The role of stressors and oxytocin upon vocalizations and social behaviour in *Octodon degus*

by

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A thesis submitted in conformity with the requirements
for the degree Master of Arts

Department of Psychology
University of Toronto

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Abstract

Stressors such as social deprivation and painful stimuli are salient motivating factors for social interaction. It remains unknown, however, whether social behaviours elicited by different stressors such as social separation and aversive events can be dissociated at the behavioural and vocalization level in rodents. To address this, I used the rodent *Octodon degus*, which demonstrate a complex repertoire of vocalizations and social behaviour. I examined vocalization and grooming behaviour of pairs of adult female cage-mates when re-united after two different types of stressful events. I also investigated the effect of blockade of central oxytocin receptors on the vocalization and behaviour of degus after social separation. Degus increased the frequency of vocalizations and all social grooming behaviours after social separation while they only increased certain types of social grooming behaviour after footshock compared to controls. Furthermore, blockade of oxytocin receptors attenuated vocalizations, but not grooming, during reunion after social isolation.

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1 Introduction

Multiple factors influence socially motivated behaviour across group-living species. In particular, social isolation has been shown to elicit increases in grooming and social contact upon reunion with conspecifics (Beery and Kaufer, 2015; Panksepp et al., 1997). This behaviour is particularly evident in highly social species such as Tonkean macaques, which express increased embracing, grasping and "aroused greeting" vocalizations upon reunion with conspecifics after 2 hours and 48 hour social isolation (De Marco et al., 2011). These behavioural and vocal expressions are thought to be rewarding social interactions which function to bolster social cohesion and relationships within the group after separation. Another factor that motivates social behaviour are distressing experiences such as the presence of a predator, fighting or physical pain. After such an aversive experience, humans and non-human primates seek out physical touch from trusted others, which reduces activation of stress circuits in the brain (Dunbar, 2010; Feldman et al., 2010). For example, after a distressing experience such as fighting, female Tonkean macaque bystanders will embrace the victim and provide affiliative contact (Palagi et al., 2014). Furthermore, across several species including prairie voles, physical touch and affiliative contact provided by a conspecific results in reduced cortisol levels (Smith and Wang, 2014). Hence, distressing experiences can also act as strong motivating factors for animals to seek out social interaction.

Social buffering and social reward can be described as two distinct socio-cognitive motivation processes. In humans and non-human primates, these processes are dissociable as distinct changes in vocal social communication in addition to affiliative contact (De Marco et al.,

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2011). However, in standard laboratory rodent models, vocal communication is not assessed in the context of peer-to-peer social buffering or rewarding social interactions. This channel of social interaction has been understudied in standard rodent models of social behaviour due to the limited repertoire of vocalizations in these animals (Portfors, 2007). This highlights the need to develop a new rodent model that exhibits both affiliative contact and affective vocalizations. Such models open up a possibility to conduct detailed characterizations of neuronal mechanisms by applying established monitoring and manipulation tools. To this end, the present study used a social and vocal rodent species to investigate how different stressors affect socially motivated behavior. In this review I will discuss literature on the behavioural and neural mechanisms underlying 1) social buffering of stress 2) socially rewarding interactions and the possible role of oxytocin in each of these behaviours. In addition, the use of a social and vocal rodent species to understand how different stressors affect socially motivated behaviour will be discussed.

1.1 Social Buffering of Stress

1.1.1 Behavioural evidence

In humans, evidence suggests that verbal and non-verbal social interactions can ameliorate stress responses (Ditzen et al., 2007). In particular, social interaction in the form of physical touch has been shown to reduce blood cortisol levels after a stressful experience (Ditzen et al., 2007; Light et al., 2005). In animal studies, physical touch is studied as allogrooming (allo=other) between individuals. Allogrooming behaviour has been documented in insects, birds and mammals (Clutton-Brock, 2016; Radford, 2012; Zhukovskaya et al., 2013). While many animals often groom one another to remove parasites, dirt and debris from skin, fur or feathers, allogrooming has also been implicated in the maintenance of social bonds and the reduction of stress (Smith and Wang, 2014; Wittig et al., 2008). Evidence from behaviour in chimpanzees, suggest that allogrooming reduces self-grooming behaviour which is an indicator of stress. After

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a conflict, chimpanzees who received the least amount of allogrooming from peers showed increased self-grooming behaviour (Fraser et al., 2008). However, those chimpanzees that received higher levels of allogrooming showed a significant reduction in self-grooming after a stressful conflict, indicating a potential stress reducing function of allogrooming in this primate species. Furthermore, many rodent studies have indicated that the presence of a familiar conspecific after a stressful experience drastically reduces blood cortisol levels (Armario et al., 1983) and lateral amygdala activation (Kiyokawa et al., 2014). In addition, after chronic isolation reunion in sibling mice has been shown to increase pain thresholds (D'Amato and Pavone, 1996). However, allogrooming has not been documented as having a stress reducing function in standard lab rats and mice. The behavioural reduction of another's stress through allogrooming has been only documented in animals with close long lasting bonds with peers and mates (Sullivan and Perry, 2015). For example, a recent study in prairie voles- a highly social and monogamous rodent, revealed consolation behaviour towards conspecifics. In this study, naive voles were reunited with stressed conspecifics and displayed increased licking and grooming behaviours towards the distressed vole. Furthermore, oxytocin receptors in the anterior cingulate cortex were found to modulate the targeted grooming response (Burkett et al., 2016). Physical contact between familiar voles has previously been shown to reduce cortisol levels, presumably this allogrooming response functions to reduce stress (Smith and Wang, 2014). In marmoset monkeys, young marmosets reunited with their families after social isolation are highly stressed as indicated by high blood cortisol levels, however those marmosets that receive increased grooming from family members display enhanced HPA axis recovery (Taylor et al., 2015). Altogether these behavioural findings demonstrate that allogrooming significantly reduces physiological and behavioural markers of stress depending on the species. The specific neural

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mechanisms and specific types of stress stimuli that elicit different types of grooming behaviours in rodents or primates, however is a relatively new area of study.

1.1.2 Neural mechanisms

Several studies have examined how social interactions buffer stress by suppressing corticosteroid secretion. Investigations of the neural mechanisms underlying social buffering of stress indicate that oxytocin and opioids play essential roles in attenuating HPA axis activity. Specifically, oxytocin released from the paraventricular nucleus has been shown to blunt HPA axis activity through interactions with the adrenal gland by inhibiting corticosteroid secretion (Legros et al., 1987, 1988). Furthermore studies have revealed that oxytocin infused peripherally in male rats inhibits adrenocorticotropic hormone (ACTH) release from the pituitary gland (Neumann et al., 1998). Another mechanism by which oxytocin may underlie social buffering of stress is by inhibiting hypothalamic corticotropin-releasing factor (CRF) activation. For example, infusions of synthetic oxytocin into the brain of rats resulted in decreased CRF mRNA response to physical stress (Windle, 1997). In addition to stress reduction in rodents, oxytocin also increases social motivation to interact. Hence, it may interact with other neurotransmitter systems in initiating behaviour while also reducing stress. One particular neurosignaling process thought to underlie social buffering is the opioid system. The release of opioids and endorphins have been shown to occur during social attachment behaviors in voles (Burkett et al., 2011) and between mother and infants in primates (Kalin et al., 1995). The release of opioids is a strong reinforcing and rewarding stimulus that may interact with oxytocin to attenuate HPA axis activity. Preliminary studies demonstrate that oxytocin and opioid systems modulate each other as chronic oxytocin treatment reduces stress and has analgesic properties. However, the analgesic property is blocked only by the opioid receptor antagonist naloxone and not an oxytocin antagonist (Uvnäs-Moberg, 1997). These results point towards oxytocin's role in increasing

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endogenous opioids which may underlie social buffering of stress, however this link requires further investigation (Douglas et al., 2004; Kikusui et al., 2006; Uvnäs-Moberg, 1997). Overall evidence suggests a complex interaction between opioid and oxytocin signaling underlying social buffering of stress behaviour (Kikusui et al., 2006).

1.2 Isolation stress and Social Reward

1.2.1 Social Reunion

Healthy social interactions and group living are key to the well-being of a wide range of animals. Social separation and isolation can induce stressful states across social species. After temporary social isolation, motivated social interaction has been shown to be a physiologically rewarding event leading to social attachments and amelioration of separation anxiety (Panksepp et al., 1997). In order to study the neural mechanisms underlying rewarding social interactions, "reunion' behaviours are especially important. In a reunion scenario, rodents are temporarily deprived of social interaction (~24 hours) , this social deprivation which has been shown to be an aversive emotional state, increases intrinsic motivation for social interaction (Matthews et al., 2016; Panksepp et al., 1997). Once the animal is reunited with a conspecific the resulting increase in social interactions can be used to study the neural mechanisms specifically involved in social rewarding interactions. Various rodent studies also employ social conditioned place preference task (SCPP) as a behavioural paradigm to study social reward (Dölen et al., 2013; Panksepp et al., 2007). In SCPP, socially housed rodents are isolated for 24 hours prior to conditioning. During conditioning, rodents are placed in one of two contextually distinct sides of a two room chamber connected by a hallway. In half of the trials the rodents are reunited with a conspecific for 30 minutes in one contextually distinct chamber. In the other half, rodents are placed in the second chamber alone for 30 minutes. On test days, the rodent is allowed to explore all chambers with no social stimuli, and the preference for any chamber is quantified as the most

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time spent. Using this behavioural paradigm, studies have investigated the neural mechanisms underlying social reward.

1.2.2 Neural basis of social reward

Social motivation theory proposes that social interaction involves the same processes as motivation for pleasure and reward (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012). In particular, the processes of seeking, liking and learning all underlie social motivation (Berridge, Robinson, & Aldridge, 2009; Chevallier et al., 2012). Furthermore, the overlapping neural circuitry for encoding reward and social reward have been established. In particular, the dopaminergic signaling in mesocorticolimbic circuit is a core circuit in the reward and addiction process (Kelley and Berridge, 2002). The projections from the DA neurons in the ventral tegmental area (VTA) to the nucleus accumbens (NAc), amygdala and prefrontal cortex are key components of this circuit and each have distinct function in encoding reward properties. By extending the thorough understanding of drug action and reward processes, many groups have theorized the involvement of this circuitry in more ethologically relevant behaviour such as social behaviour (Insel, 2003). In a more general sense, theories suggest that the mesocortiolimbic circuit may be encoding the rewarding properties of social interaction (Insel and Fernald, 2004). Evidence for the involvement of the reward system in social reward have come from classic studies using mating, pup care and pair bonding as model behaviours.

Several groups have tested the hypothesis that mesocorticolimbic pathways mediate mother –infant interactions in rats. The evidence suggests that dopamine is released and fos is activated in the nucleus accumbens following pup exposure in maternal females (Lonstein et al., 1998). Further, VTA or nucleus accumbens lesions disrupt maternal behaviour and specifically reduce the females approach and interaction

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with pups (Numan and Smith, 1984). Studies using pair-bonding in monogamous prairie voles have implicated the interaction between oxytocin receptors within the nucleus accumbens and dopamine signaling in pair bond formation (McGraw and Young, 2010). Although, rodent studies investigating the neural mechanisms underlying maternal care and pair bonding have contributed to the understanding of social behaviour, relatively few studies have focused on naturally rewarding social behaviour between same-sex conspecifics. These peer interactions are particularly relevant for understanding the neural circuits that go awry in disorders such as autism and schizophrenia.

Building off the importance of the mesocortiolimbic circuit in pair bonding and maternal care, studies have focused on the neural dynamics in these regions during conspecific social interactions. In a pioneering study using fast-scan cyclic voltammetry, Robinson et al., found increased DA transients in regions downstream from the ventral tegmental area (VTA) specifically the nucleus accumbens (NAc) during interactions with a same-sex conspecific. However, the question of causality and identifying the specific cells and projections that drive social interaction were still relatively unknown. To answer this question, Gunaydin et al., developed new techniques to understand social reward related VTA activity and elucidate the specific downstream projections from the VTA that drive or inhibit social behaviour. The new Fiber Photometry technique developed by this group, uses a highly sensitive and temporally precise single optic fiber to detect genetically encoded fluorescent Ca2+ signals from cell bodies and axonal fibers (Gunaydin et al., 2014). By injecting a cre-dependent adeno-associated virus carrying the GCamp5g (fluorescent Ca2+ indicator) gene into the lateral VTA of transgenic TH (tyrosine-hydroxylase):: Cre mice, they aimed to record specifically from VTA dopaminergic neurons. Upon introduction to a conspecific, a marked increase in activity of DA-VTA neurons was observed. As a control, activity was recorded during interactions with a novel object.

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Interestingly, the increase in peak activity was similar between social and object interactions, however the timing in bursts was characteristically different. It was found that object interaction elicited an increase VTA activity at the end of the interaction with the object, whereas the social interaction elicited activity at the onset of the interaction. These findings are very interesting, in that they show a neural dynamic specific to social interaction at the level of the reward circuit. To test the sufficiency of this projection in driving social behaviour, VTA-DA neurons were injected with a Cre-dependent AAV encoding ChR2-eYFP into the same lateral VTA region. Using an optic fibre, the region was stimulated using 473 nm light during a homecage social interaction assay in which the test mouse is exposed to two different stranger mice, one paired with light stimulation and the other without light stimulation. The results suggest that phasic stimulation of ChR2-eYFP VTA-DA neurons sufficiently increased social investigation of the novel mouse. By inhibiting these neurons using enhanced halorhodopsin, a significant decrease in social investigation was observed. Thus, these experimental findings demonstrate that phasic activity of VTA-DA neurons are both sufficient and necessary for social approach behaviour in mice. The specific projection from the VTA-NAc but not the VTA-mPFC projection favoured social behaviour. Gunaydin et al., found that by stimulating the projection from the VTA to the NAc sufficed to increase social interaction, whereas activation of the VTA to mPFC projection had no effect on social interaction. Furthermore, electrophysiological experiments in combination with optogenetic stimulation of the VTA, resulted in increased NAc cellular activity in response to stimulation of the VTA, mirroring NAc activity during natural social interaction.

Finally, NAc medium spiny neurons (MSN) can be divided in two categories based on dopaminergic receptors (D1 and D2). In order to determine which NAc MSNs are involved in

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this specific behaviour, the social modulatory effects of D1 and D2 receptor antagonists in the NAc prior to optical stimulation of the VTA was tested. It was found that the D1 receptor antagonist specifically attenuated social interaction but not D2 receptor antagonism. To fully test whether temporally precise stimulation of D1 receptors are sufficient to increase social interactions, a channelrhodopsin was virally incorporated into the NAc D1 receptor MSNs. Direct stimulation of these D1 cells were sufficient to produce a significant increase in social interaction compared to controls. The results from this study are ground-breaking and shed considerable insight on the projection dynamics in the mesocorticolimbic circuit and the exact dopaminergic mechanism involved in social interactions.

