured. Injection with 3 mg/kg of L-368,899 resulted in a significant decrease ($P < 0.05$) in time spent in trapped familiar conspecific compartment compared to the injection of saline (0 mg/kg L-368-899). Injections of 0.1, 0.3 or 1 mg/kg of L-368,899 had no significant effect. * - significantly different from saline.

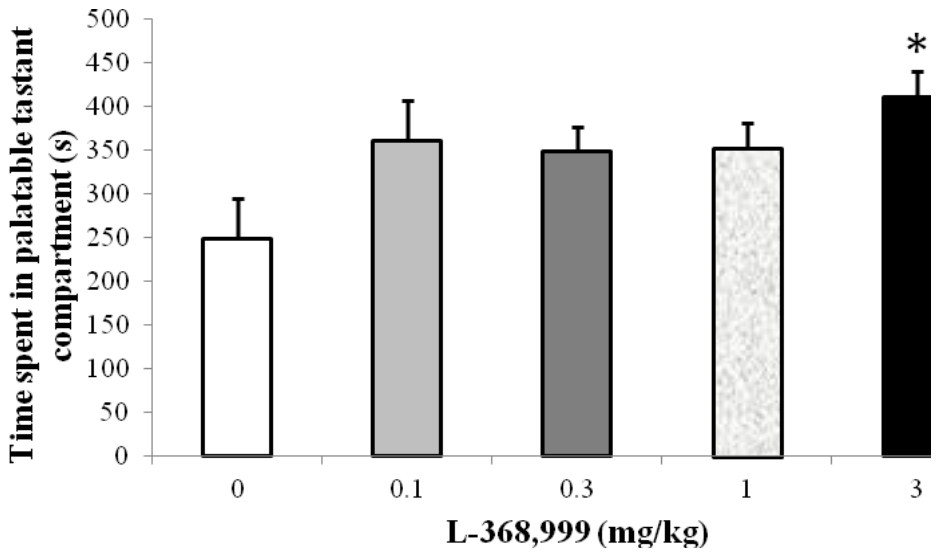

Fig 3.3. Time spent in the palatable tastant compartment in a trapped familiar conspecific vs palatable tastant paradigm. Mice were injected with either saline (0), or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a trapped familiar conspecific and another compartment with a bottle of 10% sucrose solution for 10 minutes. The time in each compartment was measured. Injection with 3 mg/kg of L-368,899 resulted in a significant increase ($P < 0.05$) in time spent in palatable tastant compartment compared to the injection of saline (0 mg/kg L-368-899). Injections of 0.1, 0.3 or 1 mg/kg of L-368,899 had no significant effect. * - significantly different from saline.

The time the free mice spent in the linking neutral compartment was brief compared to the time spent in the other compartments. OT-R blockade by injection of L-368,899 at any of the tested doses had no significant effect at any tested dose on time spent by the free mouse in this neutral compartment (Figure 3.4).

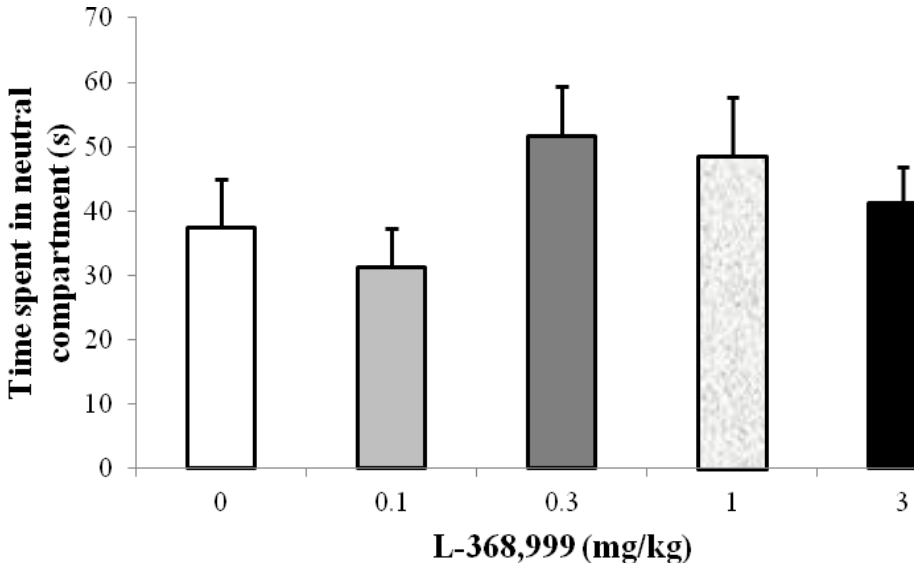

Fig 3.4. Time spent in the linking neutral compartment in a trapped familiar conspecific vs palatable tastant paradigm. Mice were injected with either saline (0), or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a trapped familiar conspecific and another compartment with a bottle of 10% sucrose solution for 10 minutes. Injection of L-368,899 at any dose did not result in any significant effect ($P > 0.05$) on time spent in neutral compartment compared to the injection of saline (0 mg/kg L-368-899) * - significantly different from saline.

OT-R blockade by L-368,899 at any of the tested doses had no significant effect on consumption by body weight of the 10% sucrose solution in this experimental paradigm compared to saline controls (Figure 3.5).