1.2.3 Oxytocin and social reward

Oxytocin has been found to strongly modulate social behaviour in humans. However, its exact role in social reward is just beginning to be uncovered. Peripheral administration of oxytocin has been correlated with increased performance on social conditioned place preference, and modest increases in interactions with non-social objects (Douglas et al., 2004). However, the problem with peripheral or intraperitoneal administration of oxytocin is that it binds to the oxytocin receptors in the adrenal cortex of the adrenal gland. This binding is thought to reduce the levels of cortisol in the bloodstream (Dölen, 2015; Gibbs, 1986). Hence, a major criticism of social behavioural effects attributed to systemic administration of oxytocin or oxytocin antagonists is that the effect may actually be due to a reduction in stress (Dölen, 2015).

Therefore, systemic injections of oxytocin antagonists are not considered a truly valid method to understand how neural oxytocin works. Hence, an in-depth investigation by Dolen et al, aimed to uncover the role of NAc oxytocin in social reward behaviour. In a first set of experiments, an oxytocin antagonist injected directly into the NAc during the conditioning for SCPP, abolished the SCPP compared to controls. The same oxytocin antagonist applied to the

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NAc during cocaine or novel object CPP, had no impact on place preference. Using retrograde tracing, the direct oxytocinergic projection from the PVN to the NAc was confirmed. Further, slice physiology experiments revealed that oxytocin induces long term depression (LTD) in NAc medium spiny neurons. This LTD was shown to be blocked using an oxytocin antagonist (Dölen et al., 2013).

In addition to the PVN-NAc projection identified via retrograde labelling, it was found that three other brain regions expressed oxytocin receptors that projected to the NAc. These were the anterior cingulate cortex, the ventral subiculum and the dorsal raphe nucleus (Dölen et al., 2013). In order to understand which afferent to the NAc was necessary for social reward behaviours, oxytocin receptors were selectively deleted using a line of oxytocin receptor conditional knock out mice (cre-recombinase system). An injection of AAV-Cre-eGFP into each afferent region (ACC, V. Sub and D.R) ablated site specific oxytocin receptors in separate groups. Interestingly, deleting oxytocin receptors in the ACC and ventral subiculum had no influence on social CPP, the mice preferred the social domain and their preference was not statistically significant from controls (Dölen et al., 2013). On the other hand, deletion of oxytocin receptors in the dorsal raphe nucleus completely abolished social CPP. Furthermore, experiments revealed that it was not the oxytocin receptors within the NAc that were actually necessary for SCPP but the oxytocin receptors on the presynaptic boutons of dorsal raphe projections to the NAc that was responsible for the LTD necessary for SCPP. Findings also revealed substantial overlap between oxytocin and serotonin 5HT1B receptor expressing cells in the dorsal raphe. The role of serotonin in this circuit was investigated by blocking 5HT1B receptors in the NAc. It was found that the blockade of 5HT1B receptors also prevented social CPP. Using slice physiology, it was found that the

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blockade of oxytocin receptors did not influence the LTD induced in NAc medium spiny neurons if serotonin was present. Hence, the results point to oxytocin playing a modulatory role on 5HT1B receptors on dorsal raphe projections to induce LTD in the NAc, which has been shown here to be necessary for social CPP.

Altogether, these results point to a highly complex and multi-neurotransmitter dependent circuit for social reward requiring coordinated activity between oxytocin and serotonin on dorsal raphe projections in addition to D1 receptor activity in the NAc. Current theories on the etiology of autism implicate polymorphisms in dopamine receptors, serotonin receptors and oxytocin receptors as potential causes for the varied social deficits observed in autism. Based on the current literature, any mutations in any of the three neuromodulator receptors would drastically alter the reward value and motivation for social interaction, particularly after social separation.

1.3 Affective communication

1.3.1 Neural basis of vocal production

Recent investigations into the neural and social basis of rodent vocalizations reveal that many factors influence vocal production. As communication deficits are a prominent symptom in many disorders, studying the underlying circuitry and neurotransmitter systems can provide answers as to how these deficits arise. In this section, literature on the vocal production pathway from human, primates and rodent studies will be discussed and the potential role of oxytocin in the production and maintenance of vocalizations.

Research conducted in non-human primates and rodents have revealed key neural regions involved in the production and patterning of vocalizations. Based on stimulation, lesion and single-unit recording studies conducted in squirrel monkeys, Jurgens (2009) proposes a hierarchical model of vocal control. Specifically, this model focuses on two distinct pathways: 1)

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a limbic cingula-periaaqueductal pathway controlling voluntary vocalizations and 2) motor cortex to midbrain pathway involved in the production of learned vocal patterns.   In the limbic cingula-periaqueductal pathway, it is proposed that the anterior cingulate cortex controls the motivational aspect of vocalizations, whereas the PAG has a gating function and the midbrain coordinates motor output. This theory is supported by stimulation and lesioning studies in squirrel monkeys, by which stimulation of each of these regions elicits vocalizations. Interestingly, lesioning the PAG and midbrain results in mutism across species however lesioning the ACC results in a marked decrease in vocalizations but not mutism. Similarly, case studies in humans reveal that lesions in the ACC result in transcortical motor aphasia, which is characterized by severely reduced motivation to speak, however the quality of speech and grammar remains unchanged (Jürgens and von Cramon, 1982). Further evidence towards the role of ACC in the voluntary initiation of vocalizations comes from research in macaque monkeys. Macaques trained in operant tasks, requiring the production of vocalization for a reward stimulus has been shown to require the ACC. Specifically, lesions in the macaque ACC resulted in an inability to vocalize for reward stimulus, however the monkeys were not mute and were still able to vocalize to threatening stimuli (Jürgens, 2009; Sutton et al., 1974). Further, combined stimulation and inactivation experiments in squirrel monkeys demonstrate that inactivation of PAG prevents vocalizations when ACC is stimulated, however does not prevent vocalizations when the midbrain is stimulated, indicating hierarchical circuit. As the limbic cingualperiaqueductal gray pathway has been proposed to control the motivational and voluntary control of vocalizations, a separate pathway has been shown to be responsible for the specific vocal patterns associated with different calls in the squirrel monkey. Specifically, this pathway is a feedback loop across the reticular formation of the brainstem to the motor cortex, which is

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thought to control fine motor movement underlying different types of vocalizations (Jürgens, 2009).

1.3.2 Hormonal modulation of vocal production

As described, evidence from primate studies indicates multiple levels of neural control over the production of vocalizations. Research spanning across species has also investigated the role of peptides such as oxytocin and vasopressin in the production of vocalizations in different social contexts. Vasopressin and oxytocin are closely related peptides which modulate many social behaviours across species. Interestingly, vasopressin has been shown to modulate the motivation to vocalize, duration of vocalization and rate of vocalization in seven species of frogs (2013). Furthermore, homologues of vasopressin and oxytocin (vasotocin and isotocin) have been shown to modulate the sex-specific vocalizations in teleost fish (Goodson and Bass, 2000a) and plainfin midshipman fish (Goodson and Bass, 2000b). In very specific species of birds (Japanese quail and male field sparrows), central infusions of vasotocin have been shown to modulate bird song (Castagna et al., 1998; Goodson, 1998). These cross-species investigations in birds, amphibians and fish point towards species-specific hormone modulation of social communication.

In mammals, oxytocin's role in social communication has not been investigated as thoroughly as vasopressin systems in amphibians and fish. Very few studies have investigated the possible role of oxytocin in vocalization modulation in mammals. Most evidence in mammals, comes from studies focusing on pup distress vocalizations (Insel and Winslow, 1991) and calls motivated by sexual reproduction (Floody et al., 1998), hence generalizability to adult peer interactions is limited. In an early study conducted by Insel and Winslow (1991), oxytocin was centrally administered via I.C.V infusion in pups isolated from their mothers. Isolating rat

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pups 6-8 days from mothers has been shown to reliably produce distress ultrasonic vocalization calls. Insel and Winslow found that oxytocin administration reduced distress calls and infusion of an oxytocin antagonist after infusion of oxytocin blocked the decrease in distress calls (Insel and Winslow, 1991). This indicates that oxytocin attenuates distress calls via oxytocin release when being groomed by the mother. Hence, distress vocalizations in pups seem to be modulated by oxytocin activity. By contrast, in a different species and social context, the hamster demonstrates increases in ultrasonic vocalizations during courtship between male and female hamsters when oxytocin is directly infused in the medial preoptic-anterior hypothalamus (Floody et al., 1998). These results indicate that oxytocin may play a multifaceted role in the production of vocalizations dependent on the valence of social context. Additionally, oxytocin knockout mice emit fewer ultrasonic vocalizations than their wild type counterparts in addition to severe social deficits (Takayanagi et al., 2005). However, the roles of oxytocin in the production of vocalizations during peer-to-peer interactions have not yet been investigated. Altogether, these studies point towards oxytocin's role in vocalization production, however an exact mechanism has not yet been uncovered.

1.4 Degu as a model for socio-affective research

In order to disseminate the neural basis of higher order social processes in humans, components of social cognition are broken down in attempts to study them in rodents. Currently, much of the neural work on social buffering and social reward is conducted in mice and rats. These standard lab animals pose a challenge in modelling naturalistic social behaviors that are generalizable to humans. Current literature suggests that mice and rats display limited forms of social behaviour, that do not go beyond short-term maternal care, pup retrieval, mating, aggression and some forms of social learning (Donaldson and Young, 2008; Insel, 2010). Although use of mice and rats allow for

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larger sample sizes, less laboratory cost and can easily rely on established brain atlases, their use in social neuroscience is relatively limited. Mice and rats particularly show weak responses to social isolation, no clear preference to caregiver, and highly variable prosocial responding (Panksepp, 1992, 2005). Currently, many groups are turning their focus towards more socially complex rodents such as prairie voles (McGraw and Young, 2010), ground squirrels (Lahvis et al., 2015), naked mole-rats (Holmes et al., 2007) and degus (Colonnello et al., 2011). Social rodents used for neural research are often chosen for a distinctive social behaviour that may provide clues as to how a particular social cognitive process occurs in humans. Here, the use of *Octodon degus* will be outlined as a valuable animal model to understand social reward and social communication as a result of their unique social behaviour and abilities to vocalize.

Octodon degus are diurnal social rodents' endemic to the Andes region of Chile. Degus are unique in that they have a wide range of audible vocalizations used to communicate varying levels of affect, signals for affiliative approach, pleasure, aggression, annoyance, alarm and distress (Long, 2007). Each of these vocalizations have been characterized based on their correlations with distinctive behaviour and their unique frequency components (Long, 2007). Degus are not a monogamous species; this is an advantage as an animal model for social neuroscience as it allows the dissociation of peer social relationships from reproductive attachments (Beery et al., 2016). Although, differing in their social capacities, degus also have similar brain/skull structure to rats, therefore well-established pharmacological and electrophysiological techniques can be used with minor modifications (Kumazawa-Manita et al., 2013a).

Much of the neural work with degus has focused on how biparental upbringing of degus differentially affect monoaminergic connectivity in the degu forebrain (Helmeke et al., 2001; Ovtscharoff Jr and Braun, 2001). As degus are unique rodents in which females and males share

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the responsibility of upbringing through an extended period of ‘childhood’. They are strong model organisms to study social development and epigenetic mechanisms in early childhood experiences (Poeggel et al., 2003a). Interestingly, research suggests profound changes in the development of vocalizations in the absence of a father during development (Braun et al., 2003; Poeggel and Braun, 1996). Further, cognitive behaviours and neural volumes are also altered as a result of paternal deprivation in young degus (Braun et al., 2003; Poeggel et al., 2003a, 2003b). In addition, the effects of social isolation in young degus show profound behavioural deficits as adults (Ferdman et al., 2007). As an emerging animal model for social neuroscience, the role of peptides oxytocin and arginine-vasopressin are of special interest in relation to affiliative behavior. However, as a novel animal model, the oxytocin and arginine-vasopressin receptor distribution in the degu brain is only beginning to be uncovered. In a preliminary investigation by Beery et al., (2016), oxytocin receptor distribution in the cortex and temporal lobe of degus was assessed. High binding was noted in the prefrontal cortex, endopiriform nucleus, CA3 of the Hippocampus and specific amygdalar and hypothalamic nuclei (Beery et al., 2016). While these studies only investigated the degu forebrain, further investigation of midbrain oxytocin receptors and whole-brain investigation of arginine vasopressin receptors are still necessary for accurate targeting of these receptors. However, the unique oxytocin receptor distribution described here provides insight into the utility of degus for socio-affective research.

Findings from previous studies in our lab with degus have shown that they are suitable subjects for studying the social transmission of emotion via vocalizations. Our findings show that naïve degus observing a familiar conspecific in distress develop a vicarious fear response to a context, and that this fear learning is mediated by

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vocalizations emitted by the distressed conspecific (Lidhar et al., under review). These results indicate that degus have complex communication and can socially transmit emotional state based on familiarity. Additionally, social reunion behaviour has been studied in degus between infants and dams. Infant degus were found to emit distress vocalizations when isolated from the mother, as has been found in rats, however upon reunion infant degus also emit chirps in the 4 kHz range thought to be associated with positive affect. Furthermore, this study revealed that degu mothers emit a nursing call to regulate nursing bouts (Fuchs et al., 2010), interestingly, this behaviour has only been documented in socially complex animals such as monkeys and pigs (Colonnello et al., 2009, 2011; Kalin et al., 1995). Overall, degus offer unique advantages over standard lab species by virtue of their lifelong bonds, complex social behaviour and communication.

1.5 Present Study

The overarching hypothesis of this study is that the motivation behind social interactions differ depending on the type of stressor experienced by the individual. An important question here, is how stressors such as social isolation and physical stress motivate social behaviour. To test this idea, I designed a series of experiments that characterized the difference in social motivation as a difference in allogrooming and vocalization in degus. In Experiment 1, degus were exposed to different social isolation times, distress conditions and reward to observe effects upon expression of vocalizations and types of social affiliative behaviour. I was specifically interested in the behavioural and vocal characteristics of reunion between adult conspecifics. Based on literature and observation, I hypothesized that degus would demonstrate dissociable social behaviour and vocalizations after exposure to the two different types of stressors. Specifically, based on the rich and complex social behaviours documented in degus (Colonnello et al., 2011; Long, 2007) I predicted increased positive vocalizations and grooming behaviours after 24h social separation and increased affiliative grooming behaviour after exposure to

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footshock. I was also interested in whether degus display specific vocalizations during specific types of grooming behavior. Hence, the temporal co-occurrence of vocalizations and behaviours were explored. Furthermore, I tested whether degus display allogrooming towards a distressed conspecific during the observed distress condition. Based on degus high sociality, I hypothesized that degus would display increased allogrooming towards a distressed conspecific.

In Experiment 2, I examined the role of oxytocin in these social behaviours and vocalizations. Oxytocin's modulation of social behaviour has been documented in many species. Vast literature suggests that oxytocin's mechanisms are influenced by social context and does not influence social behaviour in a straight-forward manner (Dölen, 2015; Shamay-Tsoory and Abu-Akel, 2015). Furthermore, oxytocin has been found to enhance cortical processing of stimuli and to underlie motivational processes of social approach. In Experiment 2, I investigated whether blocking central oxytocin receptors in degus had any effect upon social reunion behaviour or vocalization production after a 24-hour isolation. As no manipulation of oxytocin receptors in degus has been done previously, this is the first investigation of its kind in this social species. Based on previous rodent studies, I hypothesized that if behaviours and vocalizations demonstrated after 24-hour isolation are based on social reward neural processes, then, blocking oxytocin receptors may attenuate these voluntary behaviours and vocalizations. Altogether this study aims to understand some of the motivational and hormonal factors underlying social reunion behaviour in degu adult conspecifics.