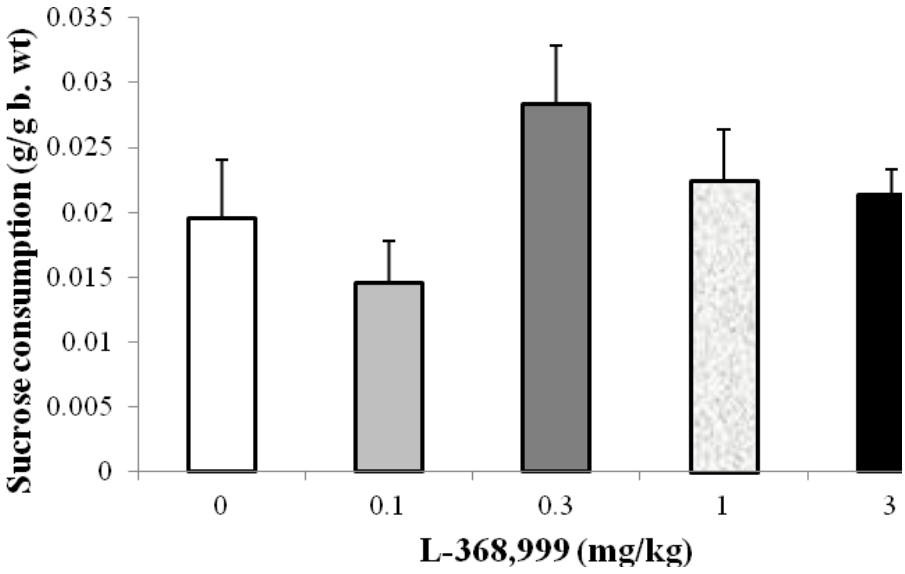

Fig 3.5. Consumption of sucrose by weight in a trapped familiar conspecific vs palatable tastant paradigm. Mice were injected with either saline (0), or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a trapped familiar conspecific and another compartment with a bottle of 10% sucrose solution for 10 minutes. Consumption of the sucrose solution was measured. Injection of L-368,899 at any dose resulted in no significant effect ($P < 0.05$) on sucrose consumption compared to the injection of saline (0 mg/kg L-368-899). * - significantly different from saline.

3.2.2. Experiment 4: Effect of OT-R antagonism on time spent with a trapped novel (unfamiliar) mouse versus a palatable tastant

In a two-choice paradigm between a compartment containing a trapped novel (unfamiliar) conspecific and a compartment containing a palatable 10% sucrose solution, OT-R blockade by injection of L-368,899 had no significant effect at any tested dose on time spent by the free mouse in the compartment containing the

trapped novel mouse or the compartment containing the palatable tastant, compared to saline controls (Figures 3.6 and 3.7).

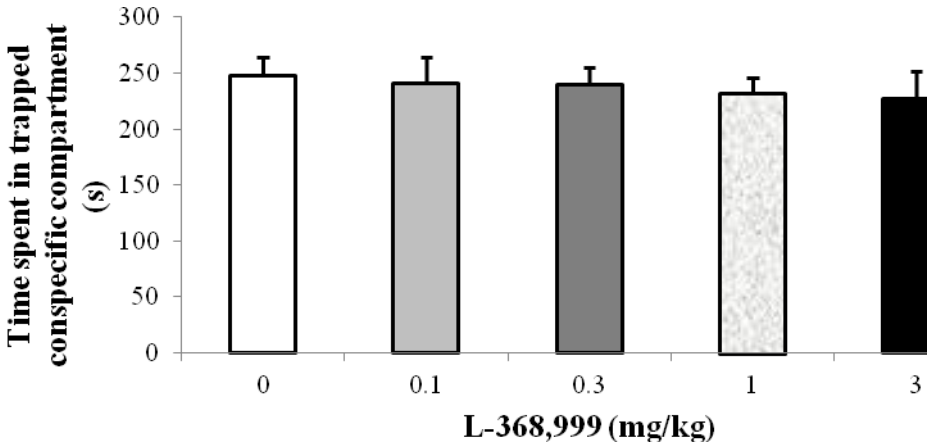

Fig 3.6. Time spent in the trapped novel conspecific compartment in a trapped novel conspecific vs palatable tastant paradigm. Mice were injected with either saline (0), or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a trapped novel conspecific and another compartment with a bottle of 10% sucrose solution for 10 minutes. The time in each compartment was measured. Injection of L-368,899 at any dose did not result in any significant effect ($P > 0.05$) on time spent in the trapped novel conspecific compartment compared to the injection of saline (0 mg/kg L-368-899) * - significantly different from saline.

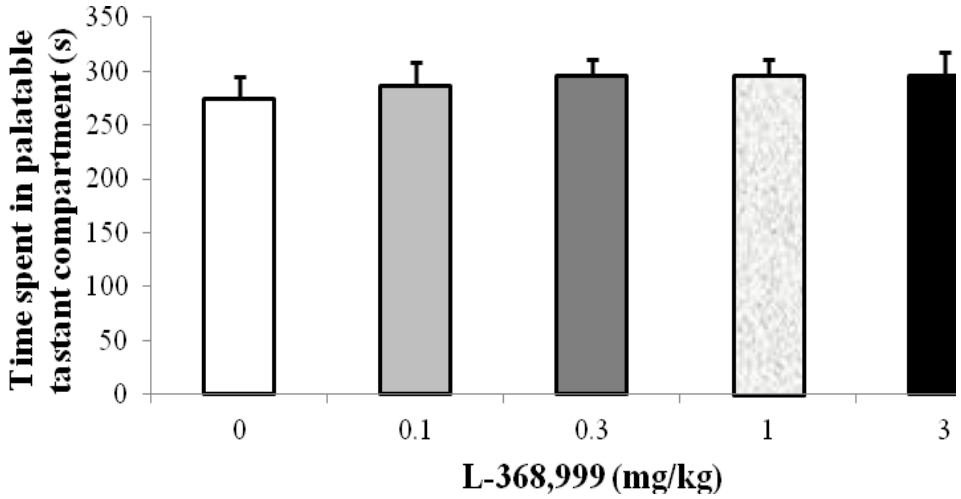

Fig 3.7. Time spent in the palatable tastant compartment in a trapped novel conspecific vs palatable tastant paradigm. Mice were injected with either saline (0), or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a trapped novel conspecific and another compartment with a bottle of 10% sucrose solution for 10 minutes. The time in each compartment was measured. Injection of L-368,899 at any dose did not result in any significant effect ($P > 0.05$) on time spent in the palatable tastant compartment compared to the injection of saline (0 mg/kg L-368-899) * - significantly different from saline.

The time the free mice spent in the neutral compartment was brief. OT-R blockade by injection of L-368,899 at any of the tested doses had no significant effect at any tested dose on time spent by the free mouse in this neutral compartment (Figure 3.8).