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2 Experiment 1: Characterization of allogrooming and vocalization after social isolation and physical stress in *Octodon degus*

2.1 Introduction

The present study sought to determine how different stressors such as social separation and aversive events, motivate social behaviour during reunion in degus. To address this question, pairs of degus were subjected to 24-hour social isolation, 45-minute social isolation, exposure to food reward, exposure to repeated footshocks (distress) and exposure to a conspecific receiving repeated footshocks (observed distress) all before reunion in the homecage. Social grooming behaviours and vocalizations emitted during each condition were compared to a 1-minute isolation control. Here, I hypothesize that degus will show dissociative social behaviour and vocalizations after a social separation and the distress/observed distress conditions compared to the 1-minute control. Given that social isolation and pain stimuli are both different types of stressors, I predicted that resultant behaviour will be dissociable at the level of vocalization and grooming in these highly social species.

2.2 Materials and Methods

2.2.1 Subjects

Eighteen female degus co-housed in pairs were used for this experiment. Females were used here due to colony constraints; however future experiments will replicate this in males. Degus were pair-housed in 15 x 19 x15 polycarbonate cages in a breeding colony at the biological science facility at the University of Toronto, St. George campus. Degus were kept on a 12:12h light/dark cycle with food and water available ad libitum. All degus were tested after

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reaching adulthood, ages ranged from 6 months to 1-year-old. All protocols were approved by the local animal care committee.

2.2.2 Apparatus

Social reunion took place in the degus homecages, placed inside a 15''x19''x 60''plexiglass and MDF chamber (Fig. 1). The roof of the social reunion chamber was embedded with a Sony webcam and a Sony microphone providing video and audio feed to the bandicam multi-media recording software (Bandisoft, South Korea). This software ensured timestamped coordination between the microphone recording and the video recording.

Personal/observed distress conditions occurred in a 18" × 17.5" × 38 cm box in which a transparent wall segregated two contextually distinct chambers (Fig. 2). This wall ensured that degus inside had a direct view of each other. The transparent wall prevented physical interactions, however small holes in the wall and space underneath the shock grid floor allowed for auditory and olfactory stimulus to travel. The shock grid floor (Colbourne instruments, Holliston, USA) consisted of 5 mm thick stainless steel rods spaced 1 cm apart. Sony webcams were embedded in the roof of the distress chambers to provide video feed to the Bandicam multi-media recording software. The Sony microphone was embedded in the wall of the shock chamber. Shock administration was coordinated using an Arduino (www.arduino.cc) running custom written Arduino software, controlled by a custom-written GUI interface built in MATLAB (MathWorks; Natick, MA). Additionally, the two conditioning chambers were differentiated using laminated patterned wall paper. The shock chambers and wallpaper were thoroughly

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cleaned before and after each trial with 70 % ethanol to prevent any residual olfactory cues from affecting the animals' behaviour.

2.2.3 Testing Procedure

This experiment took place over eight days. During each day, degus underwent specific procedures illustrated in (Fig. 1).

Habituation: Degu pairs were brought to the conditioning room and their homecage was placed into the sound proof recording chamber. The pair spent 1 hour undisturbed in the chamber over 3 consecutive days.

1-minute isolation: In order to control for any stress associated with being removed from the homecage during isolations, each pair was placed in separate isolation cages, in different areas of the room for a 1-minute period. Pairs were then reunited in the homecage within the sound proof chamber for 20 minutes.

45-minute isolation: The pair was placed in separate isolation cages in different areas of the room for a 45-minute period. Pairs were then reunited in the homecage within the sound proof chamber for 20 minutes. The *45-minute isolation* and *24-hour isolation* were counterbalanced across groups to control for any order effects.

24-hour isolation: The pair was placed in separate isolation cages in different areas of the colony room for a 24-hour period. The pairs were then reunited in the home cage within the sound proof chamber for 20 minutes.

Reward: The pair was placed in separate isolation cages in different areas of the room for a 45-minute period. Unlike the *45-minute isolation* condition, degus were each exposed to a positive stimulus; a preferred food of 8 sunflower seeds in the isolation cage. This condition was used as

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an additional control for aversive stimulus + *45-minute isolation* experienced in the subsequent *distress* condition.

Distress: To compare the effect of physical stress/fear against social isolation prior to reunion, both degus experienced a 'distress' condition. After a 5-minute baseline period in a shock chamber, each degu received 10 2s 1.0 mA footshocks over a 2.5-minute period. After the shock period, the degus were placed in separate cages for 15 minutes. The degus were then reunited in the homecage within the sound proof chamber for 20 minutes. Level of shock was determined based on our previous investigation of observational fear learning in the degu (Lidhar et al., under review. To reduce the extreme aversiveness experienced in that paradigm, we reduced the shock numbers to half. Based on the number of pain vocalizations emitted during the shock period, this condition was determined to be sufficiently aversive.

Observed Distress: In order to test whether observing a conspecific under physical distress prior to reunion elicits a behavioural change in the observer, one degu was subjected to 10 2s 1.0 mA footshocks over a 2.5-minute period while a conspecific observed in an adjacent chamber. After the observed distress period, both degus were placed in separate cages for 15 minutes. The degus were then reunited in the homecage within the sound proof chamber for 20 minutes.

2.2.4 Vocalization analysis

To identify and analyze vocalizations emitted by the degus the sound files were processed using a specific protocol. First, each sound file was listened to and corresponding spectrograms were investigated for vocalizations using playback audio software (Audacity v.2.1.2 (Pittsburg, USA)). In order to remove background noise and amplify the vocalization signal, Audacity' "noise reduction" feature was used. To do this, a portion of background noise within each file was used as a 'noise profile' by which the

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software then uses as a filter to remove noise. In this case, noise from air vents were filtered out from each file. Then, two experimenters (myself and an undergraduate student) independently listened to sound files and identified portions of the file containing vocalizations. In order to correctly identify vocalizations, Long's (2008) description/analysis of degu vocalizations was used as a reference. The portion of the sound file containing the vocalizations was then clipped into separate audio clips. Each scorer was blind to the subject and condition. Next, each clip was then analyzed using Sound analysis pro (SAP)- a vocalization detecting software developed for segmenting syllables and vocalization structure in birdsong (Tchernichovski et al., 2000).

Next, spectral analyses were conducted on isolated vocalizations to explore vocalization structure such as; frequency components, duration, amplitude and pitch. Parameters such as frequency, amplitude, frequency modulation and duration, were used to identify degu vocalizations (Long, 2007). SAP's outline syllable feature was used to highlight syllables identified using the set frequency and amplitude parameters, then the blind scorer manually listened to each highlighted syllable and accepted or rejected its inclusion as a vocalization. Due to large amounts of digging noises, certain vocalizations contaminated with digging noise were manually selected on the spectrogram for inclusion. Once the syllable was selected for inclusion, the scorer would categorize the syllable based on spectral features and sound likeness to degu vocalizations outlined by Long (2007). Scorers were blind to subject and condition. Syllables identified by the scorer were then inputted into a database along with the corresponding information on the syllable's mean frequency, duration, amplitude, frequency modulation, amplitude modulation, entropy and start time.

Vocalization data were analyzed using MATLAB, to identify the raw numbers of syllables, duration, and co-occurences with different behaviours.

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2.2.5 Behaviour analysis

Each video recording was analyzed using BORIS (Behavioural Observation Interactive Research Software, Torino, Italy) software which is used to log events while observing animal behaviour. It allows for coding of state behaviours (distinct start and stop time) and point events which are occurrences'. It also allows scoring of each individual degu so that individuals within each dyad had unique scores. Behaviours scored are described in Table 1. Each video was analyzed independently by two researchers (myself and an undergraduate assistant). Event logs produced by the BORIS software were analyzed using MATLAB to calculate duration, frequency and the time sequence of different behaviours across the session.

2.2.6 Statistical Analysis

All statistical analyses were performed by using SPSS (IBM, V22, USA). Where behavioural data were deemed non-parametric using a Kolmogorov–Smirnov test, data was analyzed using non-parametric tests. Data on the duration of reunion vocalizations was analyzed using rank-transformed One-way repeated measures ANOVA (also known as Friedman test, described by Weunsch (2013)), followed by post hoc pairwise comparisons with Bonferroni correction. Specifically, time spent isolated/distress condition was set as a repeated measures variable and the duration of vocalizations and behaviours as the dependent variables. As all behaviour and vocalization data from experiment #1 were non-parametric all data were rank-transformed. Specifically, each pairs vocalization and behaviour scores were ranked from 1-6 and analyzed using a repeated-measures ANOVA, then all comparisons were corrected for using the Bonferroni correction. Independent samples t-test was used to analyze behavioural differences between observers and demonstrators during reunion after the observed

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distress condition. Statistical significance of co-occurring vocalization and behaviours were calculated using chi-square tests.

2.3 Results

2.3.1 Behavioural changes across conditions

The duration of each social interaction was calculated and compared across conditions. Time spent in face to face grooming was significantly different across conditions (Fig. 3A; $F(5,48) = 4.09$, $p = 0.004$). Post hoc analyses revealed a significant increase in face to face grooming at 24 hours compared to 1-minute ($p = 0.012$), however all other conditions were not significantly different from the 1-minute control (1 minute vs. 45-minute $p = 1.00$, 1 minute vs. Distress $p = 0.167$, 1 minute vs. Observed Distress $p = 1.00$)

Degus demonstrated significant differences in body-grooming across conditions (Fig 3E., $F(5,48) = 4.08$, $p = 0.004$). Post-hoc analyses revealed that body grooming was significantly higher at 24 hour compared to 1-minute ($p = 0.009$), all other comparisons yielded non-significant results (1-minute vs. 45-minute $p = 1.00$, 1-minute vs. reward ($p = 1.00$), 1-minute vs. Distress $p = 0.387$, 1-minute vs. Observed Distress $p = 1.00$)

Degus also displayed significant differences in nose-to-rear contact across conditions (Fig 3C., $F(5,48) = 4.66$, $p = 0.002$). Post hoc analyses revealed significant increases in nose-to-rear contact between 1-minute and 24-hour isolation ($p = 0.001$), and between 1-minute and distress ($p = 0.016$) all other comparisons to the 1-minute control revealed non-significant results (1-minute vs. 45-minute $p = 0.67$, 1-minute vs. observed distress $p = 1.00$).

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2.3.2 Vocalizations changes across condition

During reunion across conditions, many different vocalizations were emitted (Fig.4-5). Degus exhibited significantly different numbers of total vocalizations during reunion after varying isolation times and distress conditions, (Fig 6., $F(5,48) = 6.06$, $p<0.001$). Higher variability within the 1-minute condition led to a trend towards significance when compared to 24 hours ($p= 0.16$), however, 24 hour was significantly increased compared to all other conditions (45 minute vs. 24 hours ($p =0.013$), reward vs. 24 hours ($p= 0.013$), distress vs. 24 hours ($p= 0.005$) and observed distress ($p=0.0001$).

2.3.3 Temporal relationship between social behaviour and vocalization

Temporal co-occurrence of vocalizations and behaviours were determined by calculating the number of vocalizations emitted during specific types of behaviours. Significant increase in vocalizations particularly chitter vocalizations emitted during face to face interaction after 24 hour isolation were found (Fig. 7 p, $\chi^2(25, N=36) = 215.8$, $p <0.0001$)

2.3.4 Behavioural differences between observers and demonstrators

After observed distress, no significant differences were observed between number or duration of grooming behaviours initiated by the observer compared to the demonstrator (Fig 8., $t =1.187$, $p= 0.2526$).

2.4 Discussion

Degus are highly social animals and express a variety of vocalizations. Consequently, social separation and other stressful experiences likely influence affiliative behaviour and vocalizations in these animals. It is clear from previous work that grooming behaviour has a social buffering effect upon stress (Burkett et al., 2016; Taylor et al., 2015). Literature suggests that social separation and aversive events such as

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footshocks act as stressors in rodents (Burkett et al., 2016; Matthews et al., 2016; Panksepp and Beatty, 1980). If degus express increased social behaviour and vocalizations after any type of stressful experience, then both social separation and footshocks should elicit comparable numbers of vocalizations and grooming during reunion. However, the data suggest that isolation stress and physical stress differentially motivate social behaviour and vocalizations. These experiments indicate that degus display increased vocalizations and social interactions in the form of all types of grooming specifically after being socially isolated for 24 hours compared to control (1-minute isolation). In addition, after 24 h separation, high number of chitter vocalizations were found to occur specifically during face-to-face interaction (Fig. 9). An observational study conducted by Long (2007) showed that chittering in degus mainly occurred between closely bonded conspecifics and during prolonged nose-nose contact. Based on their observations, these chitter vocalizations are likely to bolster and maintain social relationships. These findings and those from this experiment point towards chittering during face-to-face interaction as an expression of a positively rewarding social interaction. Because these vocalizations do not occur after experiencing a physical stressor or experiencing a rewarding condition, we can rule out that chitter vocalizations are emitted as a result of stress. Rather, given the motivational context associated with social deprivation, we can use chitter vocalizations to study the neural basis rewarding interactions among conspecifics.

In contrast, after experiencing stress in the form of footshocks, degus did not exhibit increased vocalizations however they did exhibit increased rear grooming, but not face-to-face or body grooming as seen during reunion after 24 h. These results indicate that degus may display distinct forms of interactions for varying purposes. It is possible that social grooming in degus serves as a buffer for pain/stress whereas the emission of vocalizations (particularly chitters) signals a rewarding social interaction especially after social separation. Furthermore, it is unclear

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whether rear grooming exhibited by degus in this study included licking and grooming of each other's hindlegs, where footshocks were applied. From the angle of the camera in this study, ano-genital grooming and hindleg grooming cannot be distinguished. Future studies may further determine the type of grooming behaviour by collecting behavioural data from underneath reunited degus. Grooming of a stressed conspecific has been described as 'consolation' behaviour which reduces or buffers the stress of a conspecific(Burkett et al., 2016; Fraser et al., 2008; Palagi et al., 2014). Indeed these results suggest that degus exhibit targeted social grooming towards the source of pain in conspecifics, potentially a behavioural display of consolation.

These results are important, as they are the first rodent investigations of specificity of vocalizations during a socially rewarding interaction in adult conspecifics. Studies using rats and mice have not yet demonstrated specific vocalization types during social contact with adult conspecifics. In rats, 50 kHz ultra-sonic vocalizations have been generally associated with positive affective state. However, these same USVs have also been demonstrated during receipt of pharmacological reward, human handling or 'tickling' of rats and during rough and tumble play (Burgdorf et al., 2011; Knutson et al., 1998). A specific syllable or vocalization type has not yet been attributed to specific types of positive social behaviours in rats. In mice, ultrasonic vocalizations have been shown to be emitted during mating, pup isolation and juvenile play behaviour but positive and negative affect has not yet been attributed to the vocalization type (Portfors, 2007), however modulation in "prosody" of mouse vocalizations are theorized to express affective state but remain to be characterized (Lahvis et al., 2011). In comparison, degus have a range of audible vocalizations types that are uniquely associated with positive and negative affect dependent on co-occurring behaviours (Long, 2007). Here, it is shown

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that although degus emit a wide range of vocalizations during social reunion, our data suggest that degus selectively emit increased chitter vocalizations during face to face interaction when reunited after a 24 h separation (Fig. 9).