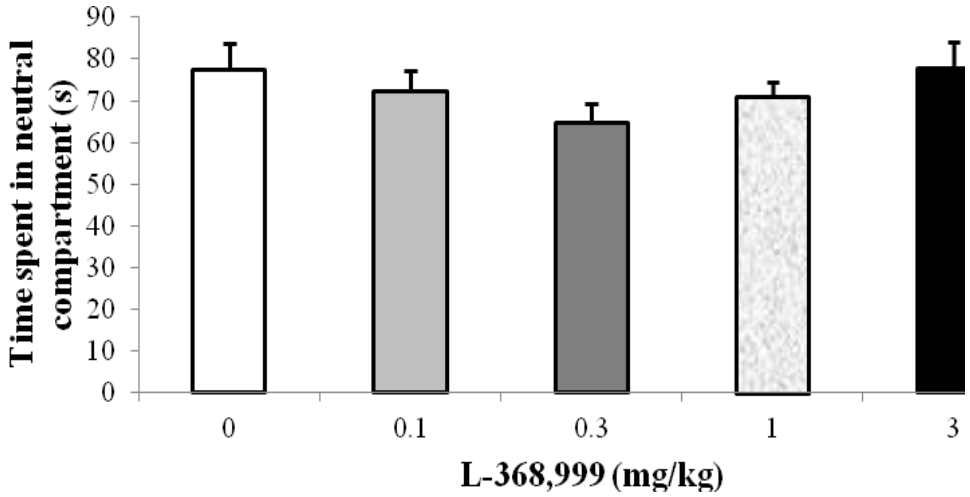

Fig 3.8. Time spent in the linking neutral compartment in a trapped novel conspecific vs palatable tastant paradigm. Mice were injected with either saline (0), or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a trapped novel conspecific and another compartment with a bottle of 10% sucrose solution for 10 minutes. Injection of L-368,899 at any dose did not result in any significant effect ($P > 0.05$) on time spent in neutral compartment compared to the injection of saline (0 mg/kg L-368-899) * - significantly different from saline.

OT-R blockade by L-368,899 at any of tested doses had no significant effect on the consumption by body weight of the 10% sucrose solution (Figure 3.9).

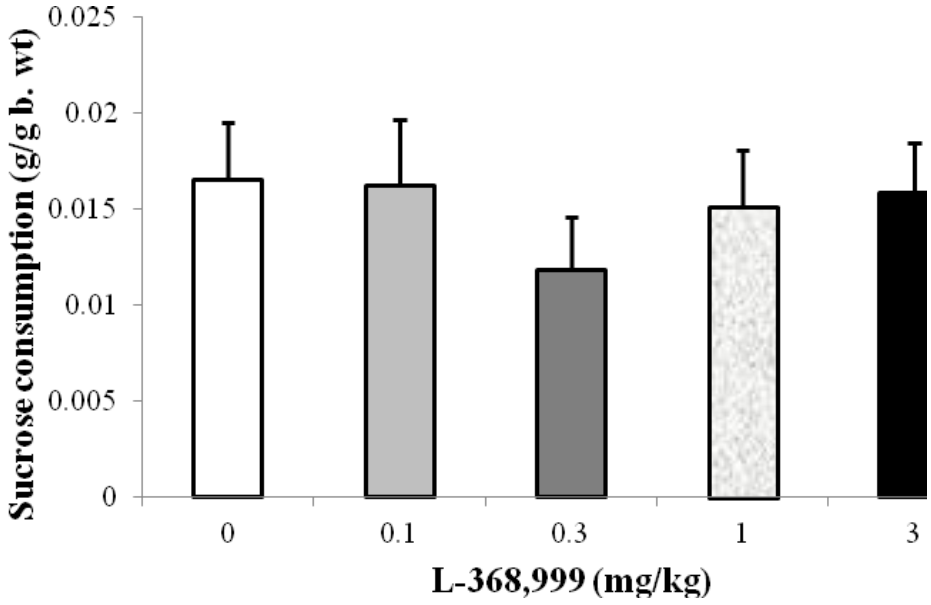

Fig 3.9. Consumption of sucrose by weight in a trapped conspecific vs palatable tastant paradigm. Mice were injected with either saline, or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a trapped unfamiliar conspecific and another compartment with a bottle of 10% sucrose solution for 10 minutes. Consumption of the sucrose solution was measured. Injection of L-368,899 at any dose resulted in no significant effect ($P < 0.05$) on sucrose consumption compared to the injection of saline. * - significantly different from saline.

3.2.3. Experiment 5: Effect of OT-R antagonism on time spent with a palatable tastant versus an empty restrainer

In a two-choice paradigm between a compartment containing a palatable 10% sucrose solution and a compartment containing an empty restrainer, OT-R blockade by L-368,899 at any of the tested doses had no significant effect on the time spent in the compartment containing the palatable tastant or the compartment

containing the empty restrainer compared to saline controls (Figures 3.10 and 3.11).