During the observed distress condition, a degu observes their conspecific undergo footshocks in a distinct context. After this period both observer and demonstrators are reunited and resultant behaviour is recorded. I hypothesized that observers would display increased grooming behaviour towards the distressed demonstrator. However, our data indicates a non-significant difference between the grooming (body, rear and face-to-face) behaviour initiated by the observer compared to the demonstrator. However, given the sample size limitations, all degus in this condition had experienced personal distress prior to the observed distress condition. Recent studies investigating grooming directed towards a distressed conspecific exclusively used naïve non-distressed observers (Burkett et al., 2016). This may be a key limitation in our experiment as observers may be experiencing high levels of stress due to the fear memory of the context. Studies have shown that stressed observers are less likely to exhibit emotional contagion or elementary levels of empathy (Martin et al., 2015). However, increased rear grooming elicited after the distress condition point towards the likelihood that this social grooming buffers stress in degus. In order to assess whether this grooming functions to reduce one's own stress or the conspecific's stress, future studies should utilize naïve observers.

Overall, this experiment reveals a specific pattern of behaviour and vocalizations induced by reunion after social isolation but not physical distress. This is an important finding because it elucidates that stressors can elicit different behaviours and vocal expression in degus. These behavioural expressions indicate that degus may be able to express affective state during a positive social interaction and modulate its behaviour in response to a conspecific in distress.

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These findings also suggest that degus are suitable animal models for studying complex neural processes that underlie empathy such as neural representation of others, emotional contagion and affective social communication. Particularly, remaining questions are the neural factors that modulate this highly specific social behaviour and vocalizations emitted during reunion after 24 hour isolations. A key modulating factor of degu vocalizations and affiliative behaviour may be the peptide oxytocin.

3 Experiment 2: The role of oxytocin receptors in neural processing of social reward

3.1 Introduction

Oxytocin receptors are G-protein coupled receptors that are expressed centrally and peripherally. There are no known subtypes of oxytocin receptors, however a few polymorphisms in the oxytocin receptor gene have been identified (Gimpl and Fahrenholz, 2001). Oxytocin itself also binds to AVP1a vasopressin receptors with moderate affinity (Song et al., 2014). Also, oxytocin receptors have a highly variable species-specific distribution (Beery et al., 2016). Mammals in particular exhibit differences in expression of oxytocin receptors in both cortical and subcortical circuits. In particular, cross-species investigations of oxytocin receptor distribution revealed functional correlations within specific brain regions for species-specific adaptive behaviours (Beery et al., 2016). While oxytocin receptor expression profiles have been associated with species-specific behaviours, overall numbers of central oxytocin receptors do not confer the sociality of a species. Several highly social species of rodents express variations in receptor distribution however it does not dictate whether a rodent is group-living or solitary (Beery et al., 2016).

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Research focusing on the production and release of oxytocin has identified that the nonapeptide is synthesized in the paraventricular nucleus and supraoptic nucleus of the hypothalamus and secreted through the pituitary gland (Neumann et al., 1993). The synthesis of oxytocin occurs in two distinct types of hypothalamic neurons; parvocellular and magnocellular neurons (Gurdjian, 1927; van den Pol, 1982). In a recent review, Dolen, (2015) outlined three modes of oxytocin transmission thought to exist in the brain. (1) Endocrine transmission; secretion of oxytocin directly into the blood stream, this mode of transmission has been found to be the result of oxytocin released from magnocellular neurons at the axon terminals of the posterior pituitary. (2) Paracrine transmission/ volume transmission; oxytocin secreted into the CSF, slow diffuse bathing of the brain. This mode of transmission has been attributed to the magnocellular large vesicle release directly from the cell bodies. (3) Synaptic release of oxytocin; axonal release onto cells particularly in the reward circuit (Dölen, 2015). The small size of the oxytocin neurons projecting to the NAc suggests that parvocellular neurons may be responsible for synaptic release of oxytocin. Synaptic release of oxytocin is faster than volume transmission and is proposed to have a neurotransmitter-like action rather than large-scale hormonal modulation (Dölen, 2015).

Overall, literature suggests that oxytocin plays a major role in motivating socially rewarding interactions. Studies investigating social reward, have elucidated species-specific mechanisms of oxytocin's modulation of social approach and grooming behaviour (Burkett et al., 2016; Dölen et al., 2013). While hormonal modulation of vocalizations emitted during socially rewarding interactions have not been investigated in adult rodents, evidence suggests that oxytocin may modulate motivation for vocal social communication (Seltzer et al., 2010; Theofanopoulou, 2016). Based on this body of evidence, I investigated how blocking central oxytocin receptors in both members of the pair of adult degus would influence their social

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grooming, emission of vocalizations, and vocalization types. Furthermore, based on behavioural findings from Experiment 1 in which degus exhibit increased social grooming and affective vocalizations after 24 h separation, I hypothesized that blocking central oxytocin receptors would attenuate both social behaviours and vocalizations exhibited during social reunion.

3.2 Materials and Methods

3.2.1 Subjects

Fourteen female degus co-housed in pairs were used for this experiment. Degus were pair-housed in 15” x 19” x 15” polycarbonate cages in a breeding colony at the biological science facility at the University of Toronto, St. George campus. Degus were housed on a 12:12h light/dark cycle with food and water available *ad libitum.* All degus were tested after reaching adulthood, ages ranged from 6 months to 1-year-old. All protocols were approved by the local animal care committee.

3.2.2 Apparatus

The reunion apparatus as described in section 2.2.2 and fig. 1.

3.2.3 Stereotaxic cannula implantation

According to standards published by the animal care committee at the University of Toronto, all surgeries took place under aseptic conditions in a surgical suite. Degus were anesthetized via 4 % isofluorane at induction and maintained at 1.5% with a flow rate of 1.5 L/min. Once placed in the stereotaxic frame, the skull was exposed and stainless steel screws were screwed into the skull to adhere to dental acrylic resin.

As no studies have been published showing successful cannulation in Degus, the coordinates for cannula placement were initially chosen by translating a typical cannula location

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in rats to a location within a brain atlas for degus (Kumazawa-Manita et al., 2013b). The coordinates were further adjusted based on the outcome of pilot experiments (n=12). Given that this was a novel technique employed in degus, we implanted bilateral cannulae to increase our chance of correct placement. In order to bilaterally target the lateral ventricles, small holes were drilled into the skull and guide cannulae (Plastics One, Roanoke, VA, USA) were lowered at a 15° angle bilaterally (-1.20 mm Anterior-posterior, +/- 3.2 mm Mediolateral and -2.3 mm Dorsoventral from Bregma). The guide cannulae were secured to the skull with dental acrylic resin and the guide cannulae were closed with a dummy cannula wire and a brass obturator (Plastics One, Roanoke, VA, USA) to prevent damage chewing and scratching when the degus were placed with their cagemate.

Degus were housed singly for 3-5 days after surgery for recovery during which degus received daily doses of ketoprofen administered via intraperitoneal injection during the first three days of post-op. After the recovery period, degus were then housed with their cagemate for at least 6 days before testing. Each degu within the pair was implanted with bilateral guide cannulae.

3.2.4 Pharmacological manipulation

In this study, the oxytocin antagonist (ornithine vasotocin analog) desGly–NH2,d(CH2)5[Tyr(Me)2, Thr4]OVT (Bachem, Torrance, CA) was utilized, as it has been reported to be highly selective for oxytocin receptors through in-depth analysis and comparisons of pharmacological properties of different types of oxytocin receptor antagonists such as affinity and binding potential (Manning et al., 2008). OTR-A was dissolved in artificial cerebrospinal fluid to a full concentration of 0.15 ug/ul as used in Lukas et al. (2011) and a half-dose

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concentration of 0.075 ug/ul to investigate a dose-dependent effect. The same aCSF was used for the vehicle infusions.

3.2.5 Intercerebroventricular microinfusions

Many studies have opted towards microinfusing animals either using a restraint device or anesthesia. However, as the experiment requires multiple days of infusion, to avoid adverse effects such as stress, anxiety or lethargy from anesthesia I opted to allow the degus to move freely on the experimenters' lap while receiving a limited amount of preferred food (sunflower seeds). During habituation days 1-3, degus were handled and experienced the removal of the brass cap and guide cannula, as well as insertion of the infusion needle. This allowed for smooth infusions during the experiment and reduced stress to the animal.

Twenty minutes prior to reunion, an infusion needle (Plastics One, Roanoke, VA, USA) was inserted into the guide cannula. The infusion needles extended 1.00 mm beyond the guide cannula into the ventricle. Due to thickness of corpus callosum, the second batch of experimental animals received an infusion needle that extended 1.5 mm beyond the tip to ensure penetration into the cerebral ventricle. Using a microinfusion pump (Harvard Apparatus, South Natick, MA), 5 ul of aCSF or OTR-A was infused at a rate of 0.5 ul/minute into the ventricle. After the infusion the degu was placed in its homecage for at least ten minutes prior to reunion. Each degu was infused with aCSF, full-dose OTR-A and half dose OTR-A unilaterally and counterbalanced for ventricle side infused first.

3.2.6 Testing Procedure

In this experiment, OTR-A, half-dose OTR-A and aCSF were infused prior to reunion after a 24 hour isolation. To avoid effects of chronic isolation and to allow for a drug wash-out period, degus were co-housed for 24 hours in between each testing day (Fig.9).

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3.2.7 Histology

According to previous studies utilizing I.C.V infusions in non-standard rodent models (Burkett et al., 2016; Mooney and Holmes, 2015), India ink was used to examine the spread of ventricular infusion (n = 3 pairs). 1-2 days after testing, 5 ul of 40% India Ink (v/v in aCSF) was injected unilaterally in 6 degus. Brains were quickly extracted and post-fixed in 4% PFA for 4 hours. Brains were then cryoprotected in 30% PBS sucrose until saturated. Brains were then sliced coronally (40 um), mounted and stained with cresyl violet. Cannula placement was confirmed after viewing brain slices under a light microscope (Fig. 10)

3.2.8 Statistical analyses

Normality of all data were determined using a Kolmorgov-Smirnov test. Where data was distributed normally, a one-way repeated measures ANOVA was used. Otherwise, non-normal data was analyzed using rank-transformed one-way repeated measures ANOVA described in section 2.2.6. Significant differences in overall ANOVA were followed by post hoc pairwise comparisons with bonferroni correction. Specifically, aCSF conditions were compared to half-dose OTR-A and full-dose OTR-A administration.

3.3 Results

3.3.1 Histology

Cannula locations varied moderately across subjects. Correct placement was determined by measuring if the guide cannula tip was within 1 mm of the dorsal portion of the lateral ventricle. Inspection of India ink infusion revealed that the ink diffused throughout the ventricle into the forebrain and also minor staining in the third ventricle. In this experiment, all cannulas were deemed to be correctly placed.

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3.3.2 OTR-A effects on social behaviour

Degus exhibited no significant differences in overall time spent body grooming (Fig.11A, $F(5,30)= 0.2566$, $p = 0.8167$), face to face interaction (Fig. 11C, $F(5,30)= 1.228$, $p = 0.329$) and rear grooming (Fig. 11D, $F(5,30) = 1.051$, $p= 0.388$) during reunion after 24 h isolation, between aCSF, OTR-A and half-dose trials. In addition, no significant difference was found in the number of bouts (frequency) of body grooming (Fig. 11B, $F(5,30)= 0.3695$, $p=0.6716$), face-to-face interaction (Fig. 11D, $F(5,30)= 0.5638$, $p=0.6424$) and rear grooming (Fig. 11F, $F(5,30)= 0.9005$, $p=.4590$). Furthermore, no significant difference was observed across all non-social behaviours including self-grooming, freezing, digging and eating (Fig. 13, $F(5,30)= 1.223$, $p=.3305$).

3.3.3 OTR-A effects on vocalizations

Degus showed a significant change in the number of vocalizations emitted between OTR-A and aCSF conditions, (Fig.14 &17, $F(5,30) = F(5,36) = 3.651$, $p = 0.009$). Specifically, significant reductions in all vocalizations were observed during OTR-A #1 compared with aCSF #1 control ($p = 0.01$) and OTR-A #1 vocalizations were significantly reduced when compared to aCSF #2 control, ($p = 0.026$), however vocalizations did not differ significantly between OTR-A # 1 vs. Half-dose #1 ($p=0.11$) and Half-dose #2 ($p=0.593$). Further, no significant differences were observed between OTR-A#2 and aCSF #1 ($p=0.870$), OTR-A #2 vs. ACSF #2, ($p = 1.00$), OTR-A #2 vs. Half-dose #1, ($p = 1.00$) or OTR-A #2 vs. Half-dose #2 ($p =1.00$).

3.3.4 Lateralized OTR-A function

When vocalization data were analyzed according left or right ventricle infusion, significant differences in vocalizations were observed (Fig 15, $F(5,36)= 3.21$, $p=0.027$).

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Particularly, when OTR-A was infused in the left ventricle compared to left infusion of aCSF ($p=0.071$) and left OTR-A vs. right aCSF ($p= 0.031$). No significant differences were found between right OTR-A infusion and left aCSF ($p=0.535$), and right OTR-A vs. right ACSF ($p=0.795$).

3.4 Discussion

The aim of this experiment was to investigate whether oxytocin plays any role in vocalizations or grooming behaviour exhibited during a socially rewarding interaction. Our results indicate the OTR-A has an inhibitory effect upon production of vocalizations emitted during social reunion after 24 h isolation (Fig. 14). These results indicate that oxytocin plays a modulatory role in the production of vocalizations during a socially rewarding encounter in female degus. Further analysis revealed that the oxytocin antagonist seemed to attenuate positive and neutral vocalizations but not negative types across conditions (Fig. 17 & Fig. 18). This indicates that oxytocin may modulate the production of voluntary vocalizations but not reflexive vocalizations such as those expressing pain or distress. In contrast, blocking central oxytocin receptors had no effect on the frequency or duration of any type of grooming behaviour compared to the aCSF control (Fig.11). In addition, while locomotion behavioural data were not collected, no change was observed in non-social behaviour (including digging, freezing, inactivity and eating) across conditions (Fig.13), indicating that the change in vocalizations are not due to reduced motor output. These findings suggest that oxytocin specifically modulates vocalization production in degus, but not social grooming behaviour.

Accordingly, these data do not fall in line with rodent literature implicating oxytocin as having an integral role in motivating social approach behaviours (Burkett et al., 2016; Dölen et

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al., 2013; Ross et al., 2009) A potential explanation may be that oxytocin is necessary for the degus motivation to vocalize, or express social reward but not for social grooming. Furthermore, the unique oxytocin receptor distribution in degus may be specialized for vocalization production (Beery et al., 2016). Autoradiographical analyses of oxytocin receptor distribution in the degu brain revealed high concentrations of oxytocin receptors in the medial prefrontal cortex (infralimbic and anterior cingulate) regions but virtually no expression in the nucleus accumbens (Beery et al.,2016). The nucleus accumbens oxytocin receptors have been shown to motivate social behaviour in voles and mice (Dölen et al., 2013; Olazábal and Young, 2006). However, the prefrontal cortex is known to have volitional control over the production of vocalizations in monkeys (Hage and Nieder, 2013), birds and frogs. Hence, it is possible that the social approach and social grooming behavioural circuit may not be dependent on oxytocin in degus but the vocalization pathway is modulated by oxytocin. Alternatively, the role of arginine-vasopressin (AVP) in socially motivated behaviour also has species-specific effects (Bachner-Melman and Ebstein, 2014; Donaldson and Young, 2008; Hammock and Young, 2006). Investigating the species-specific effect of AVP on social grooming in degus may be an important future investigation.