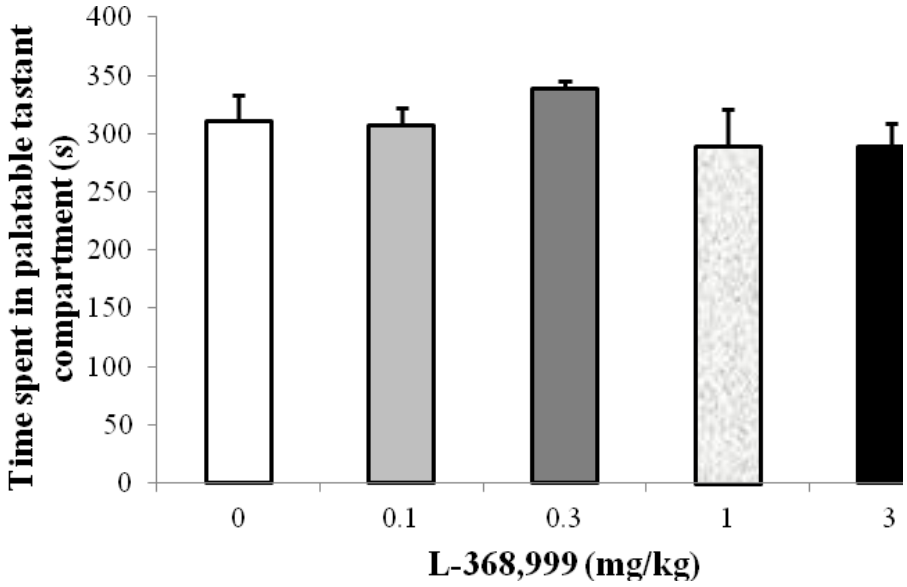

Fig 3.10. Time spent in the palatable tastant compartment in a palatable tastant vs empty restrainer paradigm. Mice were injected with either saline, or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a palatable tastant (bottle of 10% sucrose solution) and another compartment with an empty restrainer for ten minutes. The time in each compartment was measured. Injection of L-368,899 at any dose did not result in any significant effect ($P > 0.05$) on time spent in the palatable tastant compartment compared to the injection of saline * - significantly different from saline.

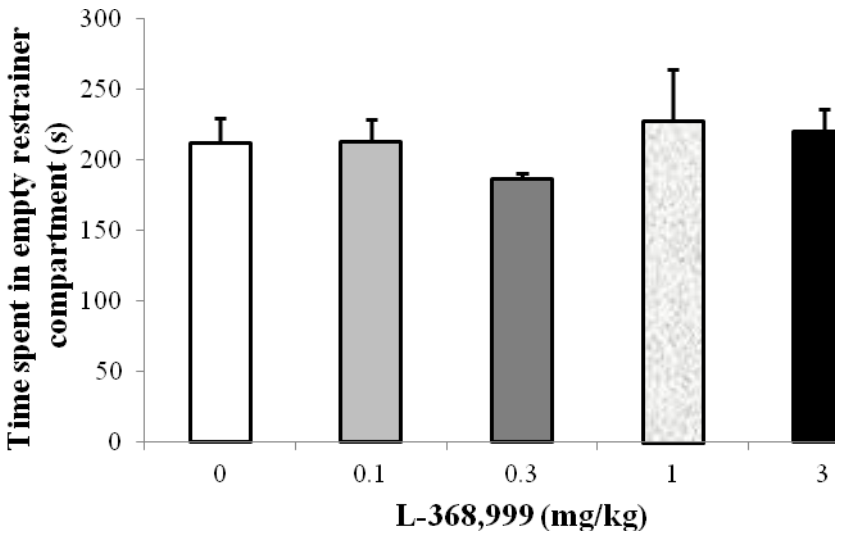

Fig 3.11. Time spent in the empty restrainer compartment in a palatable tastant vs empty restrainer paradigm. Mice were injected with either saline, or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a palatable tastant (10% sucrose solution) and another compartment with an empty restrainer for ten minutes. The time in each compartment was measured. Injection of L-368,899 at any dose did not result in any significant effect ($P > 0.05$) on time spent in the empty restrainer compartment compared to the injection of saline * - significantly different from saline.

The time the free mice spent in the neutral compartment was brief. OT-R blockade by injection of L-368,899 at any of the tested doses had no significant effect at any tested dose on time spent by the free mouse in this neutral compartment (Figure 3.12).

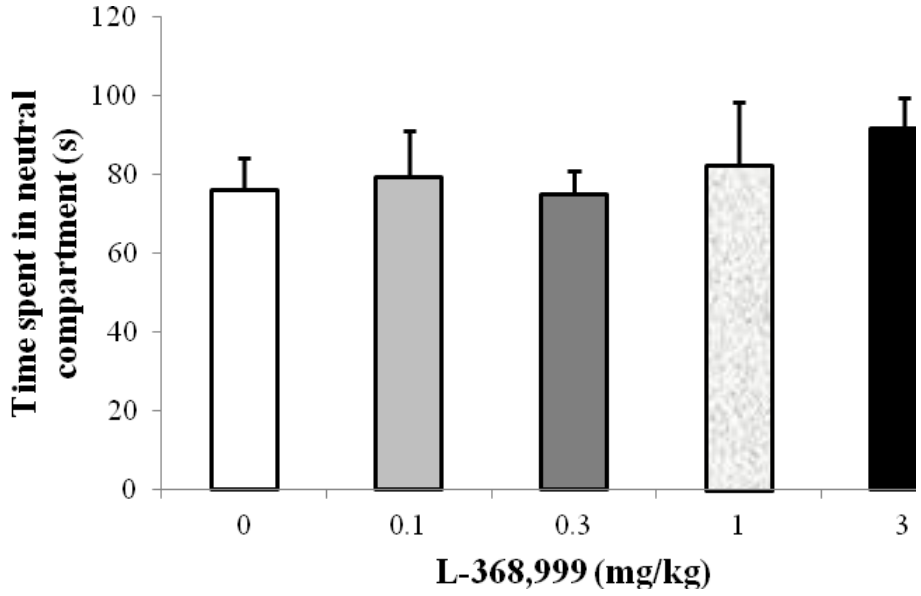

Fig 3.12. Time spent in the linking neutral compartment in a palatable tastant vs empty restrainer paradigm. Mice were injected with either saline (0), or 0.1, 0.3, 1 or 3 mg/kg L-368,899 and placed in an experimental arena with a compartment containing a bottle of 10% sucrose solution and another compartment with an empty restrainer. Injection of L-368,899 at any dose did not result in any significant effect ($P > 0.05$) on time spent in neutral compartment compared to the injection of saline (0 mg/kg L-368-899) * - significantly different from saline.

Discussion and Conclusions

The immunohistochemical data from Experiment 1 shows that neuronal OT circuits respond differently to the familiar versus novel exposure. Mice exposed to a familiar conspecific showed a significant increase in the percentage of activated OT neurons in both the PVN and SON, compared to exposure to a novel, unfamiliar conspecific. As the PVN and SON are both known to be major sites for the secretion of OT, this increased activity of OT neurons may also suggest that familiarity in a social interaction is associated with increased secretion of OT (Calcagnoli et al., 2014). Studies show that capacity for social recognition, i.e. the ability to recognise a familiar mouse, is abolished in OT knockout mice, despite repeated social exposures (Ferguson et al., 2001). OT-R antagonism similarly impairs the duration of memory of a conspecific, which is reversed by icv OT treatment (Lukas & Neumann, 2013). Therefore, the increased activity of OT neurons in response to a familiar, but not novel, conspecific suggests that social recognition of a familiar conspecific involves activation of OT neurons in the PVN and SON.

AVP is also associated with social memory: similarly, icv administration of AVP also prolongs social memory of same-sex juvenile conspecifics in both males and females; antagonism of the AVPR1a diminishes this. As the PVN and SON are also heavily involved in the synthesis of AVP, it is plausible that increased activity of AVP neurons may also be simultaneously involved and differentially activated in familiar versus novel exposures. The social memory effects of AVP

are also believed to be localised to the olfactory bulb and septal regions, as male rats exposed to juvenile male conspecifics in the social discrimination test show elevated endogenous release of AVP in the medio-lateral septum. Furthermore, AVP infusion directly into this region improves social memory and over-expression of AVPR1a prolongs social memory. AVPR1a antagonism in the septum and the total knockout of AVPR1a in mice results in deficient or absent social memory (Lukas & Neumann, 2013). Progesterone treatment, which decreases AVP immunoreactivity in the BST, medial amygdala and lateral septum, can also induce impairment of social recognition, which can be rescued through AVP infusion in the lateral septum in adult male rats (Bychowski, Mena, & Auger, 2013). Our study did not stain for AVP neurons, however, so at this stage this is only conjecture.

Furthermore, immunohistochemical data from Experiment 2 shows that exposure to both a familiar and novel conspecific resulted in a significant increase in the density of activated neurons in the medial anterior amygdala compared a non-social control environment, whereas exposure to a familiar conspecific resulted in a significant increase in the density of activate neurons in the medial posterior amygdala that was not observed in the group exposed to an novel conspecific. It is clear that there is differential activity in the neuronal circuits – in both pattern and magnitude – between exposure to a familiar versus a novel conspecific.

Increased neuronal activity in the medial amygdala is important in the context of OT circuits as OT-R mRNA has been shown to be expressed in the amygdala of the mouse (Takayanagi *et al.*, 2005). Both magnocellular and parvocelluar OT

neurons project from the hypothalamus to the amygdala (Debiec, 2007). In particular, OT-R activation in the medial amygdala has been shown to be both necessary and sufficient for social recognition, as the site-specific administration of OT in the medial amygdala can restore the ability to develop social recognition in OT knockout mice previously incapable of this, while administration of an OT antagonist in this site prevented social recognition, similar to OT knockout mice (Ferguson et al., 2001).

Our study allowed us to further delineate the role of the medial amygdala in the context of familiarity, potentially facilitating the development or expression of social recognition. As exposure to both a familiar and novel conspecific increased neuronal activation in the medial anterior amygdala, this site may be important for both the development of new and recollection of existing social recognition of a conspecific. However, the finding that activation of the medial anterior amygdala is significantly greater in exposures to a familiar conspecific compared to a novel conspecific suggests that activity in this area is more strongly associated with facilitating existing social recognition than developing it in a naïve animal. In the medial posterior amygdala, only exposure to the familiar conspecific resulted in increased neuronal activation; activity following exposure to a novel conspecific was no different than an entirely non-social environment. Based on this, the medial posterior amygdala may be more exclusively involved in the recollection, rather than acquisition, of social recognition.

Another potential mechanism behind the different patterns in amygdala activation between exposures is the role of OT in the amygdala in integrating chemosensory

signals, such as olfactory information, which are essential for the recognition of a familiar conspecific and, conversely, the avoidance of an unfamiliar conspecific. OT-R antagonists can abolish the gene expression in the medial amygdala that normally occurs in response to relevant chemical signals from a conspecific or heterospecific, suggesting OT receptor activity in the medial amygdala is important to process such chemosensory information (Samuelsen & Meredith, 2011). Therefore, as mice encounter a familiar conspecific, activation of the medial amygdala (both anterior and posterior) occurs as they begin to process the relevant chemosensory stimuli that enable them to socially recognise this conspecific it has encountered before. To a lesser extent, we see increased activation in the medial anterior amygdala in response to a novel conspecific, which may indicate the initial processing of this novel animal's chemosensory cues that will allow for future recognition of this animal.

Based on Experiment 1 and 2, which showed that OT neurons and pathways known to contain the OT-R responded differently to the familiar versus novel conspecific exposure, three behavioural pharmacology studies were conducted in order to substantiate the claim that a relationship exists between exposure to conspecifics that different in familiarity and OT.

This effect was tested in a scenario where mice were given the choice to spend time in a compartment in which a familiar conspecific was trapped, or in a compartment where a familiar, palatable tastant was present. Our study demonstrated that in this two choice paradigm, OT-R antagonism with 3 mg/kg L-

368,899 reduced preference for time spent with the trapped familiar conspecific and increased preference for time spent with the palatable tastant.

In contrast, when given the choice to spend time with a trapped novel conspecific versus a familiar, palatable tastant, OT-R antagonism at any of the tested doses did not affect the time spent with the novel conspecific. That OT-R antagonism exerts behavioural effects in response to a familiar but not novel conspecific is consistent with our immunohistochemical evidence that OT-related neurobiological circuits respond differently in identical social scenarios that differ only in regards to the familiarity of the conspecific. For example, given that OT neuronal activity (and potentially OT synthesis) is significantly greater during exposures to a familiar conspecific, that OT-R blockade by an OT antagonist then results in differences in time spent with that familiar, but not novel, conspecific, provides further behavioural that OT circuits are activated differently depending on the social context.

A number of mechanisms may underlie this. As stated previously, OT plays an important role in social recognition and abolishing or attenuating via OT-R blockade may impair the ability to recognise a familiar mouse or respond to its chemosensory cues. This may manifest as a reduction in time shared together as the OT-R antagonised mouse perceives this conspecific as novel and displays more social avoidance. This may also explain why OT-R blockade has no effect on exposure to novel mice where there is no existing memory of this target's chemosensory cues to recall at this initial exposure.