Some neural evidence from vocal species does suggest that oxytocin and arginine-vasopressin (AVP) may play important roles in modulating vocalization production. For example, a correlational study was conducted on the arginine-vasopressin (AVP) receptor expression in two species of singing mice (*S. Teguina* and *S. Xerampelinus*). In this study they found levels of AVP receptors in the key regions of the vocal production pathway (periaqueductal gray and anterior hypothalamus) to be significantly higher in the more vocal species of singing mice (Campbell et al., 2009). In humans, evidence suggests that social vocalizations result in a release of central oxytocin in mother-daughter dyads without any

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physical touch, hence it is likely to play some role in the modulation of social vocalizations (Seltzer et al., 2010). Furthermore, investigation of homologues of oxytocin such as isotocin have been shown to modulate vocalizations in female teleost fish (Goodson and Bass, 2000b). Similarly, isotocin receptor distributions were found to be exclusively distributed within vocal-acoustic circuitry in plainfin midshipman fish and manipulations of isotocin in this species also modulated vocalization output (Goodson et al., 2003). However, our experiment targeted all central oxytocin receptors, hence it remains unclear as to which brain region in the degu is sensitive to OTR-A during the production of vocalizations. While investigations of oxytocin receptor distribution revealed high levels of expression in the medial prefrontal cortex, other important vocal-production regions such as the periaqueductal gray and reticular formation were not assessed. Hence, investigation into the presence of oxytocin receptors in these regions are an important future direction. However, as the ACC of medial prefrontal cortex has been suggested to be an essential component in the production of voluntary vocalizations, it is likely that oxytocin receptors in the medial prefrontal cortex may modulate motivation to vocalize within this species. In our results, degus are not completely muted as a result of OTR-A infusions, they are still able to vocalize. However, their motivation to vocalize is likely attenuated due to blocking of oxytocin receptors, similarly to studies in macaques in which lesions of the ACC prevent voluntary vocalizations but not those emitted in response to a threat. Neural recordings using local field potential or single-unit recording techniques in in the three key regions (ACC, periaqueductal gray and reticular formation) combined with oxytocin receptor manipulations could reveal the site-specific role of oxytocin on the vocal production pathway of degus.

As degus are a novel animal model for neural manipulation, methods for a successful cannula implant required some trial and error. The present data suggest that degus with shallower cannula placements appear to show a larger reduction in vocalizations emitted when OTR-A was

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applied to the left hemisphere (Fig 15 & 16). This was not due to an order effect, as left and right were counterbalanced across batches. Furthermore, inspection of dorso-ventral placement revealed no differences between the left and right side. As only 4 pairs of the 7 displayed shallower cannula placements, further experiments will be conducted to determine if there is a true effect of blocking left hemispheric oxytocin receptors upon vocalization production in degus. This very preliminary finding is in line with the literature describing left-lateralized neural circuitry underlying vocalization production in humans (Blank, 2002), primates (Cantalupo and Hopkins, 2001) , birds (Chirathivat et al., 2015) and frogs (Bauer, 1993). Particularly, the left medial prefrontal cortex in humans/non-human primates and its homologues in birds and frogs have been shown to be necessary for vocalization production. Specifically, humans demonstrate left-dominant lateralization of speech production through a prefrontal cortical region known as Broca's area. Lesions in Broca's area specifically result in the loss of speech production but speech perception is intact if Wernicke's area (temporal cortex) remains intact (Blank, 2002). Furthermore, in primates such as the squirrel monkey (Jürgens, 2009), rhesus monkey (Hage and Nieder, 2013, 2015) and chimpanzee (Taglialatela et al., 2008), an area homologous to the human Broca's area is the ventrolateral prefrontal cortex (VLPFC) and is implicated in volitional control of vocalization production. Research on these primate species have also documented left-lateralized neural activity in the VLPFC underlying vocalization production. (Hage and Nieder, 2013) Specifically, lesions within the VLPFC of squirrel monkeys affect motivation to vocalize, and single unit recording within this area in rhesus monkey demonstrate that VLPFC neurons encode planning of vocal output and integrates existing auditory information (Hage and Nieder, 2015). In birds the HVC, the avian homologue to Broca's area also display functional lateralization as evidenced by research in zebra finches (Moorman et al., 2012). Altogether, this evidence across species points towards a conserved neural circuitry specifically in the left

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hemisphere for vocalization production. In-depth investigation of the role of oxytocin in vocalization production during positive peer-to-peer interactions has not yet been fully investigated in standard lab rodents let alone degus. The specificity of degu behaviour and range of vocalizations demonstrated by these experiments as well as their capacity to be co-housed with cannula implants point towards degus being an especially useful animal model for studying social communication in the future.

4 General Discussion

Overall, these experiments identify the differential effect that two distinct stressors have on degu vocalizations and social interactions, as well as the role of oxytocin in these behaviours. Experiment 1 revealed that degus exhibit increased vocalizations when reunited with a conspecific explicitly after 24-hour isolation. Whereas, degus exhibit significant increases in rear grooming behaviours both after 24-hour isolation and distress, indicating that grooming behaviour may have a stress buffering function, rather than being an explicit expression of social reward in degus. Several previous studies have discussed social grooming behaviour as a form of social buffering, a process that underlies empathy. In particular, increased social grooming after a painful stimulus has been termed “consolation behavior” in voles, where increased grooming is directed to a stressed mate (Burkett et al., 2016). While prairie voles are often used to understand sexual pair bonding, the social interactions studied in our experiments all occur in adult sibling pairs in which behaviour is not sexually or parentally motivated. Furthermore, our findings raise a possibility that allogrooming in degus is also a consolation response in which degus that have experienced the same level of pain direct allogrooming towards one another. Hence, indicating that degus can potentially display emotional contagion and affective empathy to a conspecific, as they are able to recognize distress in others and display motivated behaviour to reduce stress. However, the physiological reduction of stress in this species as a result of

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allogrooming remains to be established in future studies. Furthermore, our study highlights a key advantage over traditional lab species as our experiments are conducted in adult degus, whereas vocalization studies in rats and mice are conducted in juvenile or adolescents as they show significantly more sociality than adults (Panksepp and Lahvis, 2007). Hence, studies using degus can assess naturalistic social behaviour without selecting for certain genetic traits or restrict experiments to a specific developmental time window.

While both stressors elicited some overlap in behavioural interactions, the increased vocalizations during reunion may be a more specific assay for studying neurobiological mechanisms of socially rewarding interactions in degus. While many different types of vocalizations were emitted across conditions and across pairs, a specific temporal selectivity for chittering during face-to-face interaction after a 24 h isolation was observed. This behavioural/vocal interaction may act as a social reunion "call" or expression in degus. Expression of a socially rewarding interaction after social stimuli deprivation is an important process underlying social behaviour. This behaviour has not been investigated at the level of social communication in any other adult rodent species. This unique behaviour in degus could be used as a healthy social phenotype to investigate neural processes underlying abnormal social motivation and social communication. As these are the first investigations into socially rewarding behaviour and vocalizations in adult degus; findings from this study can be extended to understand whether these chitter vocalizations are simply due to social deprivation or act as a true reunion call between conspecifics. In order to address this question, we plan to test degus who are housed with three other cagemates. In this experiment, a litter of four would be divided into two pairs and separated for 24 hours. In this case, the degus would not be socially isolated, but would be separated from a conspecific. Next, the separated conspecifics would be reunited in single pairs. If chitters are specific to reunion with a conspecific, we expect comparable numbers

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of chittering between social isolation and social separation conditions. This experiment will yield further understanding into the specific function of chitter vocalizations in degus.

In experiment 2, our findings indicate that oxytocin modulates the production of vocalizations potentially by affecting motivational processes to vocalize or by modulating neural regions responsible for the gating and integration acoustic-vocal information. In order to understand which neural region is responsible for the observed effect, future investigations can focus on recording neural activity from regions such as the ACC, PAG and reticular formation of degus with oxytocin manipulations. These studies can help elucidate which level of the vocal production pathways are hormonally modulated as this neural process in mammals currently remains unknown.

Furthermore, as described previously, endorphin/opioid release after social contact is a strong reinforcing stimulus for social interaction (Uvnäs-Moberg, 1997). Based on our findings, a possible future investigation into whether the oxytocin system interacts with endorphin release to modulate the volitional control of vocalizations. By using naloxone These future investigations may yield important results as the role of oxytocin/opioid interactions in the production of affective vocalizations is completely unknown.

Altogether, these findings have important implications for understanding distinct neural mechanisms underlying differentially motivated social behavior. This study shows that stressors elicit different behavioural and vocal profiles in degus, which are important for understanding the neural basis of social cognition. Furthermore, the role of oxytocin in social communication has important implications for understanding mechanisms underlying vocal communication, and can be further studied in this species.

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5 Conclusion

Altogether, this study demonstrates novel findings about the potential role of oxytocin, social isolation and physical distress on social behaviour and vocalizations in the degu animal model. It was found that a longer period of social isolation results in increased social vocalizations and that there is a selectivity for chitters during face to face interactions during social reunion. Further, distress alone is not sufficient to induce vocalizations during reunion, as our data suggest that vocalizations are only significantly increased after social isolation, not exposure to a physical stressor. Physical distress does however, increase certain types of social grooming comparable to increases observed after 24 h isolation, indicating a possible social buffering function of grooming behaviours. Lastly, blocking central oxytocin receptors in degus resulted in a specific decrease in vocalizations and not grooming behaviour, indicating that oxytocin may specifically modulate the production of vocalizations in degus.

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Tables

Behaviour	***Definition***
Face to face interaction	Time spent in nose to nose contact (in seconds)
Body grooming	Time spent nuzzling/grooming other's body (in seconds)
Rear grooming	Time spent in nose-to-rear contact (in seconds)
Agonistic behaviour	Time spent biting, mounting and pushing (in seconds)
Inactivity	Time spent immobile (in seconds)
Huddling	Time spent laying on top or leaning on cagemate (in seconds)
Freezing	Time spent completely motionless with tense body posture (in seconds)
Bathing in bedding	Time spent rolling in bedding (in seconds)

Table 1: Operationally defined behaviours scored during social reunion

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Figures

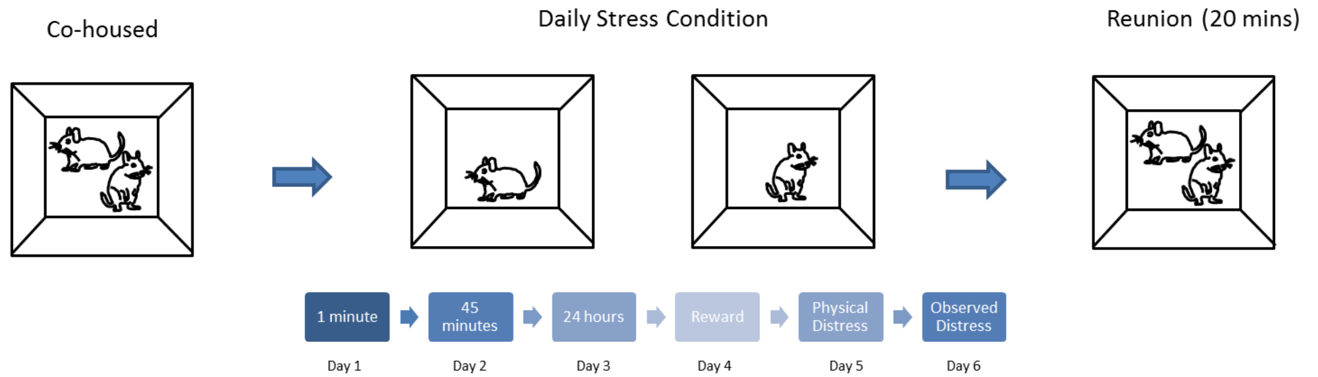

Figure 1: Reunion Timeline Degus original homecages were placed within the reunion box. Then after social isolation or distress, degus were placed individually back into their homecage within the reunion apparatus and video and audio was recorded.

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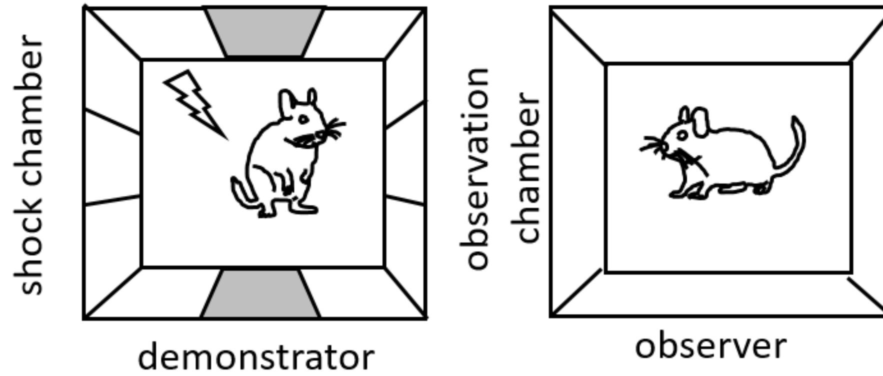

Figure 2: Distress/Observed Distress Apparatus: Degu was placed alone in shock context during distress condition and underwent the shock protocol. During the observed distress condition, the demonstrator degu underwent the shock protocol in shock context while the observer is in adjacent chamber.