An alternative explanation involves taking into account the fact that the other conspecific – whether familiar or novel – is trapped in a restrainer. The use of a trapped conspecific has been used in a number of studies investigating pro-social behaviour in rodents, particularly in experiments where free rats act to release a rat trapped in a cylindrical restrainer. It is believed that trapped rats experience distress, which motivates this liberating behaviour in other free rats. Increased ultrasonic vocalisations measured in these experiments supports that rats in these experiments are experiencing distress, although whether these distress vocalisations were originating from the trapped or free rats, or both, could not be demarcated. Furthermore, in other experimental paradigms where animals are not distressed, this liberating behaviour has been shown to not occur. Rats helped soaked conspecifics by quickly learning to open a door to allow them into a safe area, whereas rats did not open the door to help when the conspecific was dry and assumed not to be in distress (Sato et al., 2015). Therefore, rats trapped in restrainers would not be liberated by free rats unless they were in a state of distress that is readily recognisable by conspecifics.

Pro-social liberation of a conspecific trapped in some sort of restrainer, as seen in rats through learned door opening, has not yet been tested in mice. However, the capacity for emotional contagion has been demonstrated in mice, as mouse display shared experiences of distress when observing conspecific's receiving electric shocks, including freezing behaviour and the potentiation of their own experience of pain (Gonzalez-Liencres et al., 2014; Langford et al., 2006). While the mice tested in this study cannot free the trapped mouse from the restrainer, the time they spend in the compartment with the trapped animal may be interpreted as

indicative of a shared experience of distress – or emotional contagion – and/or an empathic-like desire to provide company and social interaction for the distressed mouse – potentially a rudimentary form of pro-social behaviour.

This is particularly relevant in terms of conspecific familiarity because evidence shows that emotional contagion, pro-social behaviour and empathy are also socially modulated. Empathic behaviour is generally favourably provided to familiar conspecifics, presumably as animals prefer to bestow evolutionary or adaptive advantages to kin or members of the same group (Porter et al., 2014). Manifestations of emotional contagion have been shown to occur in response to observations of familiar cagemates and not unfamiliar or stranger conspecifics (Gonzalez-Liencres et al., 2014). Familiarity influences whether rats provide helping behaviour to other trapped rats, although it has been suggested that it is familiarity with merely the strain of the trapped rat, as opposed to direct familiarity with the individual rat itself, which determines whether this help takes place (Bartal et al., 2014). OT signalling has been implicated in the regulation of human empathy: intranasal administration of OT increases scores in both self-reported and objective measures of empathy and reduces interpersonal distance in interactions (Feeser et al., 2015; Perry et al., 2014). Noting that 1) empathic-like behaviours are generally extended towards familiar members of the same group and 2) these empathic-like behaviours are likely mediated and/or regulated in part by OT, along with the possible interpretation that 3) time spent with a trapped conspecific may be a manifestation of pro-social or empathic-like behaviour, it is conceivable that OT-R blockade may disrupt these behaviours, resulting in less

time spent with the trapped conspecific, and that these effects would be most evident with a familiar conspecific.

As changes in time spent with the conspecific results in inverse changes to the time spent with palatable tastant, it is also plausible that OT-R blockade may instead be acting on mechanisms relating to intake of this palatable tastant, as opposed to the social dimension. OT is known to be anorexigenic, mice unable to produce OT or lacking the OT receptor show an obesity phenotype, and antagonism of the OT-R results in hyperphagia (Sabatier et al., 2013). In our study, it could potentially be these effects on food intake that mediate the increased time spent in the sucrose containing compartment, or even an overlapping, synergistic effect promoting preference for food and reducing sociality and empathy. However, there is evidence that this may not be the case. Firstly, while OT-R antagonism at 3 mg/kg did increase preference for time spent in the palatable tastant compartment when the alternative choice was a familiar conspecific, it had no effect on the total consumption (by body weight) of the sucrose solution. Secondly, if the effects of OT-R antagonism on time spent in each compartment were driven primarily by an effect on food intake, it would be expected that these results would be seen regardless of whether the trapped conspecific was familiar or unfamiliar to the free animal. Yet in the condition where the trapped conspecific was novel, OT-R antagonism did not significantly alter the time spent in either compartment at any dose. Finally, the control scenario, Experiment 5, showed that when mice were given the choice between a palatable tastant and an empty cage, administration of the OT-R antagonist had no significant effect on the time spent in the tastant compartment. This demonstrated

that OT-receptor blockade with the OT-receptor antagonist does not affect the time spent with the familiar palatable tastant when this tastant is presented in the apparatus, devoid of social cues.

Providing the alternative option for a palatable tastant also provides some indication of the relative value that free mice place on spending time with the trapped, familiar conspecific, as this choice requires sacrificing one's own immediate access to a valuable and rewarding resource. Similar conclusions have been drawn from experiments where rats are given the option of two restrainers containing either a trapped conspecific or chocolate chips (Bartal et al., 2011). The absence of difference between the order the restrainers were opened or latency to opening suggested that both were of equivalent value to the free rat. As mice spent significantly more time in the compartment with the trapped familiar conspecific than in the 10% sucrose compartment when injected with saline controls, it appears that this social interaction with the restrained cagemate may be equivalent or potentially more valued than a palatable food reward. The effect of OT-R blockade to reduce time spent with the trapped conspecific may reflect attenuation of the perceived value of this interaction, relative to a palatable, rewarding tastant.

Unfortunately, these behavioural studies are limited by what can be inferred from the 'time spent with a trapped conspecific' when considering pro-social or empathic behaviour. It is assumed that the trapped mouse is in distress, and that the choice to spend time with this trapped mouse over a rewarding tastant may reflect emotional contagion, pro-sociality and personal value in providing this

company. Unlike unequivocal rescue paradigms such as actually acting to liberate a trapped mouse, this time shared in the same compartment is not as concrete a demonstration of pro-social behaviour, let alone empathy. It does provide a foundation for future studies where OT-R antagonism may be used in pro-social helping behavioural paradigms, and it will be of interest to examine how OT-R antagonism or administration of OT itself modulates rescue behaviour.

In conclusion, differences in neuronal activity in OT circuits are associated with exposure to a familiar versus unfamiliar conspecific. There is greater activation of OT neurons in the PVN and SON in response to exposure to a familiar conspecific, which was not found to occur in response to exposure to a novel or unfamiliar conspecific. Furthermore, there is increased general neuronal activation in the medial posterior amygdala in response to a familiar, but not novel, conspecific. There is increased general neuronal activation in the medial anterior amygdala to exposure to both familiar and novel conspecifics, but the magnitude of this activation is significantly greater in response to the familiar conspecific. This differential response in OT circuits depending on familiarity is substantiated in the subsequent behavioural pharmacology studies, which showed that antagonism of the OT-R with 3 mg/kg L-368,899 reduced time spent with a familiar conspecific, but did not exert the same effect on a novel conspecific.

References

Bartal, I. B.-A., Decety, J., & Mason, P. (2011). Helping a cagemate in need: empathy and pro-social behavior in rats. *Science*, *334*(6061), 1427–1430. doi:10.1126/science.1210789.Helping

Bartal, I. B-A., Rodgers, D. a, Bernardez Sarria, M. S., Decety, J., & Mason, P. (2014). Pro-social behavior in rats is modulated by social experience. *eLife*, *3*, e01385. doi:10.7554/eLife.01385

Blevins, J. E., Eakin, T. J., Murphy, J. a, Schwartz, M. W., & Baskin, D. G. (2003). Oxytocin innervation of caudal brainstem nuclei activated by cholecystokinin. *Brain Research*, *993*(1-2), 30–41. doi:10.1016/j.brainres.2003.08.036

Bosch, O. J., & Neumann, I. D. (2012). Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: From central release to sites of action. *Hormones and Behavior*, *61*(3), 293–303. doi:10.1016/j.yhbeh.2011.11.002

Bychowski, M. E., Mena, J. D., & Auger, C. J. (2013). Vasopressin infusion into the lateral septum of adult male rats rescues progesterone induced impairment in social recognition. *Neuroscience*, *29*, 52–58. doi:10.1016/j.biotechadv.2011.08.021.Secreted

Calcagnoli, F., Meyer, N., De Boer, S. F., Althaus, M., & Koolhaas, J. M. (2014). Chronic enhancement of brain oxytocin levels causes enduring anti-aggressive and pro-social explorative behavioral effects in male rats. *Hormones and Behavior*, *65*(4), 427–433. doi:10.1016/j.yhbeh.2014.03.008

Camerino, C. (2009). Low sympathetic tone and obese phenotype in oxytocin-deficient mice. *Obesity (Silver Spring, Md.)*, *17*(5), 980–4. doi:10.1038/oby.2009.12

Cho, M. M., DeVries, a C., Williams, J. R., & Carter, C. S. (1999). The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (Microtus ochrogaster). *Behavioral Neuroscience*, *113*(5), 1071–1079. doi:10.1037/0735-7044.113.5.1071

Church, R. M. (1959). Emotional reactions of rats to the pain of others. *Journal of Comparative and Physiological Psychology*, *52*(2), 132–134. doi:10.1037/h0043531

Crawley, J. N., Chen, T., Puri, A., Washburn, R., Sullivan, T. L., Hill, J. M., … Young, W. S. (2007). Social approach behaviors in oxytocin knockout mice: Comparison of two independent lines tested in different laboratory environments. *Neuropeptides*, *41*(3), 145–163. doi:10.1016/j.npep.2007.02.002

Cronin, K. a. (2012). Prosocial behaviour in animals: The influence of social relationships, communication and rewards. *Animal Behaviour*, *84*(5), 1085–1093. doi:10.1016/j.anbehav.2012.08.009

Debiec, J. (2007). From affiliative behaviors to romantic feelings: A role of nanopeptides. *FEBS Letters*, *581*(14), 2580–2586. doi:10.1016/j.febslet.2007.03.095

Feeser, M., Fan, Y., Weigand, A., Hahn, A., Gärtner, M., Böker, H., … Bajbouj, M. (2015). Oxytocin improves mentalizing — Pronounced effects for individuals with attenuated ability to empathize. *Psychoneuroendocrinology*, *53*, 223–232. doi:10.1016/j.psyneuen.2014.12.015

Ferguson, J. N., Aldag, J. M., Insel, T. R., & Young, L. J. (2001). Oxytocin in the medial amygdala is essential for social recognition in the mouse. *The Journal of Neuroscience*, *21*(20), 8278-85.

Gimpl, G., & Fahrenholz, F. (2001). The oxytocin receptor system: structure, function, and regulation. *Physiological Reviews*, *81*(2), 629–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11274341

Gonzalez-Liencres, C., Juckel, G., Tas, C., Friebe, A., & Brüne, M. (2014). Emotional contagion in mice: The role of familiarity. *Behavioural Brain Research*, *263*, 16–21. doi:10.1016/j.bbr.2014.01.020

Hernandez-Lallement, J., van Wingerden, M., Marx, C., Srejic, M., & Kalenscher, T. (2015). Rats prefer mutual rewards in a prosocial choice task. *Frontiers in Neuroscience*, *8*(January), 1–9. doi:10.3389/fnins.2014.00443

Langford, D. J., Crager, S. E., Shehzad, Z., Smith, S. B., Sotocinal, S. G., Levenstadt, J. S., … Mogil, J. S. (2006). Social modulation of pain as

evidence for empathy in mice. *Science (New York, N.Y.)*, *312*(5782), 1967–1970. doi:10.1126/science.1128322

Laursen, H. R., Siebner, H. R., Haren, T., Madsen, K., & Grønlund, R. (2014). Variation in the oxytocin receptor gene is associated with behavioral and neural correlates of empathic accuracy, *8*(December), 1–10. doi:10.3389/fnbeh.2014.00423

Lee, H.-J., Macbeth, A. H., Pagani, J., & Young, W. S. (2010). Oxytocin : the Great Facilitator of Life. *Progress in Neurobiology*, *88*(2), 127–151. doi:10.1016/j.pneurobio.2009.04.001.Oxytocin