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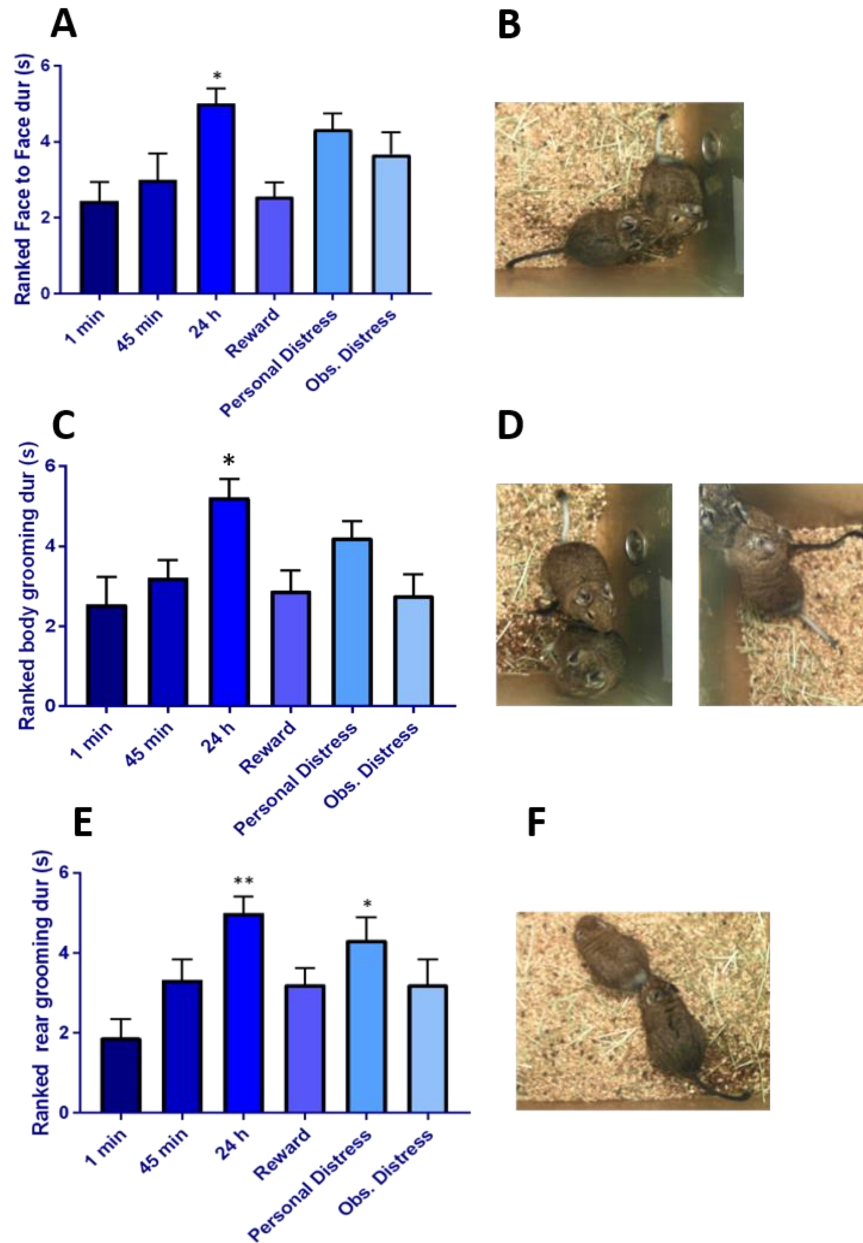

Figure 3: Duration of social behaviours across conditions (n = 9 pairs). A. There were significant differences in observed in face-to-face interaction across conditions ($F(5,48) = 4.09$, $p = 0.004$) specifically after 24 hour isolation ($p=0.01$). B. Depiction of face-to-face interaction C. Body grooming across conditions differs significantly ($F(5,48) = 4.08$, $p = 0.004$) specifically after 24 hour isolation ($p=0.01$). D. Depiction of body grooming. E. There were significant differences in Duration of nose-to-rear interaction across conditions ($F(5,48) = 4.66$, $p = 0.002$) specifically after 24 hour isolation ($p=0.001$) and personal distress ($p=0.02$). F. Depiction of rear grooming

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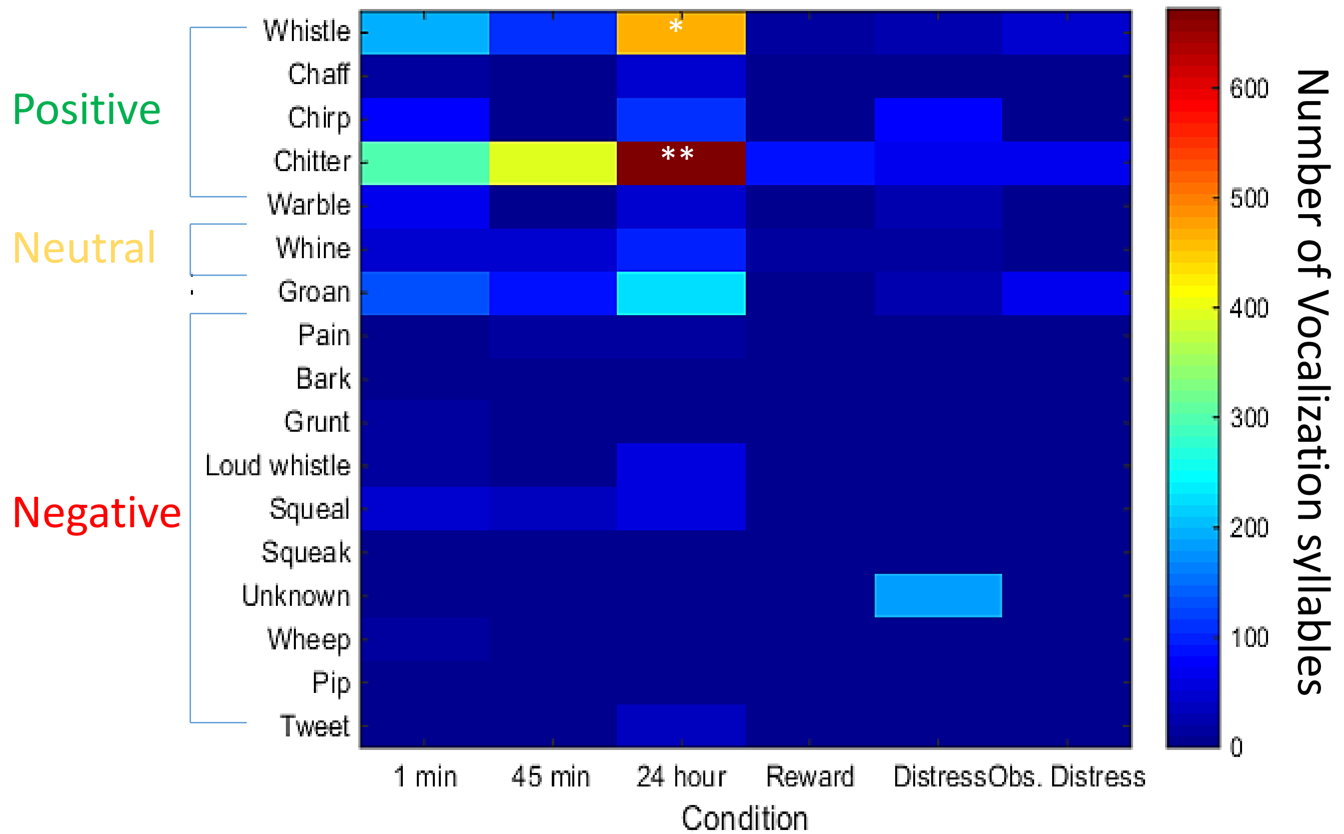

Figure 4: Distribution of each vocalization across conditions. Selective increases in vocalizations as chitters during 24 hours were observed ($\chi^2(80, N=102) = 505.378$, $p < 0.0001$) and whistles ($\chi^2(80, N=102) = 335.67$. $p<0.05$))

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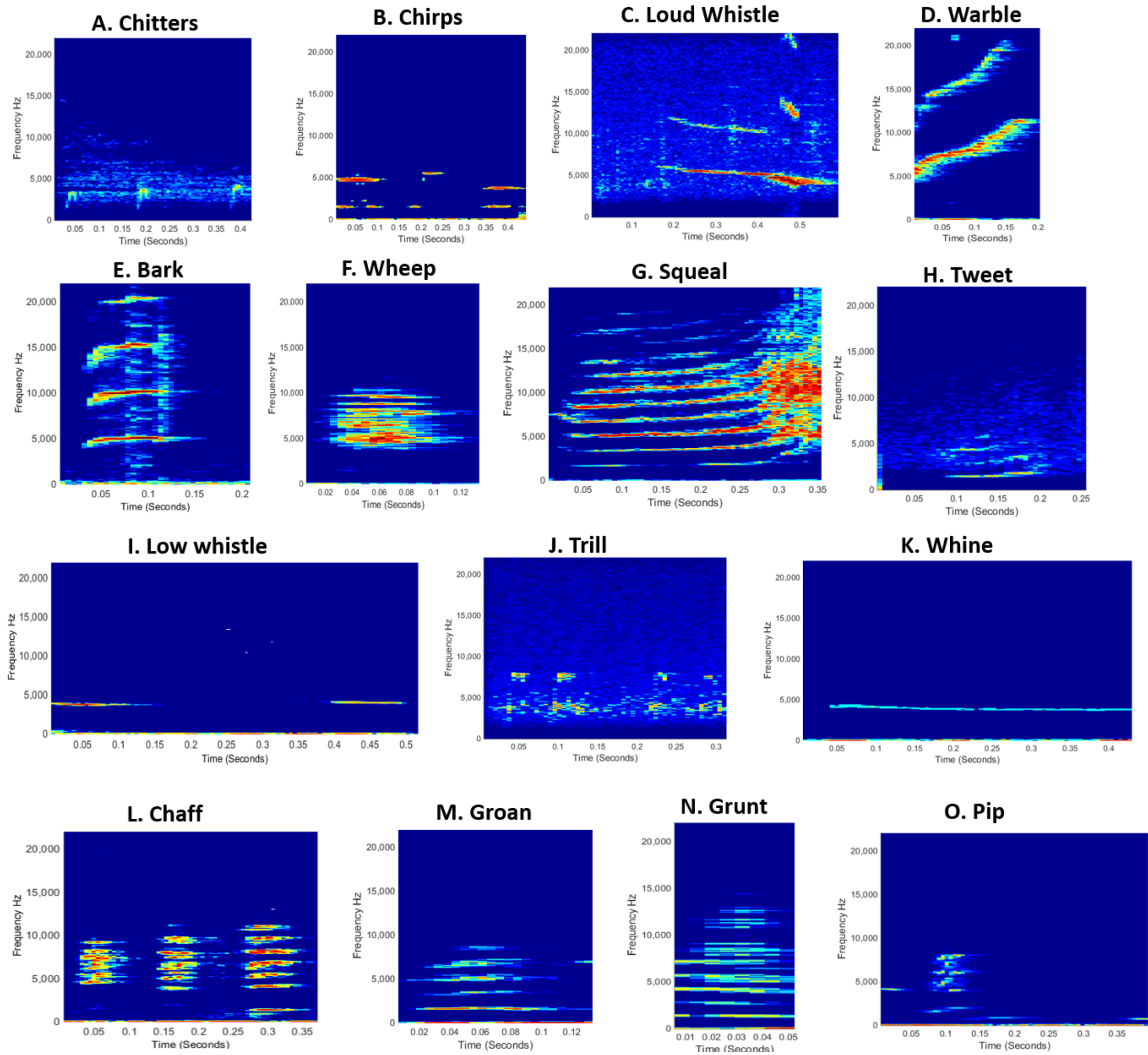

Figure 5: Vocalization types emitted across conditions (Frequency (Hz), Time (s)).

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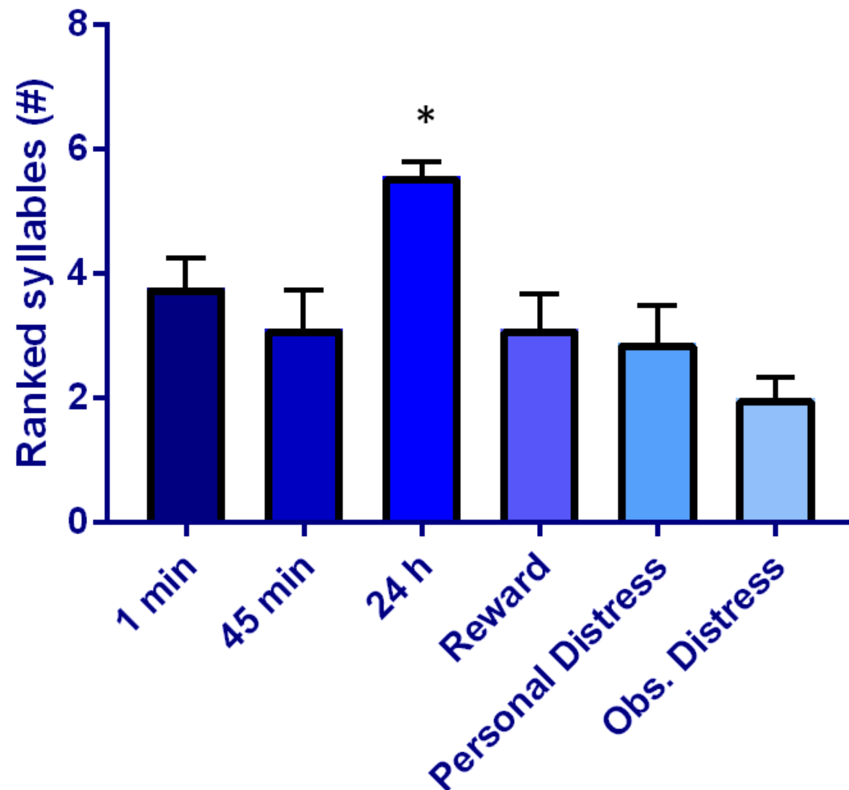

Figure 6: Number of syllables across conditions (n= 9 pairs). Overall significant differences in number of vocalizations emitted (F(5,48) = 6.053, p = 0.0001). Vocalizations were significantly increased after 24 hour isolation compared to 45 minute isolation (p = 0.013), Reward (p= 0.013), Personal Distress (p = 0.005), and observed distress (p = 0.0001) and trended towards significance against 1 minute (p = 0.16).

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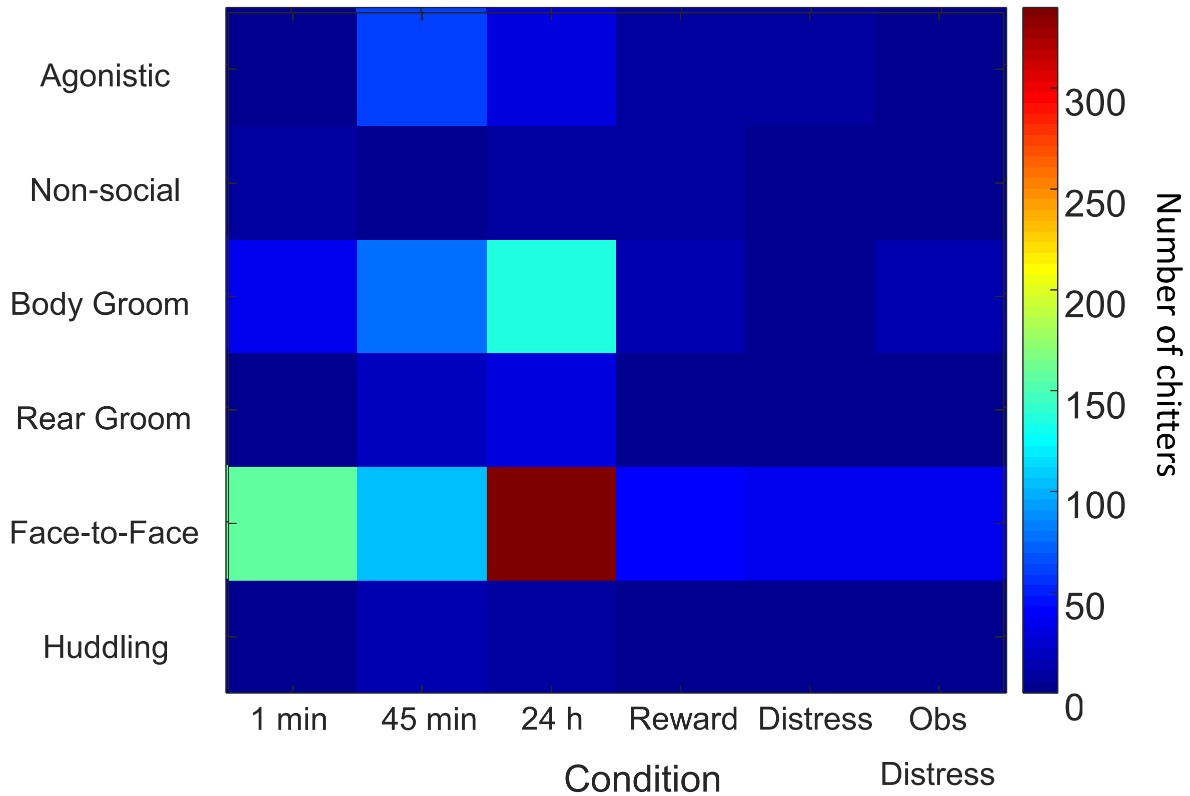

Figure 7: Temporal co-occurrence of chitters with social behaviour (n=9 pairs). Heatmap depicting temporal co-occurences of different social behaviours and emission of chitter vocalizations across pairs. (4kHz, ~20 ms) Highest co-occurences were of chitters were observed during Face-to-Face interaction during reunion after a 24 hour isolation ($\chi^2(25, N=36) = 215.8$, $p < 0.0001$)

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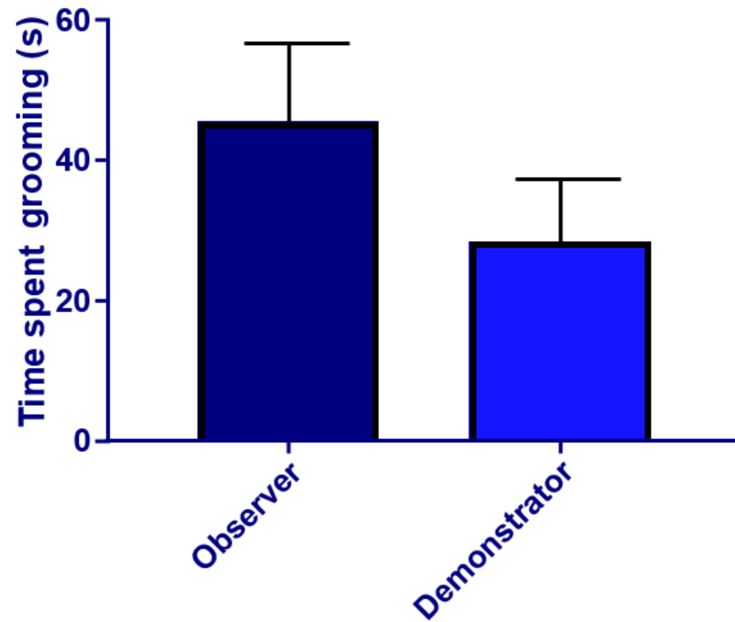

Figure 8: Duration of all social grooming initiated by observers and demonstrators (n= 9 observers n= 9 demonstrators). No significant difference between grooming behaviour initiated by observers vs. demonstrators (t =1.187, p= 0.2526)

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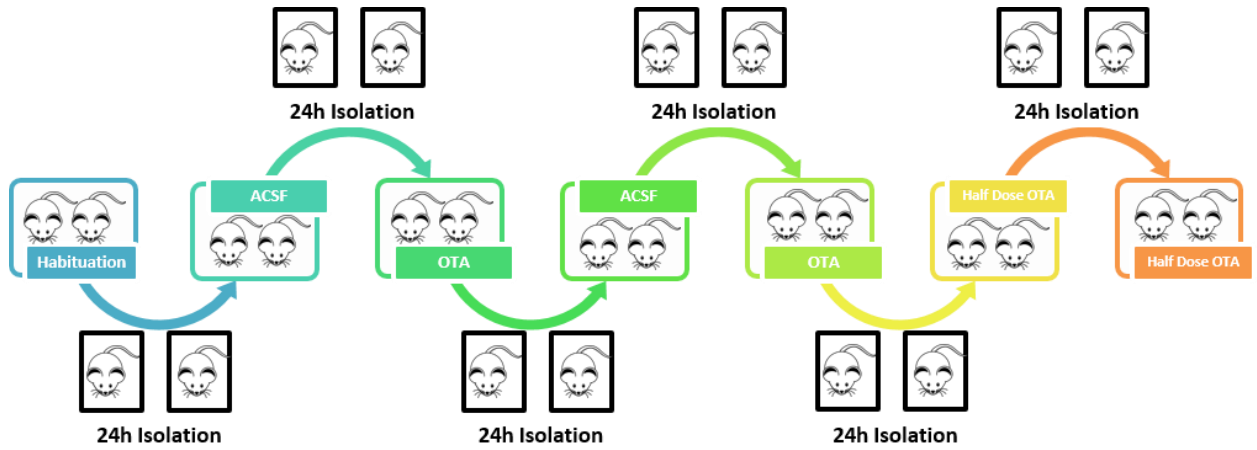

Figure 9: Infusion schedule. Counterbalanced infusions were conducted across conditions.

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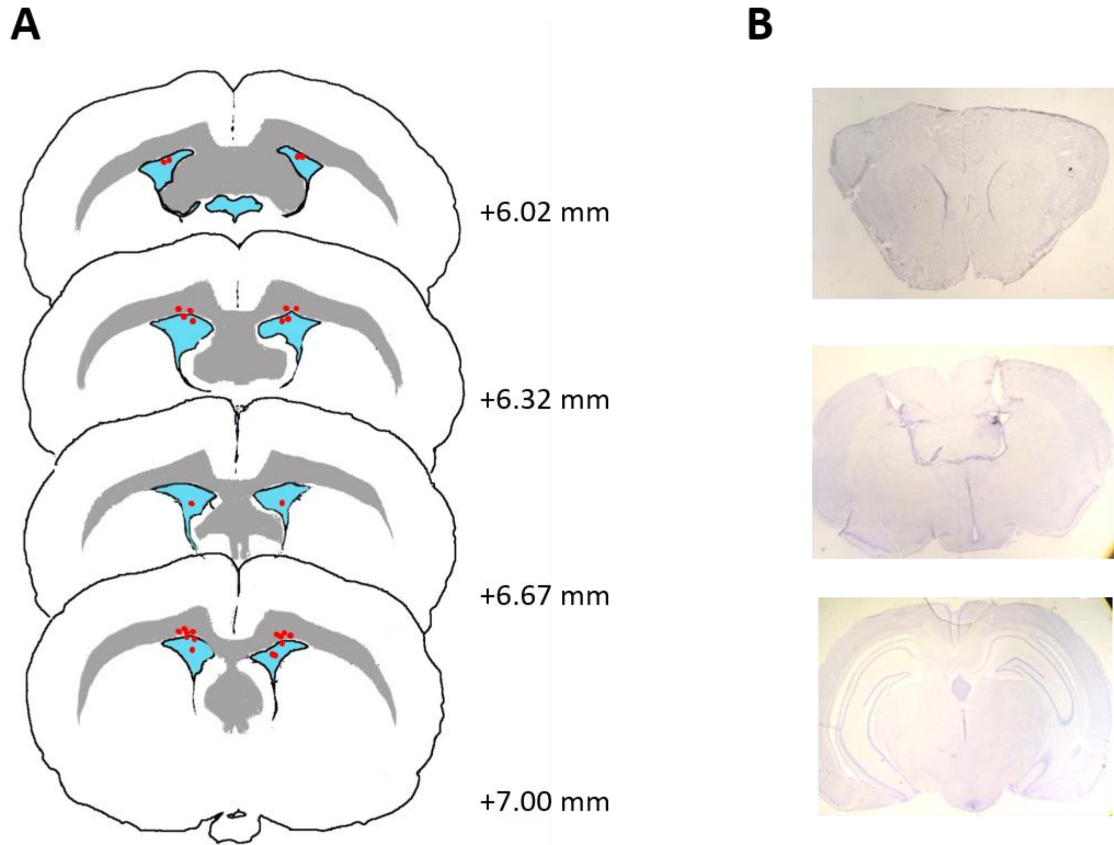

Figure 10: Bilateral Cannulae placement and Ink diffusion A Cannula were placed in the lateral ventricles (N=14). Red markers represent tip of infusion needle extending 1 or 1.5 mm from guide cannula. B India ink lining ventricles after infusion in the frontal cortex, lateral ventricle and third ventricle.

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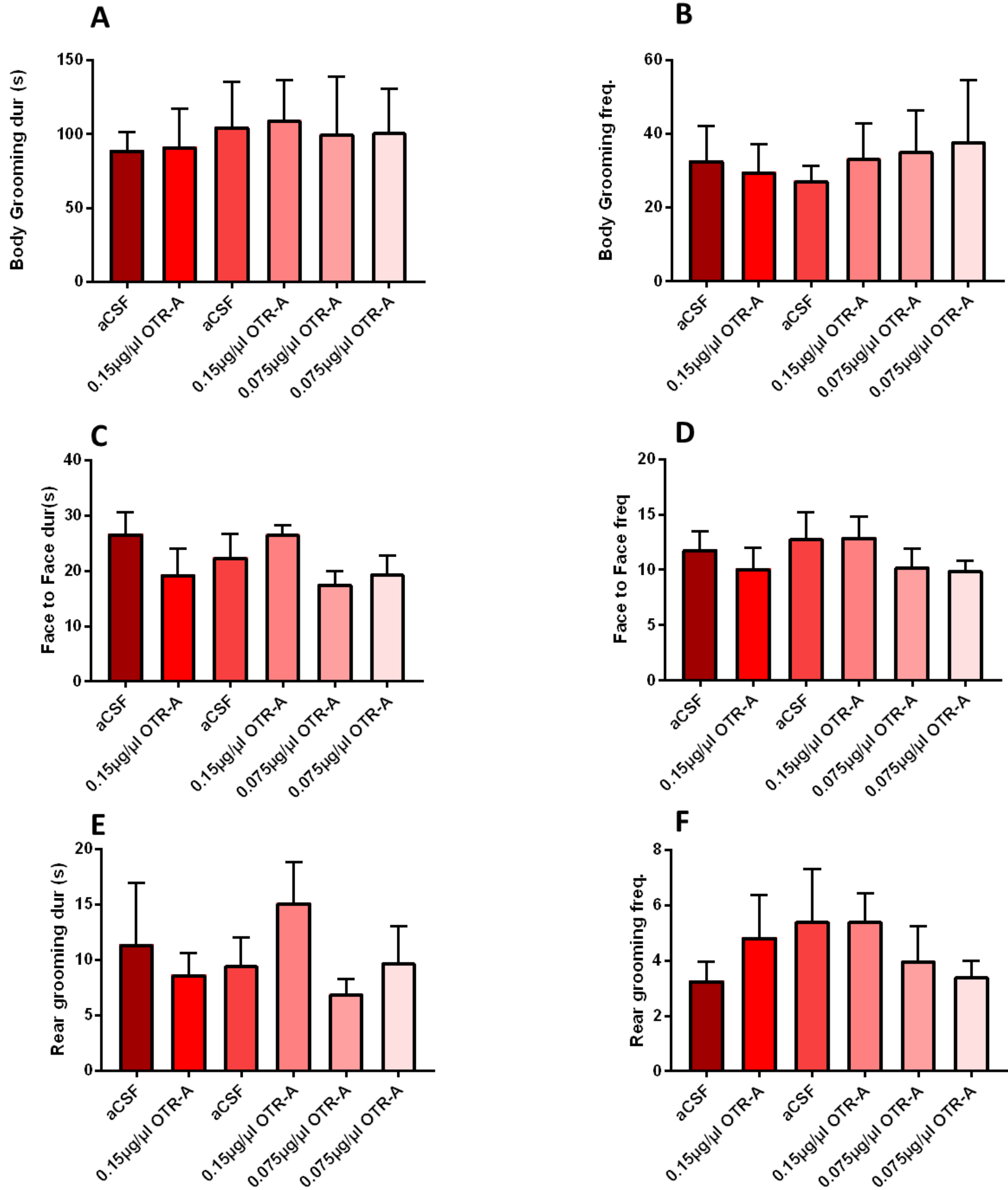

Figure 11: Duration and frequencies of grooming behaviour after infusions A-F (n=7 pairs). No significant differences were observed across durations or frequencies of social grooming behaviour across conditions.

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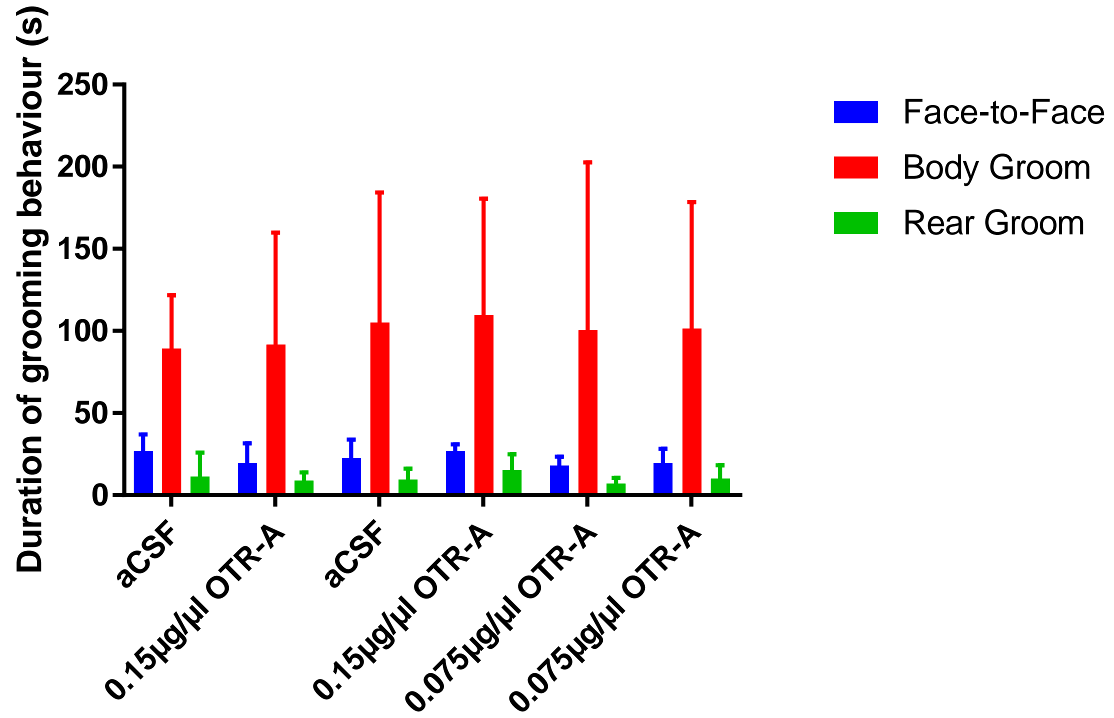

Figure 12: Relative grooming behaviours during aCSF, half-dose and full-dose OTR-A (n=7 pairs). No significant differences across conditions.