Lukas, M., & Neumann, I. D. (2013). Oxytocin and vasopressin in rodent behaviors related to social dysfunctions in autism spectrum disorders. *Behavioural Brain Research*, *251*, 85–94. doi:10.1016/j.bbr.2012.08.011

Lukas, M., Toth, I., Reber, S. O., Slattery, D. a, Veenema, A. H., & Neumann, I. D. (2011). The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *36*(11), 2159–68. doi:10.1038/npp.2011.95

Lukas, M., Toth, I., Veenema, A. H., & Neumann, I. D. (2013). Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: Male juvenile versus female adult conspecifics. *Psychoneuroendocrinology*, *38*(6), 916–926. doi:10.1016/j.psyneuen.2012.09.018

Mogil, J. S. (2012). The surprising empathic abilities of rodents. *Trends in Cognitive Sciences*, *16*(3), 143–4. doi:10.1016/j.tics.2011.12.012

Morton, G. J., Thatcher, B. S., Reidelberger, R. D., Ogimoto, K., Wolden-Hanson, T., Baskin, D. G., … Blevins, J. E. (2012). Peripheral oxytocin suppresses food intake and causes weight loss in diet-induced obese rats. *American Journal of Physiology. Endocrinology and Metabolism*, *302*(1), E134–44. doi:10.1152/ajpendo.00296.2011

Nakahashi, W., & Ohtsuki, H. (2015). When is emotional contagion adaptive? *Journal of Theoretical Biology*, 1–9. doi:10.1016/j.jtbi.2015.06.014

Olszewski, P. K., Klockars, A., Olszewska, A. M., Fredriksson, R., Schiöth, H. B., & Levine, A. S. (2010). Molecular, immunohistochemical, and pharmacological evidence of oxytocin's role as inhibitor of carbohydrate but not fat intake. *Endocrinology*, *151*(10), 4736–44. doi:10.1210/en.2010-0151

Panksepp, J. B., & Lahvis, G. P. (2011). Rodent empathy and affective neuroscience. *Neuroscience and Biobehavioral Reviews*, *35*(9), 1864–1875. doi:10.1016/j.neubiorev.2011.05.013

Perry, A., Mankuta, D., & Shamay-Tsoory, S. G. (2014). OT promotes closer interpersonal distance among highly empathic individuals. *Social Cognitive and Affective Neuroscience*, *9*(3). doi:10.1093/scan/nsu017

Porter, R. H., Moore, J. D., & White, D. M. (2014). Food Sharing by Sibling vs Nonsibling Spiny Mice (Acomys cahirinus), *8*(3), 207–212.

Sabatier, N., Leng, G., & Menzies, J. (2013). Oxytocin, feeding, and satiety. *Frontiers in Endocrinology*, *4*(March), 35. doi:10.3389/fendo.2013.00035

Samuelsen, C. L., & Meredith, M. (2011). Oxytocin antagonist disrupts male mouse medial amygdala response to chemical communication signals. Neuroscience, 180, 96-104.

Sato, N., Tan, L., Tate, K., & Okada, M. (2015). Rats demonstrate helping behavior toward a soaked conspecific. *Animal Cognition*. doi:10.1007/s10071-015-0872-2

Shamay-Tsoory, S. G., Aharon-Peretz, J., & Perry, D. (2009). Two systems for empathy: A double dissociation between emotional and cognitive empathy in inferior frontal gyrus versus ventromedial prefrontal lesions. *Brain*, *132*(3), 617–627. doi:10.1093/brain/awn279

Shirtcliff, E. a, Vitacco, M. J., Graf, A. R., Gostisha, A. J., Merz, J. L., & Zahn-waxler, C. (2009). NIH Public Access, *27*(2). doi:10.1002/bsl.862.Neurobiology

Smith, K. E., Porges, E. C., Norman, G. J., Connelly, J. J., & Decety, J. (2015). Oxytocin receptor gene variation predicts empathic concern and autonomic arousal while perceiving harm to others. *Social Neuroscience*, *9*(1), 1–9. doi:10.1080/17470919.2013.863223.Oxytocin

Stevens, J. R., & Gilby, I. C. (2004). A conceptual framework for nonkin food sharing: timing and currency of benefits. *Animal Behaviour*, *67*(4), 603–614. doi:10.1016/j.anbehav.2003.04.012

Takayanagi, Y., Yoshida, M., Bielsky, I. F., Ross, H.E., Kawamata, M., Onaka, T., Yanagisawa, T., Kimura, T. … Nishimori, K. (2005). Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(44), 16096-16101.

Uzefovsky, F., Shalev, I., Israel, S., Edelman, S., Raz, Y., Mankuta, D., … Ebstein, R. P. (2015). Oxytocin receptor and vasopressin receptor 1a genes are respectively associated with emotional and cognitive empathy. *Hormones and Behavior*, *67*, 60–65. doi:10.1016/j.yhbeh.2014.11.007

Vasconcelos, M., Hollis, K., Nowbahari, E., & Kacelnik, a. (2012). Pro-sociality without empathy. *Biology Letters*, (July), 910–912. doi:10.1098/rsbl.2012.0554

Wilkinson, G. (1984). Reciprocal food sharing in the vampire bat. *Nature*. Retrieved from http://www.life.umd.edu/faculty/wilkinson/Wilkinson84.pdf

Wolff, J., & Jr, W. L. (1981). Communal winter nesting and food sharing in taiga voles. *Behavioral Ecology and Sociobiology*, *9*(4), 237–240. Retrieved from http://link.springer.com/article/10.1007/BF00299877

Young, K., Gobrogge, K., Liu, Y., & Wang, Z. (2011). *The Neurobiology of pair-bonding: insight from a socially monogamus rodent*. *Frontiers in neuroendocrinology* (Vol. 32, pp. 53–69). doi:10.1016/j.yfrne.2010.07.006.THE

Young, L. J. (2002). The neurobiology of social recognition, approach, and avoidance. *Biological Psychiatry*, *51*(1), 18–26. doi:10.1016/S0006-3223(01)01268-9