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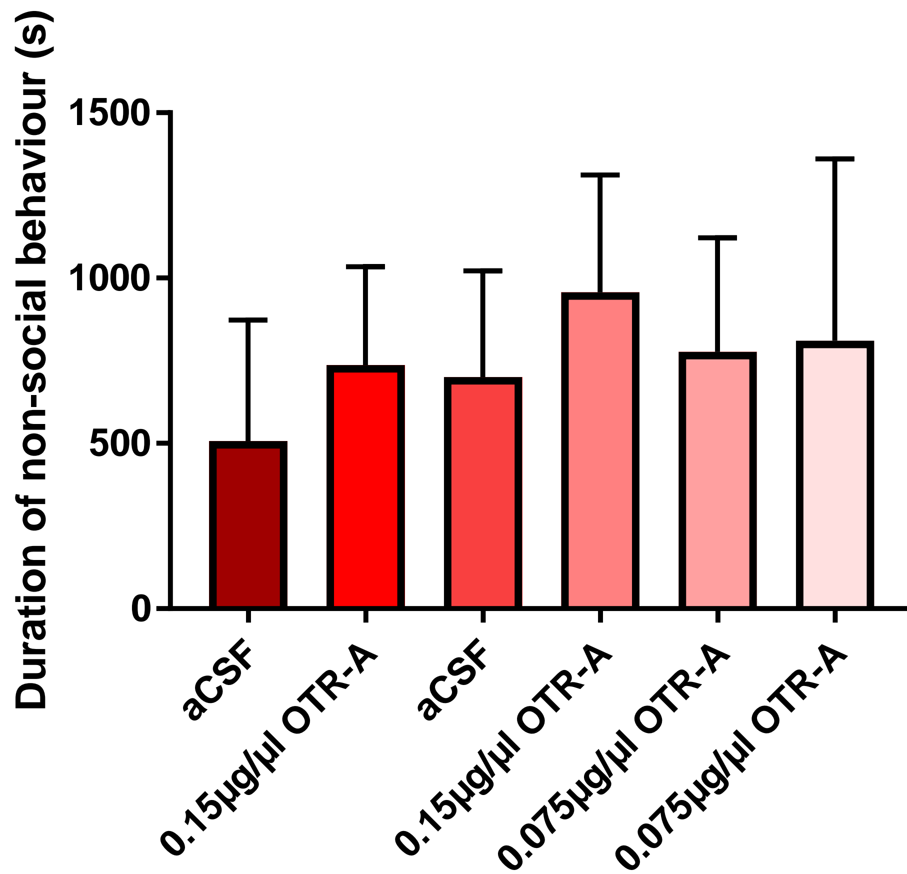

Figure 13: Duration of Non-social behaviours during aCSF, half-dose and full-dose OTR-A (n=7 pairs) No differences were observed in time spent engaging in non-social behaviours such as freezing, inactivity, eating and bathing in bedding.

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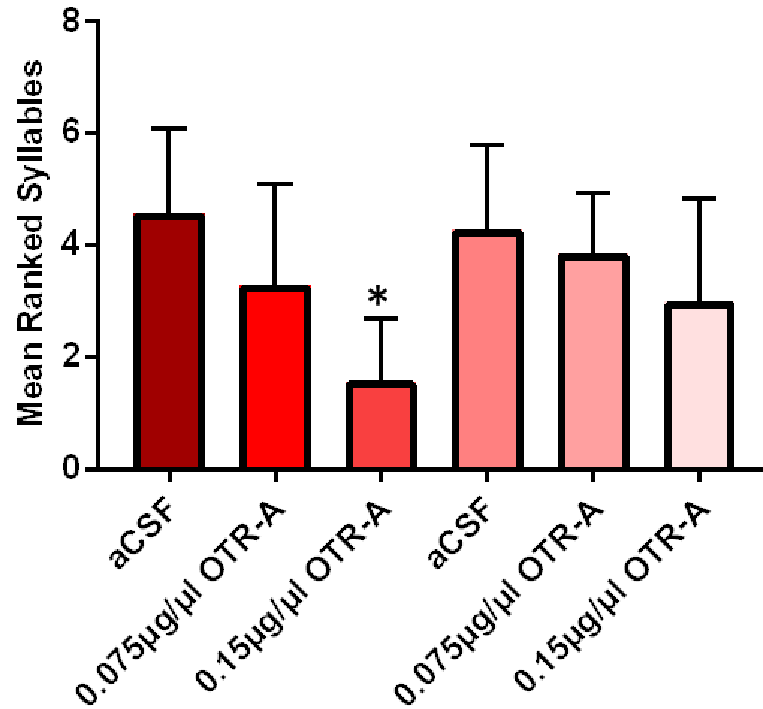

Figure 14: Number of syllables emitted during aCSF, half-dose and full-dose OTR-A (n = 7 pairs). Significant differences were observer across conditions ($F(5,36) = 3.651$, $p = 0.009$). Specifically, vocalizations were decreased at OTR-A dose 1 compared to aCSF#1 (p=0.01) and aCSF#2 (p= 0.026). OTR-A dose #2 was non-significantly different from all other conditions.

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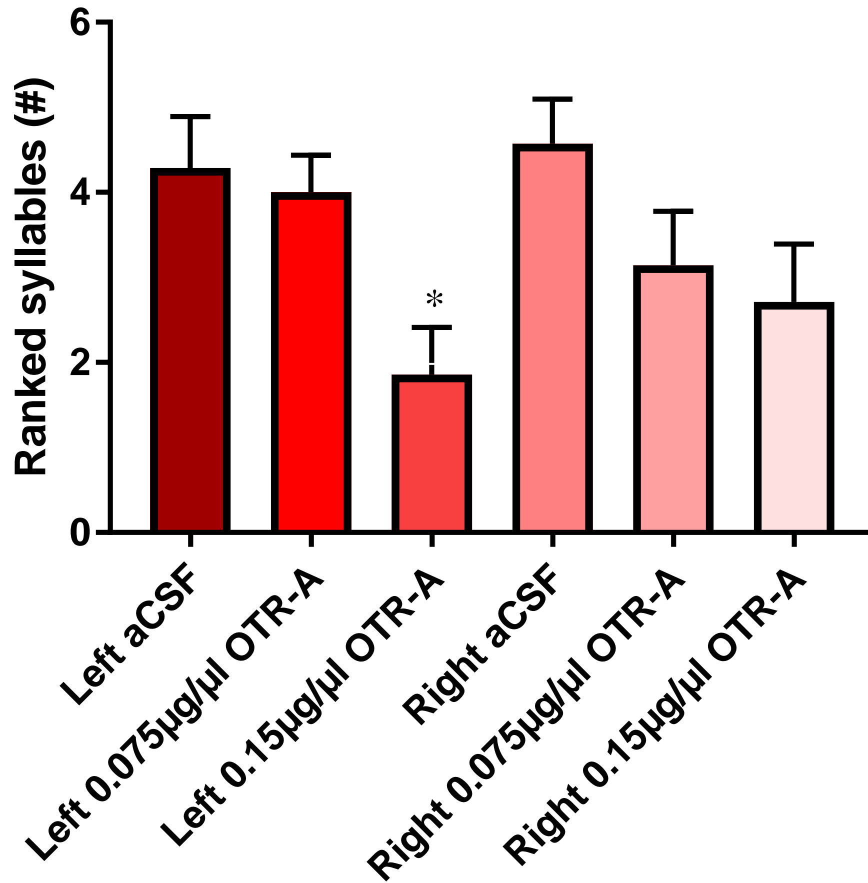

Figure 15: Syllables emitted across Left vs. Right ventricular administration of aCSF, half-dose and full-dose OTR-A (n=7 pairs). Significant differences were observed across conditions (F(5,36) = 3.651, p = 0.02). Specifically, vocalizations were significantly different between left OTA and and Left ACSF (*p*=0.003) and Right aCSF (*p*= 0.026). Right OTA was non-significantly different from all other conditions.

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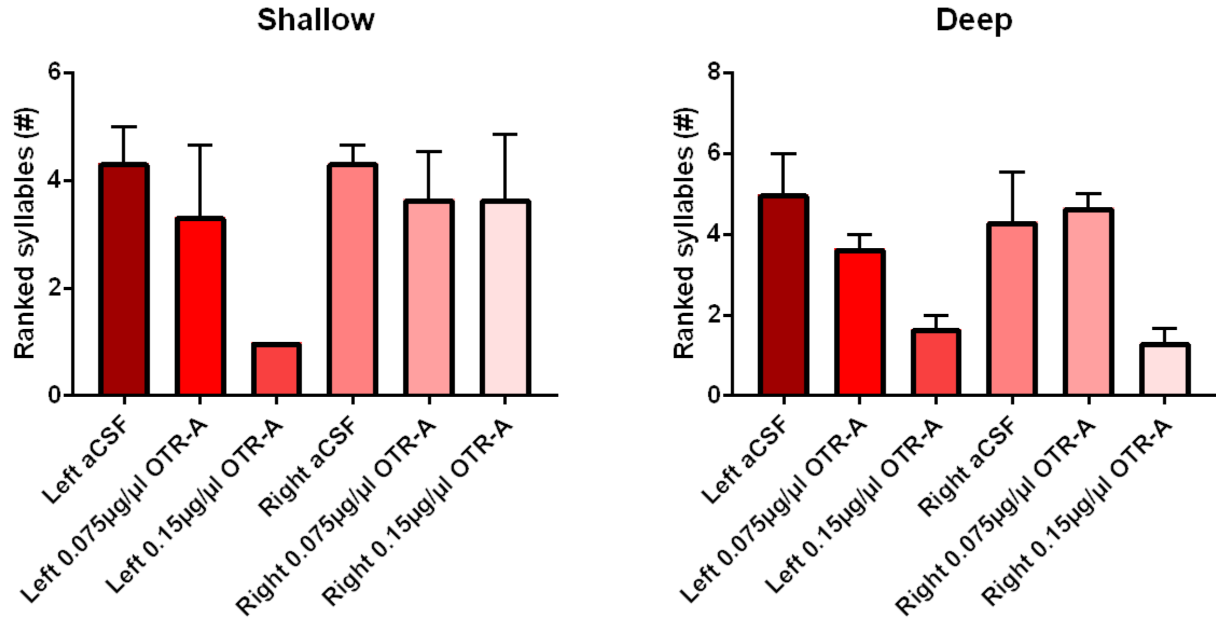

Figure 16: Syllables emitted across Shallow vs. Deep administration of aCSF and OTR-A concentrations. Spread of drug may have depended on the depth of cannula placement, leading to a left-hemisphere specific effect of OTA. Whereas deeply placed cannulas are most likely to spread to both hemispheres and show equal effect of OTA. (shallow, N= 3 pairs) (deep, N= 4 pairs)

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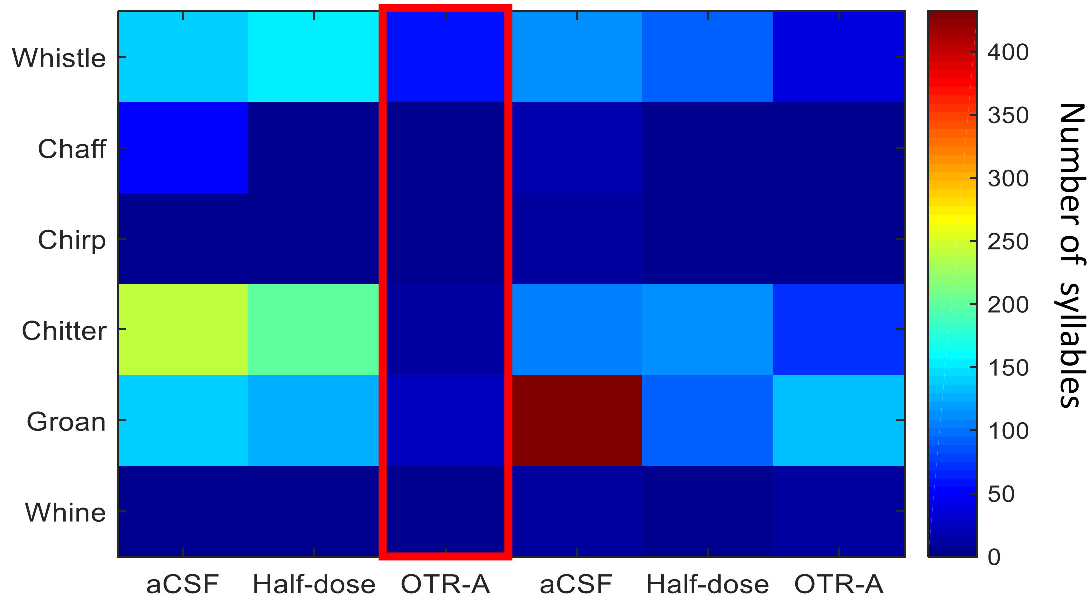

Figure 17: Positive and Neutral syllables emitted across conditions. Heat map depicting the distribution of positive and neutral vocalizations emitted during first OTR-A administration.

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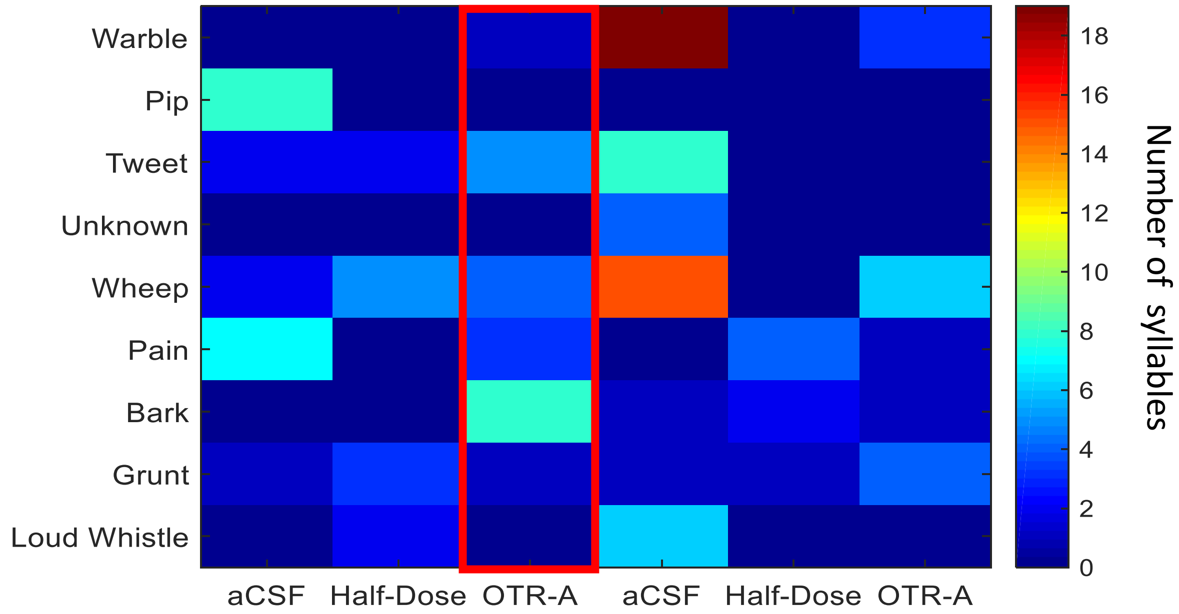

Figure 18: Negatively valent vocalizations emitted across conditions. Heat map depicting the distribution of negative vocalizations during first OTR-A administration

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